Management of Valvular Heart Disease during Pregnancy

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Heart disease complicates approximately 0.4-4% of all pregnancies.1-5 Presence of significant heart disease during pregnancy is a high risk situation; it increases the risks of adverse maternal and fetal outcome. Despite advances in the management of maternal cardiovascular diseases, heart disease during pregnancy accounts for as much as one-third of the maternal mortality.6 Furthermore, about 20% of the pregnancies in the presence of valvular heart disease are associated with poor neonatal outcomes.7,8 With the declining incidence of rheumatic fever and the significant advances in the management of congenital heart disease, the ratio of rheumatic heart disease to congenital heart disease in pregnancy has decreased to approximately 1:3 in other parts of the world. Rheumatic heart disease is still the most common heart disease complicating pregnancy in our country.5,9 In this article, we reiterate the salient aspects of management of valve disease during pregnancy.

Cardiovascular Physiology during Pregnancy

Pregnancy is associated with obligatory physiological changes that have an important bearing on the hemodynamics of the underlying heart disease.10,11 A 50% increase in intravascular volume occurs during normal pregnancy by mid third trimester. There is 50% increase in cardiac output, mainly in the first and second trimesters; the level peaks by about 24 weeks of pregnancy. The cardiac output increases by as much as 22% by 8 weeks. In the first trimester, most of the increase is accounted for by an increase in the stroke volume. The nearly 30% increase in stroke volume may be due to ventricular remodeling and a decrease in afterload due to fall in systemic vascular resistance. Later on, the increase in cardiac output is sustained by an increase in heart rate. There is an average increase in heart rate of about 10-20 beats per minute (bpm). The increase in heart rate and cardiac output has a deleterious effect on stenotic lesions.

There is a decrease of 3-5 mmHg in systolic and 5-10 mmHg in diastolic blood pressure during pregnancy, secondary to an even greater fall in peripheral resistance.

The decreased peripheral vascular tone is mediated by hormonal influences, primarily estrogen, prolactin and prostacyclins. Additionally the highly vascular placenta acts as a partial arterial venous shunt. This decreased vascular resistance has a favorable effect on the hemodynamics of regurgitant lesions. Other significant structural changes in the heart during the third trimester include myocardial hypertrophy, chamber enlargement, and mild multivalvular regurgitation. The aorta is larger and more compliant during normal human pregnancy.12 These changes in the aorta reduce ventricular afterload but at the price of increased wall stress and the risk of dissection. The gravid uterus has been reported to cause significant compression of the aorta and iliac arteries, especially in the supine position. This possibly increases the outflow resistance of the lower arterial tree. The concurrent increase in ejection of blood into the upper aorta may predispose to intimal tear, causing aortic dissection.

During labor, cardiac output increases by a further 45% above the pre-labor level, with up to 500 ml of blood being pumped into the circulation with each uterine contraction. After delivery of the baby, caval compression is relieved and after the placenta is delivered, another auto transfusion occurs from the placental sinusoids into the maternal circulation; both increase the cardiac output and stroke volume by about 80% above baseline.13 Thus the periods of greatest risk for cardiac events during pregnancy are early third trimester, delivery and immediate postpartum period.14 Blood volume is restored to baseline levels after delivery with spontaneous diuresis occurring over a period of approximately six weeks.

Diagnosis of Heart Disease during Pregnancy

Clinical diagnosis of heart disease can be difficult during pregnancy.15 Many normal women experience dyspnea, fatigue, decreased exercise capacity, palpitations, lightheadedness and pedal edema during uncomplicated pregnancy—symptoms suggestive of cardiac disease. The physical examination during normal pregnancy reveals a slightly fast resting heart rate, bounding pulses, and a widened pulse pressure with a low normal peak systolic pressure. Venous pressure is usually elevated above the normal range for non-pregnant woman but rarely in a
clearly abnormal range. The precordial impulse is hyperkinetic, and the first heart sound may be louder than normal, with prominent splitting. The second heart is usually physiologically split but may also widen and appear fixed during later stages of pregnancy. The third heart sound is heard in about 80% of pregnant women, whereas the fourth heart sound is rarely heard. The universal early ejection systolic flow murmur of less than grade 3/6 along the left sternal border is heard in 90% of pregnant women and may be enhanced by anemia. Cervical venous hum and a continuous murmur due to increased mammary blood flow may also be heard. Symptoms and signs that should raise the suspicion of heart failure include paroxysmal nocturnal dyspnea, chest pain, nocturnal cough, new regurgitant murmurs, pulmonary crackles, elevated jugular venous pressure and hepatomegaly. In the presence of diastolic murmurs, continuous murmurs, or loud systolic murmurs (> grade 2/6) or when murmurs are associated with symptoms or an abnormal electrocardiogram, evaluation by echocardiography is warranted. The increased blood flow and enhanced cardiac output associated with normal pregnancy can accentuate the murmurs associated with stenotic heart valve lesions and the murmurs associated with regurgitant lesions may actually attenuate due to decreased systemic vascular resistance. The average weight gain during pregnancy is about 1.5 kg in the first three months. In each subsequent month the average gain is 1.5 kg, being a little more in the last two months. At full term the total gain is about 10 - 12 kg. Sudden changes in weight, either gains or loss may be harmful and is a warning sign.

**Risk Assessment and Stratification during Pregnancy**

Regardless of the cardiac lesion, maternal outcome generally depends on the functional class of the patient. Patients in NYHA class I and II have less than 1% maternal mortality with pregnancy, whereas those in class III and IV have 5-15% mortality. A worsening by one NYHA class may be anticipated during pregnancy, although this is variable. In a study of 64 patients with valvular heart disease, 62% showed worsening of at least one functional class during pregnancy which occurred mostly during the second trimester. In another study, patients with mild to moderate mitral stenosis had only minor deterioration of clinical status as compared to patients with severe stenosis. All patients with severe mitral stenosis deteriorated by one to two functional class. In the recent retrospective study involving 486 pregnant patients with rheumatic heart disease, 113 (22.6%) patients were in NYHA class III-IV. There were 10 (2.1%) maternal deaths of which eight patients were in NYHA III and IV. In another Canadian study of heterogenous group of pregnant women with congenital (75%) or acquired heart disease (25%), the most significant predictor for morbidity and mortality was the presence of left ventricular dysfunction [an ejection fraction (EF)< 40%]. Other predictors of adverse outcomes included a prior cardiac event (heart failure, transient ischemic attack, stroke) or arrhythmia, an advanced NYHA functional status (class II or higher) and the presence of left heart obstructive diseases. (mitral stenosis with valve area < 2 cm², aortic stenosis with valve area < 1.5 cm²). In this study adverse outcome occurred in 13% of the completed pregnancies; 4% in women with no risk factor, 27% with one risk factor and 62% with two or more risk factors. It seems appropriate to dichotomise pregnant patients into asymptomatic (class I) and symptomatic (class II and above) for prognostic purposes as done in this study. Abnormal functional capacity (NYHA class II or higher) and left heart obstruction were also predictors of neonatal complications. In another cohort of 64 pregnant women with valvular heart disease, the predictors of adverse maternal events were moderate and severe aortic and mitral stenosis (valve area < 1.5 cm²). Congenital heart disease with severe pulmonary arterial hypertension is associated with 30-50% mortality during pregnancy. Although pulmonary artery hypertension in valve disease has not been addressed in these studies, increased pulmonary pressure (> 50 mmHg) should be a cause for concern.

**Assessment of Degree of Valve Stenosis during Pregnancy**

Doppler assessment is commonly used to evaluate the severity of valve lesions during pregnancy. But it is unclear how pressure gradients change during pregnancy with either normal or abnormal heart valves. Also calculation of valve area is not without fallacies. In a study by Rokey et al. comparing mitral valve area by pressure half time and continuity equation methods, the valve area obtained using the pressure half time method underestimated the degree of obstruction, whereas the continuity equation was more reliable and in accord with clinical impressions. Also, the area determined via the pressure half time technique tended to be greater ante partum than post-partum but the continuity equation method gave concordant values. The pressure half time method recognizes that for a given valve area, pressure half time is inversely related to driving...
pressure and directly proportional to the transported volume. In conditions with higher driving pressures or volumes, valve areas will be over- or underestimated. Similarly in aortic stenosis, evaluation of severity based on gradient has to be used with caution. The increased force of contraction from an increased ventricular volume increases the pressure on the proximal side of the valve and thus increases the pressure gradient. The decreased systemic vascular resistance on the distal side of the aortic valve during pregnancy further increases the pressure gradient. These effects may be mitigated by increased aortic diameter and compliance during pregnancy. The net influence of these events and their time course has not been fully documented. In the study by Lesniak-Sobela et al., the aortic valve gradient was greater by 20–35 mmHg during the ante-partum period as opposed to during post-partum period. Thus in the absence of appropriate studies, the clinical significance of increased systolic gradient across the aortic valve cannot be assumed to be the same in pregnant and non-pregnant patients. Assessment of the valve area will be a more appropriate indicator of severity rather than the gradients.

Medical Management
Most patients with pregnancy and valvular heart disease can be managed medically. Recommendations for patients with volume overload lesions include restricted physical activity, salt restriction and diuretics. Diuretics are given to relieve pulmonary and systemic venous congestion, but care must be taken to avoid rigorous volume depletion and uteroplacental hypoperfusion. Diuretics prescribed after the first trimester of pregnancy may interfere with normal plasma volume expansion and cause volume depletion. It is possible that electrolyte imbalance or volume depletion after prolonged diuretic therapy may have delayed neurodevelopmental effects on the fetus. Although diuretics have not been reported to have teratogenic effects, their use in pregnancy is controversial. Nevertheless, diuretics are routinely used during pregnancy for decongestive therapy. Daily weights are the best means of clinically following the degree of fluid retention since the presence or absence of visible edema is unreliable. The extracelular fluid volume can increase by 10% before edema becomes visible. Apprehension and anxiety adds to the already strained cardiac reserve. Reassurance and the use of tranquilizers and sedatives are appropriate. Inspite of oral iron medication given routinely, iron deficiency anemia is encountered frequently. In more severe anemia, intramuscular iron may be given or if the hemoglobin level falls below 9 gm, multiple small transfusion with packed cell are indicated. Infections, especially those accompanied by high fever, are very detrimental to the cardiac patient. When intercurrent infections are found they should be treated vigorously with antibiotics. Digoxin, beta blockers, adenosine, sotalol, lidocaine and procanamide can be safely used for the treatment of supraventricular arrhythmias during pregnancy. Amiodarone is best avoided because of the risk of fetal hypothyroidism. However, in high risk situations it can be used to suppress atrial and ventricular arrhythmias. Angiotensin converting enzyme inhibitors and angiotensin receptor blockers are contraindicated during pregnancy because of the risks of urogenital defects, intrauterine growth retardation and fetal death.

Open Heart Surgery
Valvular heart disease is the most common indication for cardiovascular surgery during pregnancy. Cardiac surgery can be performed during pregnancy with relative safety for the mother, with maternal mortality of 3-6% but fetal mortality of 20%-30% still occurs. Maternal deaths were reported in 9% of valvular surgical procedures and in 22% of aortic dissection repairs and pulmonary embolectomies. However the maternal mortality associated with antenatal aortic valve replacement can be as high as 11% and the risk is higher for surgery done in the immediate post partum period. The current recommendation for open heart surgery during pregnancy include (i) avoidance of open heart surgery, if at all possible, during the first trimester. The risk of teratogenesis due to drug administration and possibly cardiopulmonary bypass during the first trimester of pregnancy is always present, and any surgical procedure should be avoided during this time; (ii) use of high-flow, high-pressure, normothermic bypass during the procedure; (iii) fetal heart and uterine monitoring to allow adjustments to the blood flow and pharmacological manipulations to ensure adequate placental perfusion; and (iv) if the fetus is > 28 weeks of gestational age, to opt for cesarean section concurrently just prior to the cardiac operation.

Mode of Delivery
Delivery must be planned carefully. Patients in NYHA classes III and IV as well as many in class II should be admitted to the hospital a few days earlier in order to achieve optimum condition for labor and delivery. In the majority, spontaneous vaginal delivery is the preferred method. However, prolonged and difficult labor should be avoided and cesarian section should be considered in...
some patients electively. In patients with stable aortic dissection, Marfan's syndrome with dilated aortic root, and in whom warfarin could not be switched over to heparin 2 weeks before delivery, elective cesarian section is mandatory. Also, cesarean section may be indicated for obstetrical reasons. Cesarian section has the advantage of avoiding the physical stress of labor, but it is not free from the hemodynamic consequences related to general anesthesia and assisted ventilation. Hemodynamic fluctuations are also influenced by the volume of blood loss, which is greater with cesarian section (1400 ml) than with uncomplicated vaginal delivery (500 ml). The increase in demand on the heart imposed by the labor requires close and continuous monitoring of the mother and the fetus. Careful attention to volume status is essential. Adequate volume loading is required to avoid hypotension. Left lateral decubitus position is preferred to attenuate the hemodynamic effects of the supine position. However, intravenous fluids are risky in patients with mitral stenosis and pulmonary hypertension. Epidural anesthesia is recommended during vaginal delivery to provide adequate analgesia and thus avoiding increase in cardiac output due to pain and anxiety. It is beneficial to shorten the second stage of labor by forceps or vacuum application. Unless blood loss becomes excessive, oxytoxic drugs are withheld in cardiac patients. In patients on heparin, the drug should be withdrawn 4 hours before cesarian section or at the onset of labor and resumed 6-12 hours after either surgical or vaginal delivery. Post-partum monitoring is generally recommended up to seven days for high-risk patients.

Choice of Prosthetic Valve
Many factors determine the choice of prosthetic heart valve in a patient. The crux of the matter is the risk of reoperation in case of bioprosthetic valve versus the risk of bleeding, thromboembolism, and valve thrombosis with mechanical prosthetic valve, besides complications to the fetus. Fetal loss of about 30% is associated with pregnancy in women on anticoagulant therapy. Warfarin embryopathy occurs in about 6% of pregnancies, and the risk is highest during 6-12 weeks of gestation. The risk of thromboembolism during pregnancy in women with mechanical mitral valve has been reported to be 10-15%, which is much higher than 1.2%-5.4% annual risk in non-pregnant state and bleeding is reported in 2-4% patients per year. The maternal mortality rate for pregnancies following valve replacement surgery was 2.9%-6.1%. Bioprostheses have a risk of early structural valve deterioration during or shortly after the end of pregnancy. Moreover, at 10 years there is a high rate of structural valve deterioration (55-77%) and of valve-related reoperation (60-80%). Also the advantage of avoiding anticoagulants is lost in the long run. In the Edinburgh heart valves study, 21% of the patients required reoperation during the follow-up period of 20 years, but around four times more reoperations were performed in the bioprosthetic group compared to the mechanical group. The mortality rate was 15% at 30 days and 22% at one year following reoperations. While the risks to the mother during pregnancy are higher with mechanical valves, the reoperation rates are higher for bioprosthetic valves. Patients already requiring anticoagulation for atrial fibrillation or other indications lose advantage of bioprostheses. Yet the risks of death with mechanical valve thrombosis during pregnancy are substantial. Various aspects of anticoagulation in pregnant patients with prosthetic valves have been discussed in a recently published editorial. Individualized approach taking into account financial status of the patient, risks of reoperation, structural valve deterioration etc. is needed. There are no clear guidelines in this respect. Pregnancy is best avoided in symptomatic valvular heart disease patients.

Specific Cardiac Lesions/Problems
Mitrval stenosis: Mitral stenosis is the most common rheumatic valvular lesion seen in pregnancy due to its prevalence in young women. Approximately 25% of patients with mitral stenosis become symptomatic for the first time during pregnancy. Mortality among pregnant women with minimal symptoms is < 1%. With severe mitral stenosis, pregnancy-related mortality is about 5%. Predictors of adverse maternal outcomes include a reduced mitral-valvearea (< 1.5 cm²) and an abnormal functional class before pregnancy. Fetal mortality increases with deteriorating maternal functional capacity; it is 30% when mother has NYHA class IV symptoms. For women with mild or moderate symptoms during pregnancy, medical therapy is directed at the treatment of volume overload and includes diuretic therapy, the avoidance of excessive salt, and the reduction of physical activity. Beta-blockers attenuate the increase in heart rate and prolong the diastolic filling period, which provides symptomatic benefit. Ashcom et al. in their study of beta blockade in seven patients with severe mitral stenosis concluded that chronic beta blockade afforded some protection by blunting changes in pulmonary capillary wedge pressure and right heart pressures during exercise; however, exercise performance was not enhanced. Nevertheless, beta-blockers are recommended during pregnancy, especially if the peak pulmonary artery pressure is > 50 mmHg by echocardiogram. Atrial fibrillation
Mitral regurgitation: Mitral regurgitation is usually well tolerated during pregnancy if the left ventricular function is good. The increase in blood volume and cardiac output increases the volume overload but the decrease in systemic vascular resistance reduces the regurgitant fraction. Because of the decrease in left ventricular afterload associated with mitral regurgitation, systolic wall stress is also lowered. In patients with mitral regurgitation secondary to mitral valve prolapse, the increase in blood volume associated with pregnancy decreases the severity of mitral regurgitation. Pregnancy in patients with mitral valve prolapse follows a benign and uneventful course. The physical findings and even symptoms appear less evident and the patients usually feel better during pregnancy. However, more serious complications of mitral valve prolapse, such as arrhythmia, infective endocarditis, and cerebral ischemic events may complicate pregnancy. Patients with pre-existing ventricular dysfunction may develop progressive congestive heart failure particularly during the third trimester. They need diuretics and vasodilators to reduce the systemic vascular resistance. Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers are contraindicated. In symptomatic pregnant patients with mitral regurgitation, dihydropyridine group of calcium channel blockers, hydralazine hydrochloride, diuretics, and digoxin can be used when systolic function is impaired. In severe symptomatic mitral regurgitation, surgical mitral valve repair is a good option because it avoids the need for anticoagulant therapy. Mitral valve replacement can be done as a last resort.

Aortic stenosis: Pregnancy in patients with aortic stenosis is uncommon. The major problem in pregnant patients with severe aortic stenosis is to maintain an adequate cardiac output across a fixed aortic valve. With severe stenosis, there is a narrow window of appropriate fluid loading. Small decreases in preload due to hemorrhage or regional anesthesia may result in decreased cardiac output and dangerous clinical hypotension. Also, small increases in vascular volumemay producedramatic increasein filling pressure, resulting in pulmonary edema. The clinical consequences of the increased aortic gradient depend on the degree of pre-existing left ventricular hypertrophy and left ventricular systolic function. Women with aortic valve area > 1.0 cm² tolerate pregnancy well and have a good outcome. However, women with more severe aortic stenosis may have symptoms of left-sided heart failure. The maternal mortality in pregnant patients with severe aortic stenosis is as high as 17%. Most asymptomatic patients and those who have mild to moderate stenosis can be managed with medical therapy and close monitoring. It is important to maximize cardiac output and fetal blood flow by avoiding intense exercise, potent vasodilators, and diuretics. Termination of pregnancy should be strongly considered if the patient is symptomatic before the end of the first trimester. Women with severe aortic stenosis are more likely to experience cardiac complications during pregnancy and require interventions during the course of pregnancy. Aortic valve replacement has mortality as high as 11% during pregnancy with associated fetal risk, and only case series of successful balloon valvotomy during pregnancy is available. The association between congenital bicuspid aortic valve and aortic dissection has long been recognized. Pregnant patients with bicuspid aortic valve and enlarged aortic root should be counseled like Marfans syndrome patients. Aortic root of >5.0 cm is considered an indication for elective repair before conception. If aortic root enlargement (>4.0 cm) is first detected during pregnancy, termination with prompt aortic repair is recommended, especially if serial echocardiographic studies demonstrate progressive dilation over time. Dissection and rupture are most likely to occur during the third trimester or near the time of
delivery. The use of prophylactic β-blockade throughout the pregnancy is strongly recommended. Special care must be taken to provide adequate analgesia to prevent wide surges in blood pressure and its rate of rise (dp/dt) during labor and delivery. Obstetrical techniques to shorten the second stage of labor are recommended. General anesthesia and cesarean section may allow more optimal hemodynamic control.

Aortic regurgitation: In pregnant women aortic regurgitation, most commonly secondary to rheumatic fever is often seen with co-existing mitral valve disease. The decreased systemic vascular resistance during pregnancy reduces afterload and hence the amount of regurgitant flow across the valve. This adaptation appears to maintain the forward flow unless systolic dysfunction sets in. Therefore, as with mitral insufficiency, chronic aortic insufficiency is well tolerated during pregnancy. In asymptomatic patients, close monitoring is all that is needed. Symptomatic patients can be treated with vasodilators, as for mitral regurgitation. In the women who develop signs of congestive heart failure, physical activity should be restricted and inotropic and diuretic therapy should be initiated. Women with aortic regurgitation tolerate bradycardia poorly, as this increases the duration of diastole and the amount of regurgitation across the valve; hence maternal heart rate should be maintained between 80 and 100 bpm.

Pulmonary stenosis: Pulmonary stenosis is well tolerated during pregnancy despite the gestational volume overload imposed on the already pressure overloaded right ventricle. In three series comprising 106 pregnancies, no maternal mortality was reported. However, if the stenosis is severe, pregnancy can precipitate right heart failure, atrial arrhythmias or tricuspid regurgitation. In case of right ventricular failure during pregnancy, balloon valvotomy may be done for severe stenosis.

Tricuspid valve/Ebstein’s anomaly: Isolated tricuspid regurgitation can be managed conservatively, although greater care is required to protect against diuretic-induced hypoperfusion. In patients with Ebstein’s anomaly, the physiological changes associated with pregnancy may have appreciable adverse hemodynamic consequences. In the presence of impaired right ventricular function, the increased stroke volume may be poorly tolerated and result in worsening symptoms. Complications of pregnancy and delivery such as hemorrhage may not be well tolerated by these patients. In two series comprising 153 pregnancies in 56 women, pregnancy was well tolerated in the absence of significant maternal cyanosis or arrhythmias. Neonatal outcome was good though there was an increased risk of prematurity and dysmaturity in the babies born to mothers with cyanosis. There were no congenital anomalies in the babies. In addition, a significantly lower birth weight babies were delivered by the cyanotic mothers. Thus pregnancy is well tolerated in these patients.

Rheumatic Fever Complicating Pregnancy

Rheumatic fever and heart disease occur in younger age in our country and it is rare, but possible to have acute rheumatic fever during pregnancy. Secondary prophylaxis with injection penicillin is required and sulfonamides are contraindicated in these patients. Rheumatic fever may recur during pregnancy and carditis can be a serious complication during pregnancy. Chorea gravidarum is rarely seen now but can result in fetal death and occasionally, maternal death. More frequently chorea is seen in the primigravida, with about half of the cases occurring in the early pregnancy. Approximately one-third of these cases resolve before delivery and the remainder resolve shortly thereafter. When chorea is severe interruption of pregnancy is indicated.

Infective endocarditis: Infective endocarditis is uncommon during pregnancy and is typically limited to patients with pre-existing valve disease or a history of intravenous drug abuse. The majority of cases (74%) are caused by viridans streptococci, while enterococci and group B streptococci were uncommon except after abortion. In a recent review the maternal and fetal mortality following maternal endocarditis was found to be 22.1% and 14.7% respectively. Aortic valve endocarditis had the highest mortality rate (42%) while the tricuspid valve endocarditis had only 9% mortality rate. Infective endocarditis is a serious disease that can be missed in the early stages because of the paucity of physical signs. The low grade fever may pass unnoticed by the patient and the frequency of micturition associated with the gravid state often leads to false diagnosis of urinary tract infection and the use of antibiotic further masks the disease. Murmurs may also be interpreted as benign and of hemic origin specially in the absence of history of heart disease. However, pregnancy itself does not affect the natural history of the disease.

Medical management of endocarditis is broadly similar to the non-pregnant patient but special attention has to be given to certain aspects. The increased glomerular filtration rate and expanded blood volume that occurs in pregnancy results in shortened half life and reduced serum levels of those antibiotics that are excreted by the kidney (penicillins, cephalosporins and aminoglycosides). The serum level of
antibiotics may be reduced to <50% and hence monitoring of serum levels is appropriate to ensure adequate tissue levels and therapeutic efficacy. Penicillins and cephalosporins cross the placenta and are well tolerated by the fetus. Aminoglycosides carry small risk of fetal ototoxicity.

Currently there are no uniform guidelines for infective endocarditis prophylaxis in pregnant patients. Routine antibiotic prophylaxis is not recommended in patients with valvular heart disease undergoing uncomplicated vaginal delivery or cesarean section unless infection is suspected. Though infective endocarditis prophylaxis is indicated only for high risk patients, the effects of endocarditis can be so devastating that the need for prophylaxis should be individualized.44

Conclusions
Management of valvular heart disease during pregnancy is challenging. A thorough knowledge of the expected natural history of the disease during pregnancy and of the possible treatment options is required for clinical decision making. A meticulous individualized patient care by a team of physician, obstetrician, and anesthetist is required for optimal outcome of the pregnancy for the mother and the baby.

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Herbs have been used for medical treatment since the beginning of human civilization. Herbal medicine has made many contributions to commercial drug preparations manufactured today including ephedrine from Ephedra sinica (ma-huang), digitoxin from Digitalis purpurea (foxglove), salicin (the source of aspirin) from Salix alba (willow bark), and reserpine from Rauwolfia serpentine (snake root). With wide use of herbs in Eastern traditional medicine as well as in Western medicinal drugs, continuing research is necessary to elucidate the pharmacological activities of many herbal remedies now being used to treat cardiovascular diseases, such as congestive heart failure, hypertension, angina pectoris, atherosclerosis, and arrhythmias.

Arrhythmias have accounted for significant cardiac morbidities and mortalities over the past few decades. Although some arrhythmias may appear benign or potentially dangerous, the majority of sudden cardiac arrests are a direct result of ventricular tachycardia (VT) and/or ventricular fibrillation (VF). Although conventional anti-arrhythmic drugs have been shown to be effective in preventing arrhythmias, they themselves can also cause lethal arrhythmias in some conditions. It is known that electrical defibrillation is the only effective clinical means to treat VF. Currently, the shock strength required to successfully defibrillate is still high. A number of studies have been done to lower the defibrillation threshold (DFT). One of the means to decrease the DFT is to combine the defibrillation with the drugs. Both pharmacological and non-pharmacological interventions to prevent these lethal arrhythmias have been widely investigated. Recently, many herbal products have been shown to be anti-arrhythmic and would be useful to treat and prevent arrhythmias.

This review focuses on some herbal medicines, both crude extracts and pure compounds, that have been widely studied for their anti-arrhythmic effects in recent years. These consist of trilinolein, garlic, and certain other common herbal products. Continuing research is necessary to provide more information regarding the safety and efficacy of herbal medications now being used to treat arrhythmias.

Trilinolein and its Anti-Arrhythmic Effects

Trilinolein, isolated from the traditional Chinese herb Sanchi (Panax notoginseng), has been used for treating circulatory disorders in China for hundreds of years. Chemical structure of trilinolein consists of a triacylglycerol with the fatty acid and linoleic acid, which carries two unsaturated bonds, at all three esterified positions of glycerol. Trilinolein has been shown to have various beneficial effects, including reduction in thrombogenicity, erythrocyte deformability and having antioxidant, anti-ischemic, and anti-arrhythmic effects in various experimental models.

In a rat model study, trilinolein was reported to reduce ischemia-induced arrhythmias. In this study, trilinolein (0.01-100 ng/kg) was administered intravenously 15 min before ligation of the coronary artery. At dose of 100 ng/kg, trilinolein has been shown to completely suppress all ventricular arrhythmias. Trilinolein also reduced the incidence, rate and duration of VT and the number of ectopic beats during the first 30 min of coronary ligation. Furthermore, the effect of trilinolein on infarct size was evaluated by occluding the coronary artery for 4 hours before the infarct zone was stained and weighed. In rats subjected to 4-hour coronary ligation, pre-treatment with 10⁻⁷ g/kg trilinolein infused intravenously at 15 min prior to the coronary ligation significantly reduced infarct size.

Additionally, similar doses of trilinolein also reduced ventricular arrhythmias during 10 min reperfusion of the myocardium, following the 30 min of coronary artery ligation. Furthermore, the effect of trilinolein on infarct size was evaluated by occluding the coronary artery for 4 hours before the infarct zone was stained and weighed. In rats subjected to 4-hour coronary ligation, pre-treatment with 10⁻⁷ g/kg trilinolein infused intravenously at 15 min prior to the coronary ligation significantly reduced infarct size.

The anti-arrhythmic effects of trilinolein have been investigated in ventricular arrhythmia caused by digitalis intoxication. Ventricular arrhythmia is a frequent side effect with the use of digitalis, the mechanism underlying the arrhythmias induced by cardiac glycosides being complex. It has been shown that delayed after-depolarization (DAD) is responsible for such arrhythmia. DAD is believed to be generated by the overloading of
intracellular Ca\(^{2+}\) stores, caused by the inhibitory effect of digitalis on the Na\(^+/K^+\) pump which subsequently activates the reverse mode of the Na\(^+/Ca^{2+}\) exchanger.\(^{12}\) Treatment of guinea pigs with trilinolein (0.1-100 \(\mu\)g/kg) prior to intravenous administration of strophanthidine significantly reduced ventricular extrasystoles which were related to DADs induced by cardiac glycoside.\(^{10}\) It is known that low concentrations of trilinolein effectively reduced Ca\(^{2+}\) influx in isolated rat cardiomyocytes.\(^{13}\) Similar mechanisms may be involved in reducing strophanthidine-induced ventricular extrasystoles. Excess of Ca\(^{2+}\) and overloading of Ca\(^{2+}\) not only favors abnormal automaticity as well as triggered activity created by DADs, but also induces cell-to-cell uncoupling and block of conduction by inhibition of gap junction channels with down-regulation of intermyocyte communication which can initiate reentry.\(^{14}\) Trilinolein also dose-dependently narrowed the width of the QRS complex during VT, possibly due to the improvement of the conduction velocity between muscle fibers through the action of trilinolein on Ca\(^{2+}\) metabolism that contributed to prevention of further arrhythmias.\(^{20}\) Since VF induction and failed defibrillation have been demonstrated to be related to intracellular calcium overload,\(^{15,16}\) it is likely that trilinolein could be beneficial in this case. Further studies are needed to verify this hypothesis.

**Garlic and its Anti-Arrhythmic Effects**

Garlic (*Allium sativum*) is a perennial plant that is cultivated worldwide. Its bulb has been used as a spice or medicinal herb for many centuries.\(^{17}\) It contains a higher concentration of sulfur compounds than any other *Allium* species. The sulfur compounds are responsible both for garlic’s pungent odor and many of its medicinal effects. Dried, powdered garlic contains approximately 1% alliin (S-allylcysteine sulfoxide).\(^{18}\) One of the most biologically active compounds, alliin (diallylthiosulfinate or diallyl disulfide) does not exist in garlic until it is crushed or cut since in the bulb alliin activates the enzyme alliinase, which metabolizes alliin to allicin.\(^{19}\) Allicin is further metabolized to vinylthiines. This breakdown occurs within hours at room temperature and within minutes during cooking.\(^{20}\) Garlic oil, aged garlic and steam-distilled garlic do not contain significant amounts of alliin or allicin, but instead contain various products of allicin transformation; none appears to have as much physiologic activity as fresh garlic or garlic powder.\(^{19,21,22}\) In rats, alliin is well absorbed orally, reaching maximum serum concentrations within 10 min, and is completely excreted within 6 hours. Allicin and vinylthiines are absorbed more slowly, reaching peak levels between 30 and 120 min and persisting in the body for up to four days.\(^{23}\) There is a significant first pass effect in which allicin is metabolized to allylmercaptan, ajoene and vinylthiines.\(^{24}\) Excretion occurs renally and through hepatic breakdown, fecal excretion and exhalation.

In modern medicine, garlic and its preparations have been widely recognized as agents for prevention and treatment of cardiovascular and other metabolic diseases, atherosclerosis, hyperlipidemia, thrombosis, hypertension and diabetes.\(^{25}\) These biological responses have been largely attributed to the ability of garlic to reduce risk factors for cardiovascular diseases and cancer, stimulate immune function, and enhance detoxification of foreign compounds. It also has hepatoprotective, antimicrobial and antioxidant effect.\(^{25}\)

Previous studies have demonstrated that garlic has a significant anti-arrhythmic effect in both ventricular and supraventricular arrhythmias,\(^{25,26}\) especially its free radical scavenging activity which reduces the incidence of VT and VF induced by myocardial ischemic-reperfusion injury.\(^{27}\) However, the definite mechanism by which garlic suppresses arrhythmia is still unclear. Martin et al.\(^{26}\) demonstrated that garlic dialysate prolonged sinus node recovery time (SNRT) and effective refractory period (ERP) of isolated rat atria in a dose-dependent manner. They also reported that garlic depresses a Ca\(^{2+}\) influx which is related to prevention of DAD.\(^{25,28}\) It is known that the mechanism underlying the generation of DAD involves an overload of intracellular Ca\(^{2+}\) with oscillation of the transmembrane potential, giving rise to new activations.\(^{29,30}\) Thereafter, arrhythmia can be induced from these activations (triggered activity) if they reach a critical threshold. In a study by Kojima et al.,\(^{31}\) normalization of intracellular Ca\(^{2+}\) has been suggested to contribute to termination of VF by suppressing DAD and/or slowing ventricular conduction in the isolated rat heart model. The ability of garlic to prevent intracellular Ca\(^{2+}\) oscillation could help prevent DAD. In human studies, many clinical trials studying cardiovascular effects of garlic used dried garlic powder that contains approximately 1.3% alliin (precursor of allicin) at a dosage of 300 to 900 mg/day (5-15 mg/kg), corresponding to 0.9 to 2.7 g of fresh garlic daily.\(^{22}\) For example, in a prospective, 4-year clinical trial of patients treated with 900 mg daily of standardized garlic powder, there was a 9-18% reduction in plaque volume, a 4% decrease in low-density lipoprotein (LDL) levels, an 8% increase in high-density lipoprotein (HDL) concentrations, and a 7% decrease in blood pressure.\(^{32}\) However, in...
randomized, controlled trials, side effects in those taking garlic included heartburn, nausea, vomiting, diarrhea, flatulence, bloating, mild orthostatic hypotension, flushing, tachycardia, headache, insomnia, sweating and dizziness as well as offensive body odor. Although the exact toxicity of garlic has yet to be definitively determined, side effects are rare at the dosage mentioned above. Nevertheless, further human studies are required to evaluate the anti-arrhythmic properties of garlic, especially its definite mechanisms.

Anti-Arrhythmic Effects of Other Herbal Products

Many herbal products show their effects on inhibition of VT, VF and/or extrasystole during the period of ischemia-reperfusion injury or acute myocardial infarction in animals. Pre-treatment with magolol and honokiol, the active components of Magnolia officinalis significantly reduced the incidence and duration of ventricular arrhythmia including VT and VF in rats subjected to coronary ligation. These could in part be relevant to the vasorelaxant effects due to an increased nitric oxide (NO) synthesis. Dauricine, an active compound found in Menispernum dauricum, ameliorates acute myocardial ischemia-induced VT and VF in anesthetized dogs. The mechanism of its antagonistic effect lies in its blocking effect on inward K+ current in ventricular myocytes. Neferine, isolated from the seeds of Nelumbo nucifera, prevented the onset of re-entrant VT and sudden cardiac death after myocardial ischemic damage studied in open-chest dogs. It lengthened the ERP as well as decreased the dispersion of the ERP, and increased the diastolic excitability threshold of normal and infarct myocardium in both ventricles. All of these effects stabilize membrane potential of cardiac myocytes, and thus, could prevent arrhythmias.

Oxymatrine, an alkaloid isolated from Sophora japonica, increased the diastolic excitability threshold and lengthened ERP in dogs after myocardial infarction. However, it had no effects on the dispersion of ERP, QTc interval and VT or VF induced by programmed electric stimulation. On the other side, studies using hawthorn berry (Crataegus oxyacantha) demonstrated beneficial anti-arrhythmic effects. Using Langendorff-perfused rat heart, reperfusion arrhythmias of a 3-month oral pre-treatment with a dried Crataegus extract were reduced significantly. Crataegus, however, aggravated rather than prevented arrhythmias with its long-term application in a recent study.

Tetrandrine, an extract from Stephania tetrandra, has been shown to inhibit VT and VF during regional ischemia-reperfusion injury without further reducing ischemia-reduced heart rate and coronary artery flow. It may be a better choice for the treatment of arrhythmia and infarction induced by myocardial ischemia and reperfusion than a classical Ca2+ channel blocker, i.e. verapamil, which can reduce heart rate during ischemia. Pre-treatment with sinomenine, a compound found in Sinomenium acutum, could also reduce the incidence of VT and VF in isolated perfused rat heart subject to ischemia-reperfusion injury by lowering intracellular Ca2+ accumulation.

Other herbal products having anti-arrhythmic effects are hirsutine, EGB761 and dehydroevodiamine. Hirsutine, an indole alkaloid found in Uncaria rhynchophylla, showed anti-arrhythmic action on membrane potentials of rabbit sinoatrial node and guinea-pig right ventricle and left atrium by decreasing the slope of the pacemaker (phase 4) depolarization and prolongation of the action potential duration. EGB761, a Ginkgo biloba (maidenhair tree) extract, in combination with superoxide dismutase significantly attenuated both the formation of oxygen-free radicals and the incidence of reperfusion-induced VF and VT in isolated rat hearts. Dehydroevodiamine, an alkaloid from Evodia rutaecarpa, has been shown to depress arrhythmias in calcium-overloaded guinea-pig cardiac myocytes through its inhibitory actions on the Na+-dependent inward current, the transient inward current and, to a smaller extent, the L-type Ca2+ current. In addition, dehydroevodiamine has been shown to possess anti-arrhythmic effect similar to class III anti-arrhythmic drugs through a reduction of outward K+ currents across the sarcolemma.

Conclusions

Herbal medicines have been used for centuries and their potential benefits have been corroborated by high prevalence of their use worldwide. For cardiovascular disorders, many herbal treatments are available with limited scientific assessment of both benign and malignant arrhythmias. Some herbal medicines (crude extracts and pure compounds) that have been studied extensively for their anti-arrhythmic effects such as trilinolein, garlic and other herbal products may be beneficial as effective pharmacological therapies as well as for prevention of lethal arrhythmias such as VT and VF. Further basic and clinical studies are needed to elucidate the pharmacological effects of these herbal products and their potential impact on the prevention and treatment of arrhythmias.
Acknowledgements
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Clinical and Angiographic Procedural and Mid-term Outcome with New versus Reused Balloon Catheters in Percutaneous Coronary Interventions

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Background: To reduce procedural cost, cardiac centers increasingly use resterilized balloon catheters for percutaneous coronary interventions. Data addressing the procedural and mid-term outcome in a prospective randomized trial comparing new and resterilized balloons are not available.

Methods and Results: Percutaneous coronary interventions were performed at random in 238 consecutive patients with either new or 1–3 times reused balloon catheters. Crossing of the stenosis decreased from 96% with new balloon catheters to 93.2% (p=0.46), with 1 time reused balloon catheters to 81.8% (p=0.0056) with 2 times reused balloon catheters and to 80.8% (p=0.01) with 3 times reused balloon catheters. In all primary failures using resterilized balloon catheters, new ones of the same nominal diameter were successful. The 4.1±1.9 month angiographic follow-up rates were 96/124 (77.4%) for new balloon catheter, 35/44 (79.5%) for 1 time reused balloon catheters, 33/44 (75.0%) for 2 times reused balloon catheters, and 21/26 (80.8%) for 3 times reused balloon catheters (p for all >0.05). The late losses for new versus reused balloon catheters were 0.48±0.75 mm versus 0.73±0.79 mm (p=0.03). The percent stenosis was higher in reused versus new balloon catheters (51.9±23.2% v. 42.3±22.3%; p=0.0042) as was the restenosis rate [39/89 (43.8%) v. 31/96 (32.3%), p=0.13]. There was one death in reused balloon catheter category but no event of myocardial infarction. Rates of target lesion revascularizations were similar in stent recipients and more frequent after stand-alone balloon angioplasty with reused versus new balloon catheters [15/55 (27.3%) versus [5/59 (8.5%), p=0.01].

Conclusions: The use of two or three times resterilized balloon catheters of the type tested does not seem to be justified in stand-alone balloon angioplasty of de novo coronary stenoses and should be limited to stent procedures until data is available for other indications. (Indian Heart J 2005; 57: 114-120)

Key Words: Coronary artery disease, Angioplasty, Re-used catheters

Worldwide more than 1,000,000 percutaneous coronary interventions (PCIs) are performed annually imposing a tremendous financial and ecologic burden on the health care systems. Although manufacturers' warranties recommend single use for the majority of their products, many cardiac centers resterilize angioplasty catheters for multiple use.1-6

Investigators have not been able to document additional risks, neither of transmitting infectious diseases, nor of adverse reactions to the disinfectants when catheters are reused after, careful cleansing1-6 and resterilization by either ethylene oxide9-13 or hydrogen peroxide.14 To date, however, only limited data is available on the clinical performance of reused angioplasty balloon catheters with conflicting results in small cohorts of patients2,6,15 and no angiographic follow-up data. We therefore investigated acute and mid-term results of new versus resterilized balloon catheters.

Methods

Study design: The study is a prospective, randomized clinical trial comparing the acute and mid-term outcome of new and 1–3 times reused balloon catheters in patients with significant de novo coronary artery stenosis.

Ethical considerations: The trial was conducted in accordance with the Declaration of Helsinki and International Commission for Harmonization - Good
Clinical Practice Guidelines. An independent ethics committee had approved the protocol. Each patient’s written informed consent was obtained prior to enrolment.

**Characterization of catheter:** The catheter used was a standard monorail system featuring a proximal stainless steel hypotube shaft with LEAP™, a nylon derivative, serving as balloon material. In vitro data on its mechanical properties after several cycles of resterilization were published elsewhere.16

**Resterilization of balloon catheters:** The balloon catheters were cleansed by a disinfectant containing laurylpropylenediamine and dodecylbispropylenetriamine (Korsolex®, Bode Chemie, Hamburg, Germany), desalinated, and dried by air (Medikat, Hungen, Germany). After packaging, the devices were gas-sterilized for 7 hours 15 min by ethylene oxide, which well penetrates a narrow lumen.8 As assessed by an independent institute (Servis, Gilching, Germany), post-conditioning (26-34ºC/81-93ºF) for 3 days resulted in complete degasification of the catheters to escape the toxic effects of the agent,10 thus, meeting FDA requirements.17

**Study population:** Three hundred and ten male and female patients scheduled for angioplasty of a de novo coronary stenosis were eligible for participation (Fig. 1). In 18/310 (5.8%) patients informed consent could not be obtained, in 49/310 (15.8%) the required balloons were not available according to the random list, and in 5/310 (1.6%), crossing of the stenosis with the wire was unsuccessful. Therefore, 238 patients were randomized (Table 1).

Inclusion criteria comprised coronary artery stenosis of ≥ 70% and <100% of a visually estimated maximum lesion length of < 20 mm in association with angina pectoris. Exclusion criteria included angina at rest, recent myocardial infarction (< 24 hours), congestive heart failure, severe valvular heart disease, ejection fraction (EF) < 20%, unprotected left main stenosis, serum creatinine > 2.5 mg/dl, untreated hyperthyroidism, pregnant or lactating women, associated diseases precluding follow-up (e.g., malignancy), and simultaneous participation in other clinical trials.

**Primary end point:** Crossing of the lesion with balloon, and inflation of the balloon within the lesion was defined as success of device.

**Secondary end points:** Secondary end points included procedural success, myocardial infarction, emergent target lesion revascularization, rupture of the balloon, the number of balloons used per procedure, and consumption of contrast, time taken for the procedure, and exposure time to radiation, all taken after the wire had crossed the lesion.

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**Table 1. Patients’ characteristics**

<table>
<thead>
<tr>
<th>Variable</th>
<th>New balloons</th>
<th>Reused balloons</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>124</td>
<td>114</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.9 ± 9.8</td>
<td>66.3 ± 8.1</td>
<td>0.74</td>
</tr>
<tr>
<td>Male</td>
<td>96 (77.4)</td>
<td>87 (76.3)</td>
<td>0.88</td>
</tr>
<tr>
<td>Severity of coronary artery disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1- Vessel disease</td>
<td>32 (25.8)</td>
<td>34 (29.8)</td>
<td>0.56</td>
</tr>
<tr>
<td>2- Vessel disease</td>
<td>37 (29.8)</td>
<td>39 (34.2)</td>
<td>0.58</td>
</tr>
<tr>
<td>3- Vessel disease</td>
<td>55 (44.4)</td>
<td>41 (36.0)</td>
<td>0.23</td>
</tr>
<tr>
<td>EF (%)</td>
<td>56.6 ± 21.5</td>
<td>60.1 ± 16.3</td>
<td>0.56</td>
</tr>
<tr>
<td>Angina pectoris</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCS I</td>
<td>60 (48.4)</td>
<td>41 (36)</td>
<td>0.07</td>
</tr>
<tr>
<td>CCS II</td>
<td>30 (24.2)</td>
<td>27 (23.7)</td>
<td>1.0</td>
</tr>
<tr>
<td>CCS III</td>
<td>16 (12.9)</td>
<td>26 (22.8)</td>
<td>0.06</td>
</tr>
<tr>
<td>CCS IV</td>
<td>2 (1.6)</td>
<td>2 (1.8)</td>
<td>1.0</td>
</tr>
<tr>
<td>Unstable angina</td>
<td>16 (12.9)</td>
<td>18 (15.8)</td>
<td>0.58</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>100 (80.6)</td>
<td>92 (80.7)</td>
<td>1.0</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>206 ± 44</td>
<td>198 ± 48</td>
<td>0.18</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>118 ± 40</td>
<td>113 ± 41</td>
<td>0.35</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>51 ± 13</td>
<td>53 ± 19</td>
<td>0.34</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>30 (24.2)</td>
<td>29 (25.4)</td>
<td>0.88</td>
</tr>
<tr>
<td>Current smokers</td>
<td>23 (18.6)</td>
<td>17 (14.9)</td>
<td>0.49</td>
</tr>
<tr>
<td>Family history of CAD</td>
<td>49 (39.5)</td>
<td>50 (43.9)</td>
<td>0.60</td>
</tr>
<tr>
<td>Hypertension</td>
<td>73 (58.9)</td>
<td>69 (60.5)</td>
<td>0.90</td>
</tr>
<tr>
<td>Statin use</td>
<td>65 (52.4)</td>
<td>63 (55.3)</td>
<td>0.69</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages

EF: ejection fraction; CCS: Canadian Cardiovascular Society; LDL: low-density lipoprotein; HDL: high-density lipoprotein; CAD: coronary artery disease

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Fig. 1. Patient flow chart.
Interventional procedure: The procedures followed common clinical practice with the exception that only one defined brand of balloon catheter was allowed. Angiographic success was defined as a residual stenosis of < 30%, achieved either by stand-alone balloon angioplasty, stenting, or by any other means. A total of three angiograms were performed: the qualifying and the post-procedural angiograms (2 views each) and the follow-up angiogram using the same two views as for the qualifying procedure and additional projections, if required.

Only experienced operators with extensive skills in using reстерilized balloon catheters performed the procedures.18-22

Medication: Aspirin 100 mg/day was given orally at least two days preceding the procedure. During the intervention, heparin 100-200 U/kg body weight was given intra-arterially upon insertion of the sheath. In procedures lasting >1 hour, 75 U/kg body weight of heparin was supplemented. Intracoronary nitroglycerine 0.2 mg was administered before the first and prior to the last reference angiogram. Other medications were allowed when indicated.

During follow-up, aspirin 100 mg/day orally was given to all patients. Clopidogrel with a loading dose of 300 mg followed by 75 mg/day for 4 weeks was added after stent procedures.

Follow-up angiographies were performed under heparin 100 U/kg body weight upon insertion of the sheath and intracoronary nitroglycerine 0.2 mg 5 min prior to the first reference view.

Quantitative coronary analysis (QCA): The angiograms were reviewed by two blinded observers using qualitative morphologic and quantitative angiographic methods (CAAS II, Pie-Medical, Maastricht, The Netherlands) at the Angiographic Core Lab of the Institute for Clinical Research at Rotenburg an der Fulda. The contrast-filled catheters served as the calibration standard, while the reference and minimal lumen diameters were determined using an automated edge-detection algorithm. Reference contours were calculated by using a linear regression algorithm with assessment of the reference diameter at the site of the minimal lumen diameter.23 The diameters were taken from the worst view and its close orthogonal projection pre-procedure, after stent deployment, and at follow-up. The mean of both of the values determined the severity of stenosis. Obvious false assessment of the vessel by CAAS allowed for operator adjustment.

In case of the difference between the two operators exceeding 5%, a third operator made the final decision based on his evaluation blinded to the previous assessments.

Definition of complications: A thrombus was defined as a non-calcified filling defect within the vascular lumen, which was visible in several views and which could migrate into the peripheral artery. An acute thrombosis was defined by a total occlusion (TIMI grade 0) occurring within 24 hours after stent deployment whereas subacute thrombosis was the one that occurred > 24 hours and < 1 month after stenting. Q-wave myocardial infarction was diagnosed with the occurrence of new Q-waves (> 0.04 s) and rise of creatine kinase twice the upper limit of normal with significant increase in CK-MB whereas in non-Q-wave myocardial infarctions pathologic Q-waves were absent.

Statistical analysis: It was to be tested whether the procedural successes of new and 1–3 times reстерilized balloon catheters would not be different (Null hypothesis). Based on an estimated procedural success rate of 95% for new versus 80% for reused balloon catheters, a level of significance of α = 0.05, and a beta error of β = 0.10, a sample size of 2 x114 patients was calculated.

The Kolmogoroff-Smirnoff-test was used to prove Gaussian distribution allowing for calculation of the mean and standard deviation. Non-Gaussian samples were described by the median and the maximal and minimal values. Categorical variables were evaluated with the two-sided exact Fisher test. Simple or multiple logistic regression analysis and analysis of variance were used to analyze the influence of one or more parameters on primary procedural success rate or other dependent variables. The Bonferroni/Dunn test was used to analyze differences between groups post hoc, if analysis of variance was significant. For all tests the significance level α was 0.05.

Results

Out of 310 patients eligible for the study, 238 were randomized. The reasons for not randomizing a patient are given in Fig. 1. In 124/238 (52.1%) patients a new balloon was used as first choice, whereas in 114/238 (47.9%) patients lesion dilation was attempted initially by means of a reстерilized balloon. Resterilized balloons were either once [44/114 (38.6%)], twice [44/114 (38.6%)] or three times [26/114 (22.5%)] resterilized. The reason for less frequent use of the latter was non-availability of the specific balloon size (according to the random list) at the time of the procedure.

Primary and secondary end point: Crossing of the stenosis was significantly less successful with reused
compared to new balloons [86% v. 96%; odds ratio (OR) 3.89 (95 % CI 1.37-10.99), p = 0.0056]. The size of the guiding catheter used (6F or 8F) had no influence (data not shown). When the number of resterilization cycles was included in the model, primary success of the balloon catheter was only significantly decreased with catheters that had been resterilized more than once (Table 2). In all failures with the resterilized balloons, use of a new one of the same nominal diameter completed the procedure successfully.

The number of resterilization cycles remained a significant predictor of success (p = 0.0075, logistic likelihood ratio tests). However, when severity of stenosis was included in the model, there was no significant influence on success of device (p = 0.59) (Fig. 2). The success of balloon catheter of multiply resterilized catheters becomes apparent specially in higher grade (> 75 %) stenosis.

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The number of resterilization cycles had a significant impact on the total number of balloons needed to successfully complete the procedure (p = 0.015, ANOVA) (Table 2). New balloons and once resterilized balloon catheters in comparison to 2–3 times reused catheters showed a trend toward reduction in procedure and fluoroscopy time, while the exposure to radiation and use of contrast dye was unaffected (Table 3).

### Table 2. Success rate of balloon catheter according to the number of resterilization cycles

<table>
<thead>
<tr>
<th>No. of resterilization cycles</th>
<th>Success of balloon catheter %</th>
<th>Odds ratio (95 % CI)</th>
<th>p-value</th>
<th>Total no. of balloons needed for successful procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (new catheters)</td>
<td>119/124 (96)</td>
<td>–</td>
<td>–</td>
<td>1.16±0.41</td>
</tr>
<tr>
<td>1 cycle</td>
<td>41/44 (93.2)</td>
<td>1.74 (0.40–7.61)</td>
<td>p=0.46</td>
<td>1.18±0.45</td>
</tr>
<tr>
<td>2 cycles</td>
<td>36/44 (81.8)</td>
<td>5.29 (1.63-17.2)</td>
<td>p=0.056</td>
<td>1.34±0.57</td>
</tr>
<tr>
<td>3 cycles</td>
<td>21/26 (80.8)</td>
<td>5.67 (1.51-21.3)</td>
<td>p=0.01</td>
<td>1.46±0.76</td>
</tr>
</tbody>
</table>

*Logistic likelihood ratio test

*All parameters were log-transformed before statistical testing; only significant p values of post hoc tests are indicated Bonferroni/Dunn test v. 0 cycles: p = 0.012, p = 0.044, p = 0.026

### Table 3. Procedure-related parameters according to the number of resterilization cycles

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Procedure time (min)*</th>
<th>Fluoroscopy time (min)</th>
<th>Exposure to radiation (cGy cm²)</th>
<th>Contrast dye use (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 cycle (new catheters)</td>
<td>9.9±6.8</td>
<td>2.6±2.8</td>
<td>740±1060</td>
<td>44±12</td>
</tr>
<tr>
<td>1 cycle</td>
<td>9.1±4.9</td>
<td>2.4±1.9</td>
<td>904±1100</td>
<td>40±27</td>
</tr>
<tr>
<td>2 cycles</td>
<td>12.5±7.2*</td>
<td>3.2±2.7</td>
<td>690±643</td>
<td>47±26</td>
</tr>
<tr>
<td>3 cycles</td>
<td>11.5±10.6</td>
<td>4.2±5.4</td>
<td>887±1511</td>
<td>49±29</td>
</tr>
</tbody>
</table>

*p value (ANOVA) p=0.076 p=0.052 p=0.82 p=0.29

*All parameters were log-transformed before statistical testing; only significant p values of post hoc tests are indicated Bonferroni/Dunn test v. 0 cycles: p = 0.012, p = 0.044, p = 0.026

### Angiographic data

Procedures resulted in a percent stenosis reduction from 80.8±8.6% to 26.2±12.1% in the total cohort. The overall acute lumen gain of 1.66±0.56 mm showed no significant differences between new or used catheters (Table 4). The acute gain was significantly larger in stent recipients compared to patients with stand-alone balloon angioplasty (1.79±0.58 mm v. 1.53±0.50 mm, p = 0.0003). This benefit could only be observed in patients treated initially with a new or once resterilized balloon (data not shown).

After 4.1±1.9 months, the angiographic follow-up rate was 185/238 (77.7%), 96/124 (77.4%) for new and 89/114 (78.1%) for reused balloons (p = NS). The follow-up rates did not differ between the groups. In patients receiving stand-alone balloon angioplasty, late loss differed significantly between the groups (Table 4). For new balloons it was 0.42±0.70 mm and statistically higher in balloons resterilized twice (1.25±0.54 mm), which translated into late loss indices of 0.21±0.42 in new balloons and 0.93±0.58 in balloons resterilized twice (p < 0.0001). In patients receiving stents no differences were observed. Therefore, in stand-alone balloon angioplasty patients, the percent stenosis at follow-up was significantly more severe in the once resterilized catheter group compared to the new catheter group (68±23% v. 41±18%, p = 0.0001).

The overall binary restenosis rate was not significantly higher in patients dilated with reused compared to new balloons[39/89 (43.8%) v. 31/96 (32.3 %), p=0.13], being significantly more pronounced (p = 0.00097) after stand-alone balloon angioplasty [9/12 (75%)] in patients treated with twice resterilized catheters and unchanged (p = 0.97)
in stented vessels (Table 4). No myocardial infarction occurred and one death was registered (in those with the resterilized balloon catheter used first (p=NS).

In a logistic regression model using procedural success as the dependent parameter and the number of resterilization cycles, the operator skill, the age of the patient, low-density lipoprotein (LDL)-cholesterol, diabetes, smoking status, and stenting as independent parameters, only the number of resterilization cycles was a significant predictor for success (decreased after 2 and 3 cycles, p = 0.0028 and 0.069).

**Discussion**

The primary reason for using resterilized catheters in percutaneous coronary interventions is the expectation of providing the same quality of medical care at reduced cost. This expectation, however, cannot be unequivocally substantiated by the data available at present.

For the procedural success of coronary stenosis of ≥ 70% and < 100% with reused balloon catheters, similar rates for new and reused balloon catheters in the present study are in keeping with data of others, who reported comparable procedural success rates at the cost of significantly more stents required in one study.

The crossing of the lesions with reused catheters was significantly inferior compared to new balloons (OR 3.89, 95% CI 1.37–10.99, p = 0.0056) and was associated with an increased number of balloon catheters required per procedure (p=0.015). These differences reached statistical significance for 2–3 times reused balloon catheters.
opposed to once resterilized balloon catheters. The inferior performance can be attributed to the increased crossing profile secondary to the blunt rewrapping after the first inflation. For both, fluoroscopy and procedure time, trends toward more time needed were observed whereas exposure to ionizing radiation and use of contrast dye were similar.

These results are in keeping with a dual-center work in which one hospital used new balloons whereas in the second center only resterilized balloon catheters had to be chosen. For resterilized balloon catheters, significantly more balloons were required per lesion (2.4±1.5 v. 1.2±0.5, p < 0.00001), a higher incidence of initial balloon failure occurred (10.2 % v. 3.3 %, p < 0.00001), and prolongation of the procedure time (81 ± 41 v. 68 ± 32 min, p < 0.00001) and increased volume of contrast medium use (201±86 v. 165±61 ml, p < 0.0001) were observed. These results were confirmed in lesions of 90% and beyond. In yet another study comparing 53 consecutive patients with multivessel catheters to a second series of 54 patients with single use balloons the values for fluoroscopy time, the number of guiding catheters, and the number of angioplasty balloons used were similar.

The most intriguing finding of the present trial, however, is the fact that after 4.1±1.9 months of follow-up parameters such as late loss, late loss index, percent stenosis, and binary restenosis rate showed significantly higher values when stand-alone balloon angioplasty was attempted with reused balloons compared to the new ones. This holds true despite the fact that the rates of myocardial infarction and death were the same in both groups. This finding is not contradictory to the angiographic results since this trial does not provide any information whether the process of intimal proliferation was completed after four months or, more likely, will be continuing. In other series, higher rates of adverse clinical events of 7.8% v. 3.8% (p < 0.025) after 10-month clinical follow-up and of 10.0% v. 7.9% during the hospital stay were observed. The latter, however, could not be confirmed in a multivariate re-analysis.

Possible mechanisms of the enhanced restenosis rate encompass more mechanical vascular trauma induced by the higher crossing profile and the less smooth balloon surface. While leaking of the balloons is very unlikely, alterations in the balloon material could have induced more inflammation.

Limitations of the study: There is possibility of bias of the procedural results by the participating operators. The use of resterilized balloon catheters used to be the common approach in this center for the years preceding this study. Therefore, it is not very likely that the operators would jeopardize their current way of performing percutaneous interventional procedures. Since patients with restenosis were excluded, it may be assumed that the procedural results in restenotic lesions and in-stent restenosis lesions may be different. The overall importance of the present data is limited by the fact that the number of stent procedures is increasing worldwide.

With respect to the enhanced late loss and its associated parameters it must be emphasized that this trial was randomized for the primary and not for the secondary end points; therefore, some caution is recommended. Post hoc analysis, however, disclosed a power of 80% for a level of significance α=0.05 for the late loss index suggesting that the enhanced mid-term proliferation following stand-alone balloon angioplasty is more than an accidental finding.

The results apply for the four months follow-up period only and may not be translated to a longer period. There are also uncertainties whether balloons manufactured from different materials would result in different outcomes.

Conclusions: The findings of this study raise serious concerns with respect to reusing resterilized balloon catheters in stand-alone balloon angioplasty of de novo lesions due to their impaired procedural performance and apparently enhanced restenosis rate. Evaluation for a longer time period, i.e. for a nine months follow-up period is warranted. In non-drug eluting stent procedures of denovo coronary lesions, however, reused balloon catheters may still be used provided the altered mechanical properties of the devices are known and taken into account.

Acknowledgments
We are indebted to Mrs. Claudia Krapf and Miss Tina Ifland for their expert assistance in conducting this trial.

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17. US Food and Drug Administration. Ethylene oxide, ethylene chlorohydrin, and ethylene glycol: proposed maximum residue limits and maximum levels of exposure. Federal Register 1978; 43: 27472–27483
Role of Endothelin-1 in Genesis of Coronary Artery Disease

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Background: The endothelial cells produce the most potent vasoconstrictor known as endothelin-1. Elevated plasma levels of endothelin have been associated with coronary artery disease, essential hypertension and heart failure. The aims of the present study were, to compare the plasma endothelin-1 levels in coronary artery disease patients and healthy controls, to confirm endothelin-1 as surrogate marker for coronary artery disease and to compare the presence of endothelin-1 like immunoreactivity in aortic and internal mammary artery specimens obtained during coronary artery bypass graft surgery.

Methods and Results: The circulating levels of endothelin-1 were determined by enzyme-linked immunoassay in patients of coronary artery disease (n=145) and compared with healthy controls (n=70). Tissue endothelin-1 immunoreactivity was examined by immunohistochemical method in aortic and internal mammary artery tissue specimens obtained from 20 patients of coronary artery disease during coronary artery bypass grafting to understand the role of endothelin in atherosclerosis. Significantly higher levels (p < 0.001) of endothelin-1 were observed in all patients of coronary artery disease as compared to healthy controls. The immunoreactivity of endothelin-1 was localized to endothelial cell layer in internal mammary artery whereas in aortic specimens, in addition to endothelial cell layer, immunoreactivity was seen in the cytoplasm of smooth muscle cells of intima and media.

Conclusions: The significant increase in plasma endothelin-1 in coronary artery disease cases as compared to healthy subjects and presence of tissue endothelin-1 immunoreactivity in smooth muscle cells of intimal as well as medial layer of aorta confirm the role of endothelin-1 as a surrogate marker of atherosclerosis.

Key Words: Coronary artery disease, Atherosclerosis, Endothelial dysfunction
coronary artery bypass graft (CABG) surgery as a possible clue to understand the role of ET-1 as a surrogate marker of atherosclerosis.

Methods

Venous blood samples were collected after 14 hours of fasting in sterile, vacumed tubes containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant. Plasma was separated within half an hour, by centrifugation for 10 min at approximately 1000 × g, aliquotted and stored at ≤ -20°C. Repeated freeze-thaw cycles were avoided.

Plasma ET-1 was measured by quantitative sandwich enzyme immunoassay technique using commercial kit (R & D systems). Cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c) were measured as routine parameters on dry chemistry autoanalyzer (Vitros 750).

Male patients of CAD (n=145) in the age range 35-65 years, (confirmed by coronary angiography), before commencement of any drugs for CAD, were enrolled in the study over a period of 3 years for plasma ET-1 measurement. Out of 145 CAD subjects, 52 had single vessel disease (SVD), 45 had double vessel disease (DVD) and 48 had triple vessel disease (TVD). Seventy age-matched male subjects were enrolled as controls. The control subjects had no history of hypertension, diabetes or dyslipidemia and were not on any medication. All control subjects had negative stress test and in 15 subjects on whom coronary angiography was done due to atypical chest pain, angiograms were normal.

For the detection of tissue ET-1 immunoreactivity, tissue samples from both males (n=17) and females (n=3) undergoing CABG surgery were obtained. These 20 subjects were in addition to above 145 cases. Their plasma ET-1 levels were not measured because they were known cases of CAD and were taking medicines such as nitrates and beta-blockers which affect plasma ET-1 levels.

Tissue ET-1 immunoreactivity was studied from aortic and internal mammary artery specimens obtained during CABG surgery from 20 subjects with TVD (mean age 57.4 ± 2.95 years). The study was approved by the institutional review committee. Informed consent was taken from all the subjects prior to sample collection.

A three-step method consisting of the sequential application of primary antibody, link antibody and avidin-biotin complex (ABC) method was used (Dako). The tissues were fixed in 10% buffered formalin, dehydrated in graded series of alcohol and embedded in paraffin blocks. Serial sections of 6 µm thickness were taken for staining. Rabbit polyclonal anti-endothelin anti-serum (Pharmingen) in 1:100 dilution was used as primary antibody. A secondary antibody, biotinylated link goat anti-rabbit antibody was used followed by treatment with peroxidase-labeled streptavidin. The peroxidase activity was viewed by staining with diamino benzidine (DAB) substrate (brown staining). The slides were counterstained with hematoxylin. As controls, sections from human internal mammary artery with intact endothelial cell layers were used which gave the expected well-localized pattern within endothelial cells with negative staining of subintimal and medial layers. The stained slides were examined by the histopathologist in our institution.

Statistical analysis: The differences in categorical variables were analysed by χ² test. The comparison of continuous variables was done using unpaired student’s t test. Analysis of variance (ANOVA) followed by Bonferroni’s post hoc analysis was used for multiple comparison between different groups. Somer’s d statistical analysis was used to evaluate the correlation of ET-1 level with the number of diseased vessel. A value of p < 0.05 was considered statistically significant.

Results

The clinical features of the study population are shown in Table 1. The χ² analysis revealed no significant difference between the prevalence of risk factors such as smoking, alcohol consumption, personality type and significant family history of cardiovascular diseases (CVD) in CAD groups (SVD, DVD, TVD) as compared to controls. Hence there was no bias due to these risk factors in further analysis.

Table 2 summarizes continuous variables of the study population. The multiple comparisons using statistical method of ANOVA followed by Bonferroni’s post hoc method showed no difference in the mean levels of age between all groups (SVD, DVD, TVD).

Table 1. Comparison of categorical variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (n=70)</th>
<th>SVD (n=52)</th>
<th>DVD (n=45)</th>
<th>TVD (n=48)</th>
<th>χ²</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smokers*</td>
<td>16</td>
<td>13</td>
<td>12</td>
<td>10</td>
<td>0.499</td>
<td>0.591</td>
</tr>
<tr>
<td>Alcohol consumers**</td>
<td>26</td>
<td>23</td>
<td>15</td>
<td>19</td>
<td>1.303</td>
<td>0.728</td>
</tr>
<tr>
<td>Type A personality*</td>
<td>28</td>
<td>23</td>
<td>16</td>
<td>21</td>
<td>0.953</td>
<td>0.812</td>
</tr>
<tr>
<td>Positive family history**</td>
<td>21</td>
<td>18</td>
<td>14</td>
<td>15</td>
<td>0.308</td>
<td>0.958</td>
</tr>
</tbody>
</table>

Variables expressed as number of subjects

*Current or past users of any type of tobacco products
**Subjects consuming at least 1-2 pegs of alcohol daily
*Always remaining tense, anxious and overambitious
**Positive family history of coronary artery disease in at least one of the blood relatives (≤ 59 years)

SVD: single vessel disease; DVD: double vessel disease; TVD: triple vessel disease
the four study groups. However, ANOVA showed significant difference (p = 0.031) in mean levels of body mass index (BMI) between these groups. Bonferroni’s post hoc method showed significant increase in BMI level in DVD cases as compared to SVD subjects (p = 0.02, 95% CI 0.169, 3.064). However, there was no significant difference in BMI amongst other study groups. There was significant increase (p < 0.001) in mean ET-1 level in all the grades of CAD i.e. SVD [p<0.001, 95% CI -0.198, 0.143], DVD [p<0.001, 95% CI -0.446, 0.180] and TVD (p<0.001, 95% CI -0.456, 0.195) as compared to controls. In this single factor ANOVA study, the total sample of 215 subjects achieved 95% power to detect a difference of at least 0.23 using the Bonferroni (with control) multiple comparison test at a 0.05 significance level. The common standard deviation within a group is assumed to be 0.20. However, Bonferroni’s post hoc method showed no significant difference amongst the three groups of CAD cases (SVD, DVD and TVD). The correlation of the median level of plasma ET-1 (calculated from the total study population, 0.77 pg/ml) with number of diseased vessels i.e. with different grades of CAD with $\chi^2$ analysis using the Somer’s d statistical method showed significant positive association of plasma ET-1 levels with the number of diseased vessels. $\chi^2$ analysis using Bartholomew’s test for proportion also showed that the number of subjects having ET-1 level > 0.77 pg/ml increased significantly ($\chi^2 = 51.3$, p < 0.001) with increase in number of diseased vessels (Fig. 1). This analysis can detect increasing trend with power of 100%.

The statistical analysis performed by method of ANOVA followed by Bonferroni’s adjustment showed significant increase in ET-1 levels in CAD subjects grouped as per their clinical presentation i.e. in subjects having unstable angina pectoris (UAP) (0.89 ± 0.31, n=52, p < 0.001, 95% CI 0.231, 0.408), subjects having stable angina pectoris (SAP) (0.86 ± 0.26, n=56, p < 0.001, 95% CI 0.206, 0.373) and subjects with no angina (NA) but with symptoms of angina equivalent (0.86 ± 0.28, n = 37, p < 0.001, 95% CI 0.201, 0.379) as compared to controls. In this single factor ANOVA study, the total sample of 215 subjects achieved 95% power to detect a difference of at least 0.23 using the Bonferroni (with control) multiple comparison test at a 0.05 significance level. The common standard deviation within a group is assumed to be 0.20. However, there was no significant difference in ET-1 levels between UAP group, SAP group and NA group (Fig. 2).

Smoking, hypertension, diabetes mellitus and dyslipidemia are major risk factor for CVD. The comparison of mean ET-1 levels between smokers (n = 16) and non-smokers (n = 54) in control group using unpaired student’s t test showed significant rise (p = 0.045, 95% CI 0.005, 0.215) in plasma ET-1 levels in smokers as compared to non-smokers. Group sample sizes of 16 and 54 achieved

### Table 2. Comparison of continuous variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (n=70)</th>
<th>SVD (n=52)</th>
<th>DVD (n=45)</th>
<th>TVD (n=48)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>51.0 ±7.66</td>
<td>53.0 ±6.88</td>
<td>52.4 ±8.69</td>
<td>52.4 ±6.92</td>
<td>0.407</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.2 ±4.26</td>
<td>24.5 ±2.74</td>
<td>26.1 ±2.74</td>
<td>25.2 ±2.82</td>
<td>0.031</td>
</tr>
<tr>
<td>ET-1 (pg/ml)</td>
<td>0.57 ±0.19</td>
<td>0.84 ±0.29</td>
<td>0.88 ±0.29</td>
<td>0.89 ±0.29</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Variables expressed as mean±SD

SVD: single vessel disease; DVD: double vessel disease; TVD: triple vessel disease; BMI: body mass index; ET-1: endothelin-1

### Fig. 1. Comparison of number of subjects having ET-1 levels above median level in different CAD groups (Bartholomew’s test).

n1: number of subjects having values above median level (0.77 pg/ml)
n2: number of subjects having values below or equal to median level

Con: controls; SVD: single vessel disease; DVD: double vessel disease; TVD: triple vessel disease; ET-1: endothelin-1; CAD: coronary artery disease

### Fig. 2. Diagram showing comparison of ET-1 levels between different groups of CAD according to clinical presentation and control group.

Values expressed as mean ± SD

CON : controls, n=70; UAP : unstable angina pectoris, n = 52; SAP : stable angina pectoris, n=56; ET-1 : endothelin-1; CAD: coronary artery disease; NA : no angina, n = 37
50% power to detect a difference of 0.1. A study with larger sample size is required to achieve increase in power of detection. In CAD group, there was no significant difference in ET-1 levels between smokers (n = 35) and non-smokers (n = 110) (Fig. 3). None of the control subjects had hypertension, diabetes or dyslipidemia. When CAD subjects were sub-grouped with respect to all these risk factors, and compared using student’s t test, no significant difference in ET-1 levels was seen between diabetic versus non-diabetic subjects, normotensive versus hypertensive subjects and normolipidemic versus dyslipidemic subjects as seen in Table 3. In many subjects there were multiple risk factors, hence the comparison of ET-1 levels with respect to one risk factor may be masked by presence of other risk factors.

Tissue ET-1-immunohistochemical staining in aortic specimens confirmed that ET-1 like immunoreactivity was not only localized in endothelial cells but was also seen in the cytoplasm of the intimal and medial vascular smooth muscle cells suggesting the involvement of ET-1 in neovascularization and smooth muscle cell proliferation associated with atherosclerosis (Fig. 4). In contrast, in sections taken from internal mammary artery specimens, where there was no atherosclerosis, mild brown staining was seen only in endothelial cells, but not in the cytoplasm of the vascular smooth muscle cells (Fig. 5).

Table 3. Comparison of ET-1 levels between subjects with and without cardiovascular risk factors in CAD group

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± SD</th>
<th>t</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic (n = 103)</td>
<td>0.87 ± 0.29</td>
<td>0.199</td>
<td>0.842</td>
</tr>
<tr>
<td>Diabetics* (n = 42)</td>
<td>0.86 ± 0.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normotensive (n = 92)</td>
<td>0.87 ± 0.30</td>
<td>0.193</td>
<td>0.847</td>
</tr>
<tr>
<td>Hypertensive** (n = 53)</td>
<td>0.86 ± 0.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal lipids (n = 26)</td>
<td>0.82 ± 0.28</td>
<td>0.960</td>
<td>0.338</td>
</tr>
<tr>
<td>Dyslipidemia* (n = 119)</td>
<td>0.88 ± 0.29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Documented cases
**Systolic blood pressure ≥ 140 mmHg, Diastolic blood pressure ≥ 90 mmHg
*Total cholesterol ≥ 200 mg/dl, triglycerides ≥ 150 mg/dl, LDL ≥ 130 mg/dl, HDL < 40 mg/dl
ET-1: endothelin-1, CAD: coronary artery disease, LDL: low-density lipoprotein; HDL: high-density lipoprotein

Fig. 3. Diagram showing comparison of non-smokers versus smokers in control and CAD group. Values expressed as mean ± SD
CAD: coronary artery disease

Fig. 4. Photomicrographs of immunostaining of sections of aorta removed from subject undergoing CABG, stained with DAB substrate, as seen under magnification of 25×. Black arrows show brown immunostaining of ET-1. Note the strong staining of endothelial cells and cytoplasmic staining of intimal as well as medial smooth muscle cells. The staining suggests very high grade of endothelial cell dysfunction.
CABG: coronary artery bypass graft surgery; DAB: diamino benzidine; ET-1: endothelin-1

Fig. 5. Photomicrographs of immunohistochemical staining of sections of internal mammary artery with intact endothelial cells, stained with DAB substrate, as seen under magnification of 25×. Black arrow shows brown immunostaining of ET-1 which is seen only in endothelial cells, not in cytoplasm of smooth muscle cells.
DAB: diamino benzidine; ET-1: endothelin-1
Discussion

Endothelial dysfunction has been implicated as a major event in the pathogenesis of atherosclerosis. So, there is considerable scope for research in diagnostic assays for the assessment of endothelial function. Elevated plasma levels of ET-1 have been associated with CAD, essential hypertension and heart failure, where endothelial dysfunction is the key factor.

In the present study, plasma ET-1 levels were determined in CAD patients and compared with normal subjects. The number of subjects having high levels (higher than median) of ET-1 increased with number of diseased vessels showing significant positive correlation with grade of CAD. Our results are in agreement with the findings of other researchers who also found increase in ET-1 in different stages of CAD.7-9

Lerman et al.7 reported significantly elevated levels of ET-1 in 40 patients with atherosclerosis of various arteries (aortic, coronary, renal and peripheral) as compared to 100 normal subjects. There was a significant correlation between plasma ET-1 and the number of sites of disease involvement (r = 0.89, p < 0.001). Salomone et al.8 reported significant rise in ET-1 in cases having chronic stable angina and angiographically documented CAD and chronic stable angina cases having normal angiogram as compared to normal subjects. ET-1 was also found to be correlated with severity of the disease (r = 0.25, p = 0.04) and number of stenoses (r = 0.36, p = 0.002). Kaski et al.9 compared plasma ET-1 levels in 54 patients with angina pectoris who underwent baseline testing of electrocardiography (ECG), stress test, two-dimensional echocardiography (2D echo), thallium stress test and coronary angiography to 21 healthy control subjects. The plasma ET concentrations were significantly higher in patients compared with control subjects. Borries et al.10 determined ET-1 in patients with stable angina, unstable angina and healthy subjects. The concentration of ET-1 of the patients with SAP and UAP were significantly higher than that of healthy controls. These results were similar to our findings.

However, other researchers did not find significant difference in their studies. Yasuda et al.11 and Stewart et al.12 in their comparative study of patients with SAP with controls did not find significant difference in ET-1 levels. This could be due to their small sample size. Our sample size was much larger, hence we found significant increase in plasma ET-1 levels in CAD subjects having stable angina as compared to controls. Table 4 shows an overview of the results of studies conducted in different countries. Our results are in agreement with majority of studies (4 out of 6 studies) which point to the importance of ET-1 as a surrogate marker of CAD.

Table 4. Overview of results from different studies

<table>
<thead>
<tr>
<th>Authors</th>
<th>Country</th>
<th>Number of subjects</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lerman et al.</td>
<td>USA</td>
<td>40 atherosclerosis / 100 Con</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Salomone et al.</td>
<td>UK</td>
<td>65 SAP with CAD / 49 Con</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11 SAP (normal CAG) / 49 Con</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Kaski et al.</td>
<td>UK</td>
<td>54 CAD / 21 Con</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Borries et al.</td>
<td>Germany</td>
<td>20 SAP / 11 Con</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 UKP / 11 Con</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Yasuda et al.</td>
<td>Japan</td>
<td>10 SAP / 25 Con</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Stewart et al.</td>
<td>Canada</td>
<td>7 SAP / 22 Con</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Sainani et al.</td>
<td>India</td>
<td>145 CAD / 70 Con</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Present study</td>
<td>India</td>
<td>52 UAP / 70 Con</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>56 SAP / 70 Con</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

SAP: stable angina pectoris; Con: controls; CAD: coronary artery disease; CAG: coronary angiogram; UAP: unstable angina pectoris

Cigarette smoking is a well-known risk factor for the development of CVD. The experiment carried out by Sainani et al.14 on cigarette smoke extracts indicated that it induces oxidation of LDL which is the initial stage of endothelial dysfunction. Our study, smokers and non-smokers were compared in control group to ascertain the effect of smoke on endothelial dysfunction. Our results showed that there was a significant increase (p = 0.045) in ET-1 levels in smokers (n = 16) as compared to non-smokers (n = 54) in control group. Our results were in agreement with the work done by Orem et al.15 who measured plasma ET-1 in 24 males who had no coronary atherosclerotic lesions established by coronary angiography. They also reported higher plasma ET-1 concentration in patients who were smokers than those who were non-smokers. All these results suggest an increase in ET-1 in a very early stage of endothelial dysfunction before actual disease manifests. However, we did not find any significant difference between smokers and non-smokers in CAD cases possibly because atherosclerosis in CAD cases in both smokers and non-smokers was already established. Thus, ET-1 seems to be an important early marker of endothelial dysfunction, which is a key factor for atherogenesis.

Autocrine action of ET-1 is well established. Not only do the vascular endothelium but, under certain conditions, smooth muscle cells also generate ET-1.16

In our study, we found significant staining in endothelial cells as well as in cytoplasm of subintimal and medial smooth muscle cells in aorta specimens from subjects who had TVD, and had undergone CABG surgery. In sections taken from internal mammary artery, staining was localized only in endothelial cells and that was also not as significant as seen in endothelial cells of aorta.
Minamino et al.18 examined the expression and localization of muscle cell proliferation associated with atherosclerosis. The peptide is involved in neovascularization and smooth muscle cells. The presence of ET-1 like immunoreactivity in endothelial cells and also in vascular smooth muscle cells shows that this peptide is involved in neovascularization and smooth muscle cell proliferation associated with atherosclerosis. Minamino et al.18 examined the expression and localization of endothelin-converting enzyme-1 (ECE-1), the final key enzyme of ET-1 processing, in human atherosclerotic lesions and found that the patterns of ECE-1 localization resemble those of ET-1 localization described in previous studies by Lerman et al.6 Ihling et al.19 performed qualitative and quantitative immuno-histochemistry in normal internal mammary arteries (n = 10), in coronary arteries with adaptive intimal fibrosis (n = 10), in aortic fatty streaks (n = 10) and in distinct regions of advanced carotid plaques (n = 15). They reported that together with ET-1, ECE-1 was abundantly present in human arteries and the up-regulation of ECE-1/ET-1 system was closely linked with the presence of chronic inflammation, and was present in very early stages of plaque evolution. Their findings suggested that enhanced production of active ET-1 may substantially contribute to cell growth and the regulation of vascular tone in advanced atherosclerotic lesion as well as in the very early stages of plaque evolution, when a plaque is still imperceptible clinically.

The measurement of circulating ET-1 in our population, where prevalence of CAD is increasing day by day, would be helpful in diagnosing endothelial cell dysfunction at a very early stage, thereby helping in implementing preventive measures. Early detection of endothelial cell dysfunction, which is seen in this study, in smokers with no apparent cardiac problem, may be a useful measure to guide therapy prior to the development of symptomatic atherosclerosis.

Conclusions: Measurement of ET-1 in smokers above 35 years can give an early and useful clue about endothelial cell dysfunction if the ET-1 levels are raised. Such persons should be advised specifically regarding prevention of CAD by diet and lifestyle modifications. Many challenging issues still remain unsolved, and therefore, the endothelium at both the molecular and cellular levels must be further studied for both research and clinical interests.

Acknowledgements

We are grateful to the Managing Trustee of Jaslok Hospital and Research Centre, Seth MK Chanrai, members of scientific advisory committee, Research Director - NH Wadia and former Research Director - MG Deo for providing us with necessary facilities, the financial support for the project and valuable suggestions for undertaking the research project at this Centre.

We express our gratitude to AB Mehta, Director, Cardiology Department, Jaslok Hospital and Research Centre, the entire team of Cardiologists and the staff of Cardiology Department for their help in providing patients and coronary angiography reports for the study and to SR Khubchandani, Head, Histopathology and Electron Microscopy Department, for examining the tissue samples.

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system on the patients evaluated for coronary artery disease. Circulation 1975; 51: 5–40
Real-Time Three-Dimensional Transthoracic Echocardiography

Ravi R Kasliwal, Nagendra S Chouhan, Ashish Sinha, Pankaj Gupta, Sharad Tandon, Naresh Trehan
Escorts Heart Institute and Research Centre, New Delhi

Background: Complex anatomy of intra-cardiac structures requires spatial orientation of image in three dimensions for better understanding and enhanced image interpretation. We evaluated the feasibility and efficacy of the emerging ‘real-time three-dimensional transthoracic echocardiography’ technique for comprehensive assessment of cardiac anatomy, physiology, pathomorphology and pathophysiology in patients with structural heart disease.

Methods and Results: Patients with structural heart disease (n=152) were evaluated by conventional two-dimensional transthoracic echocardiography and real-time three-dimensional transthoracic echocardiography using standard protocol. Fifty-six cases were of rheumatic etiology with multi-valvular involvement (mitral stenosis: 32; mitral regurgitation: 29; tricuspid regurgitation: 8; aortic valve disease: 11) and 21 cases of non-rheumatic valvular heart disease. A total of 38 congenital heart disease patients were examined including 23 patients with atrial septal defect. Left ventricular function (n=20) and right ventricular function (n=10) were also assessed using dedicated software.

Conclusions: Results of real-time three-dimensional transthoracic echocardiography mitral valve area assessment by planimetry are comparable to two-dimensional transthoracic echocardiography with additional information about surface anatomy of leaflets and the subvalvular apparatus in real time with clear demarcation of commissural fusion and scallops of leaflets. Enface view of atrial septal defect with direct visualization of shape, size and number of defects, tricuspid valve area by planimetry, right ventricular shape, objective assessment of ventricular volumes and regurgitation vena contracta area are the fields where three-dimensional transthoracic echocardiography was of additive value to conventional two-dimensional transthoracic echocardiography. This study proves clinical feasibility of real-time three-dimensional transthoracic echocardiography but requires further validation of quantitative observations. (Indian Heart J 2005; 57: 128–137)

Key Words: Echocardiography, Congenital heart disease, Valvular heart disease
We report our experience of first 152 cases done on real time 3D transthoracic echocardiographic (3DTTE) imaging system in different cardiac conditions and its incremental value over conventional 2D transthoracic echocardiography (2DTTE) in assessment of intra-cardiac anatomy and clinical decision making.

**Methods**

**Study population:** Real-time 3DTTE was performed in 152 patients in our institute (age range 2-71 years) with structural heart diseases diagnosed on 2DTTE. 2DTTE was performed using a Sonos 5500 (Philips Medical Systems, USA) ultrasound system and 2.5 and 8 MHz transducer capable of providing real time B mode and color Doppler image. Clinical impression made from review of real time 3DTTE and 2DTTE obtained at the same visit were compared for final assessment by two independent, blinded echocardiographers.

**3DTTE data acquisition and analysis:** In all patients 3DTTE images were obtained from standard parasternal, apical and sub-costal windows using a Sonos 7500 Live 3D Echo (Philips Medical Systems, USA) ultrasound system. Method of data acquisition was based on recommendations of Adhoc 3D Echo Protocol Working Group endorsed by the International Society of Cardiovascular Ultrasound. Data analysis was performed using integrated tools for advanced image processing to obtain views with details of endocardial and valvular surfaces and their relationship with adjoining structures. Quantitative assessment was performed on a dedicated personal computer using dedicated software capable of measuring three-dimensional data sets.

**Qualitative analysis:** These 3DTTE images were analyzed using integrated software system by cropping in three different color-coded cutting planes perpendicular to each other (Fig. 1A). Besides these cutting planes, an additional oblique plane capable of cropping the image in any desired angulation was also used. This particular plane is advantageous in assessing the intra-cardiac structures placed at an angle other than the perpendicular one (Fig. 1B). One single 3D data set acquired in any possible view is good enough to get all the conventional 2D views [short axis (Fig. 1B), three-chamber (Fig. 1C) and four-chamber (Fig. 1D)]. The echocardiographic images were sectioned to obtain the desired cut planes. Brightness, contrast and color mapping were adjusted to obtain the optimal image quality. These acquired real-time and full volume cine images can be rotated in space to visualize intra-cardiac structures from different sides (Fig. 2A, 2B).

**Quantitative analysis:** The acquired 3DTTE data sets were stored on CD-ROM in DICOM format and transferred to a separate workstation for off-line data analysis. Quantitative analysis was done using dedicated software analysis systems (QLAB Advanced Quantification software, version 3.0, Philips Ultrasound, USA, and Tomtec 4D Cardio-view, version 1.2, Tomtec, Gmbh, Germany) for measurement of distance, area, volumes and angles.

**Quantitative assessment of valve area:** For planimetry assessment of valve area, conventional 2DTTE short-axis view and 3DTTE images were acquired from any available window. Measurements were done using off-line analysis software (QLAB Advanced Quantification software, version 3.0) which provides a 3D cine image along with corresponding 2D images in three modifiable cutting planes. These planes were aligned to the axis of valve opening plane to get short axis image in the plane of shortest valve area enface. Results were compared.
Quantitative assessment of atrial septal defect (ASD): Consecutive patients diagnosed to have ASD with 2DTTE were enrolled in the protocol of transcatheter device closure. ASD shape, number and dimensions (major diameter $D_1$, minor diameter $D_2$ and area) were determined by 2DTTE, 2DTEE and 3DTTE. During transcatheter closure, balloon occlusive diameter (BOD) which was regarded as the gold standard for selection of the size of any device,8-10 was measured using standard technique and was correlated with echo-derived measurements by different techniques.

Quantitative assessment of ventricular volumes: Left ventricular volume analysis was done using a semi-automated border detection software (Tomtec 4D LV Analysis v.1.2, Tomtec, Gmbh, Germany). The original 3DTTE pyramidal volume, acquired encompassing the complete left ventricle (LV), was used to calculate global as well as segmental volumes, actual stroke volume and ejection fraction (EF). EF was calculated off-line from time-volume curve of entire reconstructed cast. This cast can be subdivided into conventional 17 segment model of LV with estimation of individual segmental motion and volumes in time-volume curve.11 Wall motion abnormalities and LV dyssynchrony could also be detected and analyzed by the segmental time-volume curve.

Right ventricular volume and EF analysis was performed on optimal 3D images encompassing the complete right ventricle (RV) volume using Tomtec Cardio-view software. The images were cropped in three orthogonal cutting planes to obtain best possible image of RV in long axis. Endocardial borders were manually traced in eight contiguous volumetric slices of tagged end-systolic and end-diastolic frames with formation of volume-rendered holographic cast. End-systolic volume, end-diastolic volume and EF were calculated from these images.7

Statistical analysis: In patients of mitral stenosis, measurements of maximal area obtained by 2DTTE, 2DTEE and 3DTTE, and in patients of ASD maximal diameter and area obtained by 2DTTE, 2DTEE, 3DTTE and cardiac catheterization were compared using linear regression analysis, paired t test for continuous variables, and calculation of the mean difference. A $p$ value < 0.05 was considered statistically significant. Inter-observer and intra-observer variabilities for 3D measurements were determined in all studies.

Results

A total of 152 patients studied included valvular heart disease ($n=77$), congenital heart disease ($n=38$), ischemic and non-ischemic cardiomyopathy ($n=20$) and other miscellaneous cardiac anomalies ($n=17$). The average time required for data acquisition including patient preparation was $8.2\pm2.1$ min and for analysis $20.3\pm6.8$ min.

Valvular heart disease: 3D echocardiography gives additional information about surface anatomy of cardiac valves and their relation with surroundings in real time which was found to be useful in assessing the functional and anatomical integrity of the valve by partly overcoming the limitations of 2D study especially in patients with poor acoustic windows.

Mitral valve: In our study of 32 patients (12 females, mean age $36.45\pm8.88$ years) with rheumatic mitral stenosis, assessment of mitral valve area was done using conventional 2DTTE short-axis view and 3DTTE image acquired from any available window. Mitral valve area was measured with greater confidence by 3DTTE planimetry with additional information about commissural fusion and scallops of leaflets (Fig. 2) with clear demarcation of surface anatomy of leaflets and the subvalvular apparatus (Fig. 2). 3DTTE-derived planimetry mitral valve area (MVA) (Fig. 3) had a good correlation with conventional

Fig. 2: Real time 3DTTE data set taken from parasternal window showing mitral valve (zoomed) viewed from left ventricular side (A) and LA side (B) showing the surface of the thickened anterior and posterior mitral leaflet. The cropped 3D image obtained from apical window to show the thickened mitral valve with unfused commissures (arrowheads) and threescallops of anterior mitral leaflet A1, A2 and A3 (black arrows) (C). Full volume 3D data set acquired from parasternal window. Anterolateral left ventricular wall was cropped to visualize mitral apparatus with subvalvular structures. Arrowheads point to the thickened chordae (D).

3DTTE: three-dimensional transthoracic echocardiography
2DTTE methods (3DTTE planimetry MVA and 2DTTE planimetry MVA, r=0.985; 3DTTE MVA and 2DTTE pressure half time MVA, r=0.965) (Figs 4 and 5). It was also found that 3DTTE valve area correlated better with smaller MVA value derived from two different 2DTTE methods (r=0.987) (Table 1). Inter-observer and intra-observer variability was good for 3DTTE (r=0.93 and 0.96 for mitral valve area). In seven patients who underwent balloon mitral valvotomy (BMV), 3DTTE provided images with spitted commissures and it was possible to measure splitting width objectively. Leaflet tears, if any, were also better visualized.

On evaluating 29 patients with mitral regurgitation (MR) it was possible to visualize entire regurgitant jet with its varied asymmetrical shapes with actual calculation of regurgitant orifice area. It was also observed that vena contracta was not a circular structure as assumed earlier, but rather with varied geometric shapes (Fig. 6A, 6B).

![Fig. 3. Full volume three-dimensional data set has been obtained from apical window shown with three orthogonal cutting planes (D) along with contemporary two dimensional images in respective planes (A), (B) and (C). The 'C' cutting plane was aligned to the opening plane of mitral valve in 'A' and 'B' images to visualize the shortest mitral valve opening area (arrowhead) in image 'C' for measurement.](image)

Prosthetic heart valves: Ten patients with prosthetic heart valve were also studied. In 2 patients with bioprosthetic valves at mitral and aortic positions respectively, we were able to delineate actual site, size and shape of the paravalvular leak and its relation to the surrounding structures while evaluating for the feasibility of putting a

![Fig. 4. Comparison between three-dimensional and two-dimensional trans-thoracic echocardiography PHT-derived MVA.](image)

![Fig. 5. Comparison between three-dimensional and two-dimensional trans-thoracic echocardiography (planimetry)-derived MVA.](image)

### Table 1. Correlation between 3DTTE and 2DTTE mitral valve area measurements

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mean Diff. (mm)</th>
<th>r</th>
<th>SEE</th>
<th>Equation</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2D PHT MVA v. 3D planimetry MVA</td>
<td>0.129</td>
<td>0.965</td>
<td>0.051</td>
<td>y=0.874x + 0.041</td>
<td>&lt;0.0001</td>
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<tr>
<td>2D planimetry MVA v. 3D planimetry MVA</td>
<td>0.069</td>
<td>0.985</td>
<td>0.035</td>
<td>y=0.924x + 0.029</td>
<td>&lt;0.0001</td>
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<tr>
<td>2D minimum MVA v. 3D planimetry MVA</td>
<td>0.020</td>
<td>0.987</td>
<td>0.033</td>
<td>y=0.937x + 0.059</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

3DTTE: three-dimensional transthoracic echocardiography; 2DTTE: two-dimensional transthoracic echocardiography; Mean Diff: mean difference; SEE: standard error of estimation; PHT: pressure half time; MVA: mitral valve area
percutaneous closure device (Fig. 6C, 6D). In 8 cases of metallic prosthetic valve, this technique was not very useful except in 2 cases where we were able to identify left atrial appendage clots. An innate limitation of all ultrasound systems is that they cannot pass through metal.

Tricuspid valve: Tricuspid valve area was evaluated in 17 patients (9 cases of rheumatic and 8 cases of non-rheumatic tricuspid valve disease). Real time 3DTTE was clearly superior to 2DTTE and even two-dimensional transesophageal echocardiography (2DTEE) by providing enface view of all leaflets at any point in cardiac cycle by single acquisition (Fig. 7A, 7B, 7C, 7D). Planimetry of tricuspid valve area, details of subvalvular structures and tricuspid regurgitation vena contracta area were calculated as for mitral valve disease, and were highly reproducible.

Atrial septal defects: 3DTTE evaluation of 24 cases of ASD provided unique enface views of the defect from the right atrial (RA) as well as left atrial (LA) sides and its spatial orientation with information about adequacy of rims for device closure and its relation to surrounding structures (Fig. 8A). In our preliminary study of 10 consecutive patients having 11 ASDs (one patient had 2 ASDs) (9 females, mean age 31.25±4.99 years), compared with conventional echocardiographic methods, 3DTTE-derived maximum ASD diameter had best correlated with the invasively determined BOD (3DTTE r = 0.930, p ≤ 0.0001, 2DTEE r = 0.927, p ≤ 0.0001, 2DTTE r = 0.921, p = 0.0002). 3DTTE-derived ASD area also best correlated with the invasively determined BOD (3DTTE r = 0.926, 2DTEE r = 0.870) (Table 2) (Figs 9 and 10). Inter-observer and intra-observer variability were good for 3DTTE (r=0.91 and 0.94 for ASD diameter, r=0.90 and 0.95 for ASD area). Post-procedure assessment of ASD device also gives details about shape and position of device in situ and shows its proper placement (Fig. 8B).

### Table 2. Correlation between BOD and other ASD size measurements

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mean Diff. (mm)</th>
<th>r</th>
<th>SEE</th>
<th>Equation</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2DTTE dia v. BOD</td>
<td>0.670</td>
<td>0.921</td>
<td>0.126</td>
<td>(y=0.844x+0.946)</td>
<td>0.0002</td>
</tr>
<tr>
<td>2DTEE dia v. BOD</td>
<td>0.327</td>
<td>0.927</td>
<td>0.114</td>
<td>(y=0.846x+0.632)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>3DTEE dia v. BOD</td>
<td>0.027</td>
<td>0.930</td>
<td>0.115</td>
<td>(y=0.871x+0.277)</td>
<td>&lt;0.0001</td>
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<tr>
<td>2DTEE area v. Cath area</td>
<td>2.321</td>
<td>0.870</td>
<td>0.242</td>
<td>(y=1.281x+1.652)</td>
<td>0.0005</td>
</tr>
<tr>
<td>3DTTE area v. Cath area</td>
<td>1.599</td>
<td>0.926</td>
<td>0.200</td>
<td>(y=1.468x+0.148)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

BOD: balloon occlusive diameter; ASD: atrial septal defect; Mean Diff: mean difference; SEE: standard error of estimation; 2DTTE: two-dimensional transthoracic echocardiography; 2DTEE: two-dimensional transesophageal echocardiography; 3DTTE: three-dimensional transthoracic echocardiography; Cath area: balloon occlusive area.
Intra-cardiac masses: We evaluated 2 left atrial myxomas, 1 right atrial myxoma, 1 tricuspid valve tumor and 2 cases each of LV thrombus, left atrial thrombus and a large LV hydatid cyst. In 2 cases of LV thrombus it was possible to section the thrombus sequentially. Initially the thrombus was a solid structure and on follow-up, central liquefaction with gradual resolution and fibrous septa formation was observed. One patient had a large cystic mass in LV cavity, in which on systematically cropping the 3D pyramidal data set, we were able to see grand daughter cyst budding from the inner wall of the large cyst which helped us in arriving at a definite diagnosis of hydatid cyst.

Ventricular functions: In our preliminary assessment from 20 cases of cardiomyopathy for evaluation of LV function using 3DTTE, it was feasible to objectively assess EF in routine clinical practice. Off-line analysis was done on Tomec software, which required 10.2±2.4 min. LV volumes during complete cardiac cycle were depicted in a time-volume curve with sequential change with time. It also gives information about segmental contribution to the EF and segmental wall motion in a time-volume curve. By analyzing these superimposed time-volume curves of 16 conventional ventricular segments, assessment of ventricular synchrony can also be done along with identification of individual segments which contract late (Fig. 11).

Evaluation of RV volume and shape was undertaken in 10 cases of pulmonary hypertension (PHTN) (PA pressure = 67±30 mmHg). Off-line assessment of global and segmental RV cavity area along with RV end-systolic, end-diastolic volumes and RVEF was done using dedicated software (Tomtec 4D Cardio-view, version 1.2, Tomtec, Gmbh, Germany) (Fig. 12). Patients with PHTN had greater RV volumes and RV end-diastolic diameters than normal individuals.

Other congenital heart diseases: Two cases of cleft mitral valve were done on 3DTTE with clear delineation of the cleft, its edges and position (Fig. 8C) and was well correlated with intra-operative visualization. Other congenital heart disease cases studied included 3 ventricular septal defects (Fig. 8D), 2 patent ductus arteriosus, 2 sinus of valsalva aneurysms, 2 Ebstein anomaly, and 4 cases of supra-mitral ring. In these complex congenital lesions, 3DTTE had given additional information by providing the spatial orientation of the anatomical structures.
3D echocardiography and valvular heart disease:

Conventional 2D echocardiography is the most commonly used modality for assessing valvular heart disease. The conventional planimetry of mitral valve orifice by 2D echocardiography, pressure half time, continuity equation and proximal iso-velocity surface area (PISA) are the acceptable methods for assessment of mitral valve area (MVA), however they have their own pitfalls. Doppler-based methods are of limited value as they are heavily dependent on hemodynamic variables and coexisting valvular lesions. As reported by Martin et al., success rate of planimetry is said to be as low as 75%. A study by Binder et al. reported an overestimation of MVA in the range of 63% for a small deviation of 6º from the optimal plane. This error further increases with a small change of transducer position, angulations and rotation. Difficulty in defining the correct imaging plane that displays the true mitral valve orifice is a major limitation. On the contrary, 3D echocardiographic estimation has overcome most of the limitations as it measures actual anatomical valve area in an ideal short axis plane and is independent of hemodynamic variables. By using the oblique plane on the 3D acquired data set, we can correctly ascertain the plane of the opening of the valve and by cropping the image in that particular plane at a level, we get the smallest opening area of the leaflets and the actual mitral valve orifice area (Fig. 13). We found a high degree of correlation in measured valve area by this method and calculated valve area by conventional 2D and Doppler-based methods (3DTTE planimetry MVA and 2DTTE planimetry MVA, r=0.985; 3DTTE MVA and 2DTTE pressure half time MVA, r=0.965). Theoretically all these facts also hold true for aortic, tricuspid and pulmonary valves. In a previous study, we reported the comparison of MVA by reconstructed 3DTEE and mitral valve area measured at operating table with high degree of agreement (r=0.95). Applebaum et al. have also demonstrated the value of visualizing the mitral valve commissural splitting and leaflet tears in reconstructed 3DTEE which were not visualized on conventional 2D images in patients who underwent balloon mitral valvotomy (BMV) for mitral stenosis. Now with real time imaging, the correlation was found to be even better with high reproducibility. The other practical use of this new technique has been in the cases of mitral valve prolapse, which is frequently under-or over-estimated using M-mode and 2D techniques because of its non-planar leaflet-annular relations. Enface visualization of mitral valve from ventricular and atrial side along with clear demarcation of scallops of valvular leaflets incorporated in prolapse is possible in volume-rendered images acquired.
using 3DTTE. Quantification of severity of mitral regurgitation is also a challenging task while deciding for operative management. Real time 3D echocardiography has theoretical advantage over conventional methods by incorporating entire regurgitant jet area in spatial orientation. It provides unique information about direction and extent of jet which are useful in eccentric jets. Clinical feasibility of this technique for assessment of valve functions has been validated by previous studies, but for any clinical significance it requires standardization and further validation. Planimetry measurement of the area of vena contracta and mitral regurgitation area was also possible by this technique and correlates well with conventional Doppler-based methods.

Two-dimensional echocardiography was proved to be reliable in diagnosis of mitral and aortic stenosis. Their role in the diagnosis of rheumatic tricuspid stenosis is still being defined as interpretations are based on indirect evidences and not the actual measurement. With advent of 3DTTE technique, assessment of tricuspid valve area had been now possible with clear delineation of all the three leaflets in enface view (Fig. 7D). With this technology one can actually measure tricuspid valve area by planimetry along with greater details about valvular morphology, and can be considered as gold standard in absence of any other modality after standardization with autopsy studies. 3DTTE also provides reproducible images of entire tricuspid regurgitant jet along with actual measurement of regurgitant valve area but requires validation studies before being used for clinical decision making.

In aortic valve disease also 3DTEE, by overcoming the limitations of conventional echocardiography and correctly ascertaining the actual valve area (Fig. 13) and effective regurgitant valve area, can reduce the need for transesophageal echocardiography, though the paucity of validation study makes it still a hypothesis.

3D echocardiography in patients with prosthetic heart valves: 3D echocardiography gives ‘enface’ view of prosthetic valves along with position of struts and opening and closing motion in a single image, especially in patients with peri-prosthetic leak in which it was very difficult to localize the site and shape of leak by conventional echocardiography. In 2 of our cases, accurate localization and assessment of size and shape by 3DTTE was the key point in deciding for device closure of these leaks.

3D echocardiography in congenital heart diseases: Congenital heart disease evaluation requires mental conceptualization of planar 2DTTE images in 3D construct of cardiac anatomy by integrating observation from adjacent views, something that is possible only for an experienced echocardiographer. The emerging real time 3DTTE has eliminated need for imaginary reconstruction of cardiac structures and their anatomical relation by different observers. In patients with complex congenital lesions, 3DTTE had given additional information by providing the spatial orientation of the anatomical structures. In our study, 3DTTE was found advantageous in enface visualization of ASDs, their shape, size and number. 3DTTE was found to be useful in measurement of ASD and was correlated well with cardiac catheterization-derived parameters (3DTTE $r = 0.930$, $p \leq 0.0001$) used for the selection of transcatheter ASD occluder size. 3DTTE is also informative in assessment of abnormal ASD device placement as reported in previous studies. Cleft mitral valve is another condition which is sometimes difficult to diagnose by 2DTTE but on 3DTTE it has high probability of being visible. These 3D images have been useful for the surgeons in analyzing the anatomy in situ and deciding the type of intervention before opening the heart.
3D echocardiography in assessment of ventricular function: LVEF is the single most important marker for prognostic assessment in patients with LV dysfunction. Until recently, 2DTTE had widespread acceptance for serial evaluation of EF which was done by eyeballing which is subjective with limited test-retest reliability. With advent of 3D echocardiographic technology, objective assessment of quantitative measurement of dimensions, areas, volumes and function is possible. It is more convincing as compared to conventional 2D echocardiographic measurements and is highly reproducible. While quantification of ventricular volumes and mass with 2D imaging requires geometric assumptions, their determination by live 3D echocardiography is actual and is not assuming anything to calculate the EF. In our study we demonstrated the feasibility of this technique which was already validated for assessment of LV functions. By analysis of regional wall motion, LV contraction patterns, their cyclic changes and temporal differences in regional wall motion pattern, this technique with higher frame rates can be used for assessment of intraventricular dysynchrony and feasibility of biventricular pacing (Fig. 11).

Right ventricular volume measurement using 2D echocardiography is very difficult because of its complex asymmetrical shape. Real-time 3DTTE has led to a revolution in this field as it gives complete assessment of RV geometry and RVEF in a holographic 3D volume data set and provides new insights into its physiology. The in vitro data indicate that RV volume calculation of excised hearts with a high speed volumetric ultrasound system is accurate but requires further validation. In our study on patients with long standing pulmonary hypertension, actual assessment of RVEF and change in shape by analyzing a volume-rendered cast of RV cavity revealed that in response to longstanding pulmonary hypertension, the RV remodels into a more globular shape from its original shape.

Future prospects: The emerging second-generation real-time 3D echocardiography extends the benefits of more complete and precise information in real clinical decision making. However, it has certain limitations like dependence on available echocardiographic window, lower frame rate (20 Hz), and motion artifacts in full volume acquisition. Respiratory and other beat-to-beat variations also damage image quality. Acquisition angle in real time mode requires technical advances to increase the size of insonated volume and frame rate of acquisition. It appears that 3D echocardiography is the technology of future, and with wider availability and further technological advancement it has potential to replace 2D echocardiography as a tool for routine clinical assessment of heart diseases.

Conclusions: The results of this preliminary study demonstrate that: (i) 3DTTE planimetry is comparable to 2DTTE in assessment of mitral valve area with better reproducibility; (ii) 3DTTE gives additional information about surface anatomy of cardiac valves and their relation with surroundings in real time; (iii) comprehensive assessment of tricuspid valve including planimetry for valve area and regurgitant orifice area is possible using real time 3DTTE; (iv) 3DTTE is additive to conventional 2D imaging in ‘enface’ visualization of atrial septal defects with better assessment of shape, size, area and number; and (v) objective assessment of LV and RV volumes is feasible with 3DTEE and is based on actual measurements and not on assumptions.

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Increased Beat-to-Beat QT Variability in Patients with Congestive Cardiac Failure

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Background: QT interval on the surface electrocardiogram reflects the time for repolarization of myocardium. Prolongation of rate-corrected QT interval, QTc is strongly associated with sudden cardiac death. Recent studies using novel techniques on beat-to-beat QT interval variability have shown that an increase in QT interval variability is associated with increased sympathetic activity and is a predictor of sudden cardiac death. We studied QT variability in patients with congestive cardiac failure, as it is associated with an increase in cardiac sympathetic activity and also sudden death.

Methods and Results: We compared beat-to-beat heart rate and QT interval data in 23 patients with congestive cardiac failure and 19 age-matched normal controls. The electrocardiographic data were acquired in lead II configuration at a sampling rate of 1000 Hz. Heart rate variability was found to be significantly lower while QT variability measures were significantly higher in patients compared to controls. QTvi (a common log ratio of QT variability normalized for mean QT interval squared divided by heart rate variability normalized for mean heart rate squared) was also significantly higher in patients compared to controls. Clinical improvement in some of these patients is associated with a decrease in QTvi, due mainly to an increase in cardiac vagal function.

Conclusions: Our results suggest a decrease in cardiac vagal and an increase in cardiac sympathetic functions in patients with congestive cardiac failure. QTvi may prove to be a useful surrogate end point to evaluate treatment effect in these patients. (Indian Heart J 2005; 57: 138-142)

Key Words: QT Variability, Heart rate variability, Congestive heart failure
Methods

Subjects: The patients were known cases of CCF and already receiving some medications (Table 1) without much benefit. The subjects were outpatients that were consecutively recruited from the hospital with a diagnosis of congestive cardiac failure. Age-matched control subjects were recruited from the staff working in the hospital and their relatives. There were 23 patients (19 males, 4 females; age: 54±15 years) and 19 normal controls (13 males, 6 females; age: 53±13 years).

Data acquisition: Electrocardiogram (ECG) was continuously acquired for 5 min in supine posture in lead II configuration in a noise-free environment. All subjects were asked to breathe normally to auditory cues from a cassette player at 12 breaths/min. All patients included in this study were able to breathe at 12 breaths/min. The ECG signal was digitized at 1000 Hz and the data were saved on Zip diskettes for later analyses.

QT variability: All analyses were conducted on 256 s segments of data sampled at 1000 Hz. This QT variability algorithm has been described in detail by Berger et al.16 and has been used by his group as well as ours in previous studies.16,19-22 This was performed on a PC using Solaris Desktop Unix software (Sunsoft, Mountainview, USA) which uses a graphical interface of digitized ECG where the time of the ‘R’ wave is obtained using a peak detection algorithm. Then the operator provides the program with the beginning and the end of the QT wave template. This algorithm finds the QT interval for each beat using the time-stretch model. If the operator chooses a longer QT template, all the QT intervals will be biased accordingly. This algorithm is used to study QT variability and not the mean QT.

The HR [beats per minute (bpm)] time series were sampled at 4 Hz using the technique of Berger et al.23 We used HR time series free of ventricular premature beats and noise. The data were then detrended by using the best-fit line prior to the computation of spectral analyses. The mean HR (HRm), detrended HR variance (HRv), mean QT interval (QTm), detrended QT variance (QTv), spectral powers [total power (TP): 0-0.50 Hz, very low frequency (VLF) power: 0-0.04 Hz, low frequency (LF) power: 0.04-0.15 Hz and high frequency (HF) power: 0.15-0.50 Hz] of HR and QT after detrending were calculated from the instantaneous HR and QT time series of 1024 points (256 s).

Table 1. Descriptive data on patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>NYHA classification</th>
<th>Pre-LVEF%</th>
<th>JVP</th>
<th>Basal edema</th>
<th>Medications at baseline</th>
<th>Post-LVEF%</th>
<th>Clinical improvement</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>47</td>
<td>M</td>
<td>III</td>
<td>45</td>
<td>+</td>
<td>↑</td>
<td>Digoxin, furosemide, thiazide, enalapril, amiodarone, nitrate</td>
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<td>Not improved</td>
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<tr>
<td>2</td>
<td>68</td>
<td>M</td>
<td>IV</td>
<td>23</td>
<td>+</td>
<td>↑</td>
<td>Digoxin, furosemide, enalapril perindopril, L-carnitine, trimetadidine</td>
<td>37</td>
<td>Improved</td>
</tr>
<tr>
<td>3</td>
<td>58</td>
<td>F</td>
<td>IV</td>
<td>40</td>
<td>+</td>
<td>↑</td>
<td>Digoxin, furosemide, aspirin, clonidine, trimetadidine, L-carnitine, carvedilol, nitrate, pantoprazole</td>
<td>39</td>
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</tr>
<tr>
<td>4</td>
<td>40</td>
<td>M</td>
<td>III</td>
<td>36</td>
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<td>↑</td>
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</tr>
<tr>
<td>5</td>
<td>58</td>
<td>F</td>
<td>IV</td>
<td>23</td>
<td>+</td>
<td>↑</td>
<td>Digoxin, furosemide, amiodarone, lisinopril, L-carnitine, trimetadidine</td>
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</tr>
<tr>
<td>6</td>
<td>50</td>
<td>M</td>
<td>IV</td>
<td>40</td>
<td>+</td>
<td>N</td>
<td>Digoxin, furosemide, amiodarone, carvedilol</td>
<td>45</td>
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</tr>
<tr>
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<td>M</td>
<td>II</td>
<td>20</td>
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<td>N</td>
<td>Digoxin, furosemide, spironolactone, metoprolol</td>
<td>25</td>
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</tr>
<tr>
<td>8</td>
<td>49</td>
<td>M</td>
<td>III</td>
<td>40</td>
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<td>Digoxin, furosemide, spironolactone</td>
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</tr>
<tr>
<td>9</td>
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<td>Improved</td>
</tr>
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<tr>
<td>11</td>
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<td>III</td>
<td>35</td>
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<td>Digoxin, furosemide, enalapril, spironolactone</td>
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<td>12</td>
<td>65</td>
<td>M</td>
<td>III</td>
<td>38</td>
<td>+</td>
<td>↑</td>
<td>Spironolactone, enalapril, metoprolol, pituitary, glibenclamide, aspirin</td>
<td>40</td>
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</tr>
<tr>
<td>13</td>
<td>71</td>
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<td>-</td>
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<td>Digoxin, spironolactone, enalapril, L-carnitine, aspirin</td>
<td>36</td>
<td>Not improved</td>
</tr>
<tr>
<td>14</td>
<td>42</td>
<td>M</td>
<td>III</td>
<td>29</td>
<td>+</td>
<td>N</td>
<td>Digoxin, furosemide, L-carnitine, trimetadidine</td>
<td>43</td>
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</tr>
<tr>
<td>15</td>
<td>30</td>
<td>M</td>
<td>III</td>
<td>38</td>
<td>+</td>
<td>N</td>
<td>Digoxin, furosemide, spironolactone, lisinopril, L-carnitine, trimetadidine</td>
<td>36</td>
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</tr>
<tr>
<td>16</td>
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<td>III</td>
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<tr>
<td>17</td>
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<td>III</td>
<td>35</td>
<td>+</td>
<td>↑</td>
<td>Digoxin, furosemide, L-carnitine, aspirin, trimetadidine, nitrate</td>
<td>No follow-up</td>
<td>No follow-up</td>
</tr>
<tr>
<td>18</td>
<td>63</td>
<td>M</td>
<td>III</td>
<td>35</td>
<td>+</td>
<td>N</td>
<td>Salbutamol inhalant</td>
<td>No follow-up</td>
<td>No follow-up</td>
</tr>
<tr>
<td>19</td>
<td>66</td>
<td>M</td>
<td>III</td>
<td>30</td>
<td>+</td>
<td>↑</td>
<td>Digoxin, furosemide, L-carnitine</td>
<td>No follow-up</td>
<td>No follow-up</td>
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<tr>
<td>20</td>
<td>40</td>
<td>M</td>
<td>IV</td>
<td>-</td>
<td>+</td>
<td>↑</td>
<td>Furosemide, nitrate, L-carnitine</td>
<td>No follow-up</td>
<td>No follow-up</td>
</tr>
<tr>
<td>21</td>
<td>68</td>
<td>M</td>
<td>IV</td>
<td>27</td>
<td>+</td>
<td>↑</td>
<td>Digoxin, furosemide, enalapril, carvedilol, spironolactone, amiodarone, nitrate</td>
<td>No follow-up</td>
<td>No follow-up</td>
</tr>
<tr>
<td>22</td>
<td>60</td>
<td>M</td>
<td>III</td>
<td>32</td>
<td>+</td>
<td>N</td>
<td>Digoxin, furosemide, enalapril, carvedilol, spironolactone</td>
<td>No follow-up</td>
<td>No follow-up</td>
</tr>
<tr>
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<td>85</td>
<td>M</td>
<td>III</td>
<td>30</td>
<td>-</td>
<td>+</td>
<td>Digoxin, lisinopril, L-carnitine</td>
<td>No follow-up</td>
<td>No follow-up</td>
</tr>
</tbody>
</table>

LVEF: left ventricular ejection fraction; JVP: jugular venous pressure; Pre- and Post-LVEF refer to treatment effect; M: male; F: female
A normalized QT variability index was calculated as suggested by Berger et al.\textsuperscript{16}

\[
\text{QT}_{vi} = \log_{10} \frac{\text{QT}_v}{\text{QT}_m} \cdot \frac{\text{HR}_v}{\text{HR}_m}^2
\]

This index represents the log-ratio between the QT interval and the HR variabilities, each normalized for the corresponding mean.

**Spectral analyses**: QT time series (256 s at 4 Hz =1024 points) was subjected to spectral analyses and the power spectrum was computed with the Blackman Tukey method.\textsuperscript{16} The powers were integrated in the bands of VLF, LF and HF regions. HR and QT interval time series were subjected to spectral analysis and the cross spectrum between the two time series was computed from 256 s.\textsuperscript{16}

**Clinical improvement**: In most of the patients, treatment was continued with the same medications they were on and only dosages were adjusted. The eight patients that showed improvement and the six, that did not improve were retested after 4-8 weeks of continued treatment. Patients were judged to have improved clinically if there was a decrease in the severity of dyspnea, pedal edema, hepatomegaly and pleural effusion, and a decrease in jugular venous pressure (JVP). We also used the left ventricular ejection fraction (LVEF) criteria to assess the improvement (Table 1).

**Statistical analysis**: We used student’s t test to compare HR and QT variables after using analysis of variance (ANOVA) with adjustment for gender. A probability level of ≤ 0.05 was accepted as significant.

### Results

Results remained the same after adjusting for gender as there was a higher proportion of females in the control group. Table 2 shows the comparison of patients and controls in supine posture during controlled breathing at 12 breaths/min. HR spectral powers were significantly higher and QT spectral powers were significantly lower in controls compared to patients. QTvi was significantly higher in patients compared to controls (Fig. 1).

**Effect of treatment**: Table 3 shows the HR and QT variability measures of 14 patients after treatment. Eight of these subjects showed clinical improvement and 6 did not. In the group that showed improvement, there was a significant increase in HR-HF power and a decrease in QTvi (Fig. 2). There was also a significant decrease in HR-QT coherence in the band of 0-0.5 Hz in the group that did not improve.

<table>
<thead>
<tr>
<th>Table 2. Heart rate and QT variability measures of normal controls and patients with congestive cardiac failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n=19)</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>HRm</td>
</tr>
<tr>
<td>HR-TP (0-0.50 Hz)</td>
</tr>
<tr>
<td>HR-VLF (0-0.04 Hz)</td>
</tr>
<tr>
<td>HR-LF (0.04-0.15 Hz)</td>
</tr>
<tr>
<td>HR-HF (0.15-0.50 Hz)</td>
</tr>
<tr>
<td>QTm</td>
</tr>
<tr>
<td>QT-TP (0-0.50 Hz)</td>
</tr>
<tr>
<td>QT-VLF (0-0.04 Hz)</td>
</tr>
<tr>
<td>QT-LF (0.04-0.15 Hz)</td>
</tr>
<tr>
<td>QT-HF (0.15-0.5 Hz)</td>
</tr>
<tr>
<td>HR-QT coherence (0-0.5 Hz)</td>
</tr>
<tr>
<td>QTvi</td>
</tr>
</tbody>
</table>

HRm (heart rate mean) is in beats per min (bpm); QTm (QT interval mean) is in ms; Powers are in Ln (natural logarithm) of bpm squared and ms squared; total power (TP): 0-0.5 Hz; very low frequency (VLF): 0-0.04 Hz; low frequency (LF): 0.04-0.15 Hz; high frequency (HF): 0.15-0.5 Hz

Patients v. controls: a<0.05; b<0.01; c<0.001; d<0.0001.
Correlations: There was no significant correlation between ejection fraction (EF) and QTvi. There were also no significant correlations between mean QT and QT total power or QTvi either in patients or controls.

Discussion

The findings of decreased HR variability and increased QT variability in patients with CCF are consistent with previous reports.7,16 We have previously shown that an increase in sympathetic function is associated with an increase in QTvi.19,22 This is also consistent with the higher QTvi reflecting a higher sympathetic activity in patients with congestive cardiac failure. However, we did not find a significant difference in coherence between HR and QT time series in any frequency band between patients and controls.

Previous evidence suggests that an increase in HR-HF power is an important prognostic factor in these patients. However, the most important finding in this study is that after treatment, QTvi decreased only in the subgroup of patients that showed clinical improvement. Though there was no significant increase in the total power of HR or coherence between HR and QT in these patients, there was a significant increase in VLF and HF powers of HR and a trend toward a significant increase for total power of HR. HR time series usually shows three peaks in the following bands: TP (0-0.50 Hz), VLF (0-0.04 Hz), LF (0.04-0.15 Hz) and HF (0.15-0.50 Hz).24,26 HF power reflects respiratory sinus arrhythmia and thus cardiac vagal function, LF power, baroreceptor mechanisms, influenced by sympathetic as well as parasympathetic mechanisms and VLF power, peripheral vascular, thermoregulatory and renin-angiotensin systems. These results suggest a possible increase in cardiac vagal function after successful treatment. This may have been responsible for the decrease in QTvi, which suggests that QT interval fluctuations became more appropriate for the level of HR fluctuations. This did not happen in patients that did not show any clinical improvement. In their report, Bonnemeier et al.27 have shown that QT interval variability decreased after successful reperfusion by percutaneous transluminal coronary angioplasty. This preliminary report adds to the literature suggesting that QT interval variability may prove to be another useful surrogate end point in heart failure.28

Limitations: A larger number of patients and controls would have brought more reliable results although the differences in QTvi were highly significant. We had data of only 256 s during controlled breathing; further, the number of subjects that improved after treatment was only eight and those that did not improve were six. Hence, the results should be viewed with caution until studies with larger samples examine the effect of treatment on QTvi. Thus these results should be considered preliminary.

Table 3. Heart rate and QT variability measures of patients with congestive cardiac failure before and after treatment in supine posture (n=14)

<table>
<thead>
<tr>
<th></th>
<th>Improved (n=8)</th>
<th>Not improved (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-</td>
<td>Post-</td>
</tr>
<tr>
<td>Hr (bpm)</td>
<td>88±18</td>
<td>80±12</td>
</tr>
<tr>
<td>Hr-TP (0-0.50 Hz)</td>
<td>2.2±2.4</td>
<td>3.0±1.7</td>
</tr>
<tr>
<td>Hr-VLF (0-0.04 Hz)</td>
<td>0.71±1.7</td>
<td>1.9±1.1*</td>
</tr>
<tr>
<td>Hr-LF (0.04-0.15 Hz)</td>
<td>0.70±3.0</td>
<td>1.8±1.9</td>
</tr>
<tr>
<td>Hr-HF (0.15-0.50 Hz)</td>
<td>1.3±2.7</td>
<td>2.5±2.2*</td>
</tr>
<tr>
<td>Qm (ms)</td>
<td>485±101</td>
<td>405±78</td>
</tr>
<tr>
<td>Qm-TP (0-0.50 Hz)</td>
<td>4.7±1.3</td>
<td>4.6±0.5</td>
</tr>
<tr>
<td>Qm-VLF (0-0.04 Hz)</td>
<td>2.5±1.6</td>
<td>2.6±0.6</td>
</tr>
<tr>
<td>Qm-LF (0.04-0.15 Hz)</td>
<td>3.3±1.4</td>
<td>3.1±0.6</td>
</tr>
<tr>
<td>Qm-HF (0.15-0.50 Hz)</td>
<td>4.1±1.2</td>
<td>4.0±0.6</td>
</tr>
<tr>
<td>Qm coherence</td>
<td>0.23±0.11</td>
<td>0.24±0.13</td>
</tr>
</tbody>
</table>

HRm (HR mean) is in beats per minute (bpm); QTm (QT interval mean) is in ms; Powers are in Ln (natural logarithm) of bpm squared and ms squared; TP: 0-0.50 Hz; VLF: 0-0.04 Hz; LF: 0.04-0.15 Hz; HF: 0.15-0.50 Hz

P<0.05

TP: total power; VLF: very low frequency; LF: low frequency; HF: high frequency


Takayasu's arteritis (TA) or non-specific aortoarteritis (NSAA) is an idiopathic inflammatory disease of aorta and its major branches. It usually affects the young individuals and results in occlusive changes in these vessels. The etiopathogenesis of the disease is still poorly understood but an autoimmune basis is suggested. In addition, genetic and environmental factors also probably play an important role. Experimental studies were conducted on various animals in the past with an attempt to induce arteritis using various non-specific antigens, yet the exact immunological status of the animal has not been analyzed so far. The present study was designed to make an attempt to produce an experimental model of NSAA subsequent to the administration of specific antigen(s) and to analyze the sequence of events involved in the disease process using mouse as a model.

**Methods**

A total of 160 female Swiss mice were taken for induction of the disease. They were divided into various groups (A–H) on the basis of injection of different antigens. Each group had 20 mice (Table 1).

**Tabel 1. Antigens given to various groups of Swiss mice**

<table>
<thead>
<tr>
<th>Group (n=20)</th>
<th>Antigen Injected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>Mouse aorta (MA)</td>
</tr>
<tr>
<td>Group B</td>
<td>Mouse peripheral artery (MP)</td>
</tr>
<tr>
<td>Group C</td>
<td>Standard collagen (SC)</td>
</tr>
<tr>
<td>Group D</td>
<td>Aorta collagen (AC)</td>
</tr>
<tr>
<td>Group E</td>
<td>Intima (IN)</td>
</tr>
<tr>
<td>Group F</td>
<td>Media (MD)</td>
</tr>
<tr>
<td>Group G</td>
<td>Adventitia (AV)</td>
</tr>
<tr>
<td>Group H</td>
<td>Normal saline (NS)</td>
</tr>
</tbody>
</table>

**Preparation of various antigens:**

Mouse aorta and mouse peripheral arteries: 20 to 30 mice were killed. Aorta and peripheral arteries were dissected out from each mouse. They were cleaned thoroughly with normal saline, minced and homogenized in the Polytron homogenizer. The homogenate was centrifuged at 3000 rpm for 30 min separately. Protein estimation of the supernate was done by Lowry's method.
Standard collagen: It was procured from Sigma Chemical Co. The source was calf skin.

Aorta collagen: Its source was human aorta. Human aorta obtained from autopsy cases being done at mortuary of our institution were cleaned. Histopathology was done to confirm that it was normal. Then it was minced and homogenized at 4°C in 0.5 M acetic acid. Pepsin was added at 20 mg per gm weight and the tissue was digested for 3 days at 4°C with continuous stirring. The clarified extract was neutralized to inactivate peptic activity and the collagen precipitated overnight at room temperature by addition of sodium chloride to a fine concentration of 20%. The precipitate was then collected and resolubilized in 0.1 M phosphate buffer (pH 7.6) clarified by centrifugation and the collagen reprecipitated by addition of sodium chloride (15%). This procedure was repeated 3 times. The final precipitate was collected in 0.5 M acetic acid, dialyzed against 0.1% acetic acid and lyophilized. Electrophoresis was performed on standard 10% SDS-polyacrylamide gels and also 3.75% gels in tubes (tube electrophoresis). The protein bands of aorta collagens corresponded to that of standard collagen (procured from Sigma) on 10% SDS-PAGE and 3.75% SDS-PAGE which confirmed the extraction of aorta collagen.

Intima, media, adventitia: The three layers of human aorta i.e. intima, media and adventitia were separated and their extracts were prepared.

The mice were divided into various groups A-H on the basis of these antigens. Each group comprised of 20 animals. The mice were immunized with these antigens separately (emulsified with Complete Freund’s adjuvant and Incomplete Freund’s adjuvant) on day 0, day 14 and day 21. They were bled on day 30th post-immunization. The mice were given boosters once a month for 8 months and then bled after 15 days of giving each booster. The antibody titers were estimated by enzyme-linked immunosorbant assay (ELISA) at 1st, 2nd, 4th, 6th and 8th month interval post-immunization (day 0). The animals were sacrificed at 2nd, 4th, 6th and 8th month interval after post-immunization (day 0).

The antibody titer: It was estimated at 1st, 2nd, 4th, 6th and 8th month post-immunization (day 0). Mean absorbance value at 492 nm was calculated for the animals of all groups bled at above time intervals separately. The statistical analysis was done by comparing the antibody titers among all the groups at the above time periods of the sacrifice by one-way ANOVA. When the p value obtained from one-way ANOVA was found to be significant only then Student’s Newman Kaull’s Multiple Range Test (MRT) was applied to compare the individual groups with one another.

Histopathological studies: The organs such as heart, aorta and kidney were dissected out and fixed in 10% formalin. Five to six micron thick sections were processed for routine histology. Tissue sections were cut and stained with hematoxylin-eosin. Histopathology of heart, kidney and aorta was done on 2nd, 4th, 6th and 8th month interval post-immunization.

Results

Antibody titer in mice bled at various intervals after injection of different antigens: Fig. 1 represents the mean values of antibody titer in mice of groups A–H.

1st month: The antibody titer ranged from 0.412±0.04 in group F (MD) to 0.114±0.02 in group H (NS) in the order: group F (MD) >group G (AV) >group D (AC) >group E (IN) >group A (MA) >group B (MP) >group C (SC) >group H (NS). These values were statistically significant (p<0.001) by one-way ANOVA analysis. The p values after MRT analysis showed that the antibody titer of all the antigenic group F (MD) and group G (AV) were significantly higher (p<0.05) when compared to the antibody titer of group C (SC), group B (MP), group A (MA), group E (IN) and group H (NS), whereas no significant difference was seen when these two groups were compared with that of group D (AC) and with one another.

2nd month: The values ranged from 0.616±0.042 in group G (AV) to 0.106±0.025 in group H (NS) in the order: group G (AV) >group F (MD) >group D (AC) >group E (IN) >group A (MA) >group B (MP) >group C (SC) >group H (NS). These values were statistically significant (p<0.001) by one-way ANOVA. The p values after MRT analysis showed that the antibody titer of all the antigenic
groups (group A-G) were statistically significant (p <0.05) when compared with the control group H (NS). Also group F (MD) and group G (AV) had significantly high antibody titer as compared to group D (AC) (p<0.05), group A (MA), group B (MP), group C (SC), group E (IN) (p<0.001) and group H (NS) (p<0.0001).

4th month: The mean antibody titer ranged from 0.785±0.050 in group F (MD) to 0.108±0.018 in group H (NS) in the order: group F (MD) > group G (AV) > group D (AC) > group E (IN) > group C (SC) > group A (MA) > group H (NS). The antibody titer showed an increase in all the test groups. These values were statistically significant (p<0.001) as assessed by one-way ANOVA analysis.

The p values after MRT showed that the antibody titer of all the antigenic groups i.e. from group A to group G were significantly different when compared with that of control group H (NS). Also group F (MD) and group G (AV) had significantly high antibody titer as compared to group B (MP) (p<0.0001), group A (MA), group C (SC), group E (IN) (p<0.001) and group D (AC) (p<0.05). The antibody titer of group D (AC) was significantly different from only group B (MP) (p<0.001) and group C (SC) (p<0.05).

6th month: The mean values of antibody titer ranged from 0.696±0.061 in group G (AV) to 0.111±0.024 in group H (NS) in the order: group G (AV) > group F (MD) > group D (AC) > group C (SC) > group E (IN) > group B (MP) > group A (MA) > group H (NS). There was an overall increase in the antibody titer in all the groups as compared to the antibody titer in sera of mice bled at 4th month.

The p values after MRT were same as that of the 4th month except that of p values obtained after comparison between group F (MD) and group E (IN). At 6th month, the antibody titer of group G (AV) was significantly higher (p<0.001) as compared to the group E (IN) whereas antibody titer of group F (MD) was statistically significant at only p<0.05 when compared to group E (IN).

8th month: The mean values of antibody titer ranged from 0.604±0.001 in group G (AV) to 0.111±0.02 in group H (NS) in the order: group G (AV) > group F (MD) > group E (IN) > group D (AC) > group C (SC) > group A (MA) > group B (MP) > group H (NS). There was a decrease in the level of antibody titer in all the groups except group H (NS) when compared to antibody titer in sera of mice killed at 6th month interval, but it was more than the value at 1st month sacrifice in all the groups except group H (NS) where the antibody titer was more or less same at all the sacrifices.

When MRT was applied, the values of group A (MA), group C (SC), group D (AC), group E (IN), group F (MD) and group G (AV) were significant at p<0.05 compared to that of group H (NS) and group B (MP). Highly significant difference was observed between group F (MD), group G (AV) and the groups E (IN), D (AC), C (SC), B (MP), A (MA) and H (NS). The values of group D (AC) were significant at p<0.05 when compared with group C (SC) and group A (MA).

Histopathology of heart, kidney and aorta of mice sacrificed at various intervals after injection of different antigens:

2nd month: The histology of heart, kidney and aorta was normal in all the groups from A to H till this stage. Very few inflammatory cells were seen in the kidney of mouse of group F (MD).

4th month
Heart: No histological change was observed in the heart in any one of the animals killed at 4th month in all the groups from A to H.

Kidney: Few inflammatory cells were present around the arterioles of kidney of mice in group A (MA), group C (SC) and group D (AC). Inflammatory foci were seen in the parenchyma of kidney of mice of group F (MD) and group G (AV) whereas no changes were observed in mice of group B (MP), group E (IN) and group H (NS).

Aorta: Normal structure was seen in the mice of all the groups except group F (MD) and group G (AV) where few inflammatory cells were seen around the aorta in the periadventitial region.

6th month
Heart: Pericardial inflammation was observed in group F (MD) and group G (AV). The structure of myocardium was normal in all the groups.

Kidney: Extensive inflammatory foci were seen around arterioles, tubules and glomeruli in all the animals (3/3) of group A (MA), 4/4 animals of group D (AC) and 4/5 animals of group E (IN). One animal of group D (AC) showed very large inflammatory patches in the kidney (Fig. 2) and distortion of the shape of the tubules in the medulla (Fig. 3). The structure of kidney parenchyma was altered in groups F (MD) and G (AV). There was extensive inflammation, cavity formation, thinning of epithelial cells lining the tubules and collection of fluid in the lumen of kidney tubules (Fig. 4). The kidneys of group H (NS) and group B (MP) showed few inflammatory cells around arterioles.

Aorta: Peri-adventitial inflammation was seen in aorta of
group C (SC), group D (AC), group F (MD) and group G (AV). The para-aortic lymph nodes were enlarged in the media and adventitia groups.

8th month
Heart: Extensive pericardial inflammation was reported in group F (MD) and group G (AV) mice (Fig. 5). The structure of heart was normal in all the groups except few inflammatory cells seen in the pericardial zone of group D (AC).

Kidney: Kidney was infiltrated with dense patches of inflammation around arterioles and tubules in all the groups except group B (MP) where few inflammatory foci were observed around arterioles. Lumens of some of the tubules were occluded. The cytoplasm was dense and opaque. Extensive lymphocytic infiltration was also seen in the adrenal glands. Hemorrhagic spots were noted.

Morphology of tubules and ducts was altered in medulla and cortex in group F (MD), group G (AV) and group D (AC) (Fig. 6). The damage was limited to few regions in group E (IN), group A (MA) and group C (SC). No change was observed in the heart, kidney and aorta of mice injected with normal saline.

Aorta: No change was observed except scanty inflammation in peri-adventitial region in group B (MP) whereas moderate peri-adventitial inflammation was seen in group A (MA), group C (SC) and group E (IN). Extensive inflammation in peri-adventitial zone, and enlarged paraaortic lymph nodes were observed in group F (MD), group G (AV) and group D (AC) (Fig. 7). Spaces in the aortic fibers and straightening of the elastic fibers was observed in mice of group F (MD) and group G (AV).

Summary of Results: The results on antibody titer clearly
indicate that there is gradual increase in the titer from 1st month till 4th month within all the experimental groups (A-G), when compared with group H (NS). The titer then starts falling sharply from 8th month post-immunization. However, the control group H (NS) does not show much variation (Fig. 1). When each individual group was compared separately with control group H (NS), the significant statistical value was obtained.

Discussion

The presence of circulating antibodies against aortic components in NSAA (Takayasu’s) patients have been described.8-12 The role of such antibodies in the underlying immunopathological process remains obscure. Experimenting directly on humans is not ethically possible and the T cell dependence and subset requirements for autoantibody production in vivo can only be inferred from in vitro studies and animal models. Therefore, we made an attempt to induce the disease in mice by immunizing them with various antigens separately followed by estimation of the antibody titer in these animals. The phenotypic expression of T lymphocytes by immunohistochemical techniques has also been done. The histopathological changes in heart, kidney and aorta have been studied in these animals to see the extent and severity of the disease.

Various animal models have been tried in the past using homologous/heterologous aortic extracts.5,13-15 In these models histological changes similar to that of human vasculitis were studied, but were not correlated with the immunological status of the host animal. Also only the crude aortic extracts were used. The antigenicity of the three layers of aorta or its components was not analyzed. Till date, the animal models of aortoarteritis were poorly defined, hence the present experimental design was used as a prototype to investigate the pathogenesis of the disease and the mechanism of induction and regulation of autoantibody production.

Our experiments showed that there was an initial increase in the antibody titer followed by a peak between 4th and 6th month and then a decline by 8th month in all the experimental animals (Fig. 1). Ueda et al.5 also detected the presence of complement fixing antibodies against the rabbit aorta (isologous) in rabbits injected for 10 months. The peak value in these animals was observed between 2-7 months after the beginning of immunization and then gradual decline was reported. However, they did not use the heterologous aortic extract i.e. human aorta as has been used in our study and also by Scebat et al.14 However, they did not isolate the three layers of the heterologous aorta or any of its components to produce aortitis which was done in our experiments.

In our experiments, the pattern of rise and fall in the antibody titer was almost uniform in all the experimental groups. However, when the titer was compared within all the groups, it was maximum in the sera of mice immunized with human media (group F) and adventitia (group G) extract at all the time periods, followed by the titers in the mice injected with aorta collagen and standard collagen. This could be due to higher antigenicity of human media
and adventitia or the presence of any one of their basic
components e.g. collagen indicated in our study.

Findings of our experimental studies can be extrapolated
with those of human studies where we observed the higher
titer of antiaorta antibodies against adventitia and media
in sera of NSAA patients. The possible role of collagen was
suggested as the antiaorta titer in human sera decreased
when the antigen (three layers of aorta) was treated with
the enzyme collagenase (ultra pure form). Further
experiments can be designed to evaluate the possible role
of other constituents of the aorta.

The antibody titer in mice immunized with isologous
aortic extract [group A (MA) and group B (MP)] was much
less as compared to the other experimental groups (C-F)
(Fig. 1). This observation was somewhat similar to that of
Scebat and colleagues where they could detect low level
of antibodies by passive hemagglutination in the rabbit sera
injected with the rat aorta (heterologous) and not with
rabbit aorta (isologous)-injected animals.

The antibody titer in mice injected with mouse aorta
extract group A (MA) was higher as compared to the titer
in mice injected with mouse peripheral artery extract group
B (MP). It has been reported that the disease involves mainly
the aorta and its major branches as compared to peripheral
arteries. The difference in the antigenicity of the aorta and
peripheral arteries can be attributed to the qualitative or
quantitative difference in the antigen involved or the
absence of antigen in peripheral arteries. This observation
of group B (MP) in experimental mice is in concurrence
with that of human studies of Ueda et al. where they could
not detect the antiaorta antibodies against the extract of
peripheral arteries in the sera of NSAA patients.

Thus our experiments have shown the presence of antibodies in mice injected with isologous (group A) as well as
heterologous tissue extracts while Ueda and colleagues could detect antibodies against isologous aorta and Scebat
and colleagues reported these antibodies only against
heterologous tissue extracts.

Histological changes in aortoarteritis cases on autopsy
have been described by a number of workers. They
suggested that the spectrum of histological changes seen
in aorta and other affected organs in NSAA is closely related
to the stage of the disease. The disease is characterized by
early acute phase followed by late occlusive phase. The early
systemic generalized manifestations last for several weeks/
months or years and as they subside, signs and symptoms of
occlusive phase appear. Histologically the disease starts
with the inflammatory process affecting aorta, heart and
kidney (acute phase) which gradually subsides over the
years and finally gets completely resolved. The adventitia
and media get replaced by the fibrous tissue leading to
stenosis and narrowing of aortic lumen causing rat tail
aorta (chronic phase).

The histological examination in our experiments
revealed inflammation in the kidney parenchyma as early
as 4th month in group F (MD) and group G (AV) and by 6
months in mice of group D (SC), and group E (IN) (Figs 3
and 4). The lesions were severe by 8th month in the above
groups. The kidney parenchyma was destroyed and altered
morphology was observed in groups F (MD), G (AV) and D
(AC) at 8th month of sacrifice. However, moderate
inflammation in the kidney of mice of group A (MA),
minimal in group B (MP) and no changes in the control
group (NS) were noticed by 8th month.

These histological lesions in the kidney may be due to
the occlusion/stenosis of renal artery (branch of abdominal
aorta) in the affected mice. Initially the NSAA was thought
to be confined only to the aortic arch but more diffuse
involvement of aorta and its major branches is now
commonly seen. The involvement of branches of abdominal
aorta in NSAA patients was first reported by Harbitz. Danaraj
and Wong focussed the attention to the
involvement of renal arteries leading to nephrogenic
hypertension in these patients. Since then involvement of
renal arteries has been noted by several authors as the most
frequently involved branch of the abdominal aorta, although
some amount of collateral circulation develops
following occlusion of renal arteries and aortorenal bypass
in these patients appeared to result in long-term success
rates comparable to bypass performed for either
atherosclerosis or fibromuscular dysplasia.

In NSAA patients pericardial and myocardial
involvement has been reported in clinical and autopsy
cases, which is a feature of acute phase of the disease.
This may precipitate as congestive cardiac failure. However,
the involvement of the endocardium is debated.

The autopsy data indicates myocardial necrosis,
inflammatory cell infiltration, myocyte with minimal
or absent muscle hypertrophy. However, in our studies
extensive inflammation around pericardium was observed
in mice of group F (MD) and G (AV) (Fig. 5), inflammatory
disease in outer myocardium in only one animal of group G (AV)
and no change in the endocardium was seen by 8th month
of sacrifice. Talwar et al. studied the morphology of
myocardium on the biopsies taken from these patients. They
observed myocardial inflammation in 50% of these patients.

The aortic lesions in the acute stage are usually confined
to the peri-adventitial inflammation, occasional infiltration of lymphocytes/plasma cells in media, whereas fibrosis, hyalinization and collagenization of the fibrous tissue replaces the functional elements of the tunica adventitia and media in the chronic stage.

We observed extensive peri-adventitial inflammation around the aorta of mice of group C (SC), group D (AC), group E (IN), group F (MD), and group G (AV).

Patchy deposits in media as well as thickened tunica adventitia was observed in one of the animals of group G (AV). One or two animals belonging to group G (AV) exhibited spaces in the elastic fibers of media and straightening of these fibers of aorta. We could not observe the destruction of the elastic fibers in media as described in Takayasu’5 arteritis patients whereas Scebat et al.,14, Ueda et al. and Sen et al. could induce somewhat similar lesions in the rabbit aorta as those of NSAA patients. Scebat et al. observed increased number of collagen and elastic fibers, accumulation of fibroblasts along with intimal lesions, disruption of the elastic lamina and necrotic muscle fibers. Scebat and colleagues put forward a hypothesis that the lesions modify the structure of the arterial tissue which would turn into an autoantigen during the pathologic period. This hypothesis, however, does not correlate with our findings where the rise in antiaorta antibody titer preceded the lesions. Thus it can be speculated that this rise in antibody titer may not be the result, but probably the cause of the lesions seen in our experimental animals.

Similarly the experimental lesions produced in Ueda’s study on rabbits which showed the fragmentation of elastic fibers, degeneration of muscle fibers and cellular infiltration in the media were also preceded by the rise in antibody titer.

Sen et al. injected various physical agents, chemical irritants (25% hypertonic saline, 2.5% phenol in glucose, 2% phenol, 2% glacial acetic acid and 4% glycerine), antigenic and bacterial products (PPD, BCG, streptococcus and tuberculin) to produce arteritis in rabbits, but they could observe gross changes in periarterioles as well as all the layers of aorta only in animals injected with bovitubercle bacilli and human tubercle bacilli. They found minimal changes like mild peri-adventitial inflammation in animals who received chemical injury, Freund’s adjuvant and BCG. Rest of the animals showed no changes.

However, they did not perform any immunological studies in these animals. Experimental work carried out by them corroborated the hypothesis that adventitia develops the inflammatory reaction first as has also been observed in our experimental animals. This was followed by fibrous proliferation of intima leading to marked occlusion of the lumen and probably medial changes in the aorta at a later stage of the disease. Somewhat similar findings have also been seen in our study suggesting that the disease is in acute phase which will be followed by chronic phase later.

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Lipid Profile and Apolipoprotein E Polymorphism in Essential Hypertension

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Background: Studies in several populations have indicated that genetic variation at the apolipoprotein E structural locus influences atherosclerosis leading to cardiovascular diseases. The possible role of apolipoprotein E polymorphism in the development of essential hypertension has not been sufficiently investigated. In this case-control study, we aimed to determine the significance of association between essential hypertension and apolipoprotein E genotypes. In addition, apolipoprotein E genotypes were correlated with serum lipid levels in order to understand the possible interaction between the specific genotype and the lipid profiles that can contribute to hypertension.

Methods and Results: The apolipoprotein E genotypes were assayed in 185 patients and 200 controls by polymerase chain reaction followed by enzymatic digestion with Hha I. Using logistic regression analysis, the multivariate-adjusted odds of hypertension were calculated. The incidence of $\varepsilon_4$ allele was found to be significantly higher in patients (12.16%) than in controls (5.75%, $\chi^2=10.87; p<0.05$) and also in patients with positive family history (16.7%) as compared to negative family history (8.87%, $\chi^2=8.45; p<0.1$). Further, it was observed that carriers of $\varepsilon_4$ allele have twice as much risk ($p<0.05$) for developing hypertension as compared to carriers of other alleles. Patients with $\varepsilon_4$ allele had significantly higher levels of total cholesterol and low-density lipoprotein-cholesterol as compared to $\varepsilon_4$ allele non-carriers ($p<0.05$). The adjusted odds ratios for $\varepsilon_4$ and $\varepsilon_2$ alleles versus $\varepsilon_3$ allele were 2.2 (95% confidence interval 1.2 to 3.8, $p<0.05$) and 1.2 (95% CI, 0.75 to 1.77, $p<0.514$), respectively.

Conclusions: Our study revealed a strong association of apolipoprotein E locus with hypertension and lipid profile. However, large population-based studies are needed to understand the exact role played by the locus in causing the condition. (Indian Heart J 2005; 57: 151–157)

Key Words: Essential hypertension, Apolipoprotein E polymorphism, Dyslipidemia

Prevalence of hypertension (HTN) is higher in adult Indians in urban areas compared with rural areas (20-40% v. 12-17%). Patients with hypertension are usually asymptomatic and its significance derives from target organ damage. The pathologic effects of hypertension are mediated through changes in blood vessels including fibromuscular hyperplasia of vessel walls and accelerated arteriosclerosis leading to coronary artery disease (CAD), congestive heart failure, stroke and renal failure. Blood pressure acts synergistically with other risk factors such as diabetes mellitus (DM), hypercholesterolemia, and smoking and increases the risk for cardiovascular diseases (CVD) especially in the elderly. Apart from classic vascular risk factors, genetic factors are also known to play a role in the etiology of CVD.

Previously, the role of apolipoprotein E (apo E) polymorphism in causing atherosclerotic events has been reported. Apo E is an exchangeable protein which acts as ligand for low-density lipoprotein (LDL) receptors. It also has a repair function in response to tissue injury. It plays an essential role in lipid metabolism, specially in the removal of atherogenic remnants of triglyceride-rich lipoproteins and by reversing cholesterol transport in plasma and intercellular lipid transport within tissues. The human apo E gene is 3.7 kb including 4 exons and 3 introns and is mapped on the short arm of chromosome 19. The mature protein is composed of 299 amino acids, i.e. 34 KDa with several functional domains.

The presence of apo E polymorphism was first described by Utermann et al. Three common isoforms of apo E including E2, E3 and E4 have been identified. The three
The genetic variations at apo E have been shown to affect lipid and lipoprotein levels in the general population. 23-27 The E4 isoform is associated with increased levels of total cholesterol (TC) and beta lipoprotein 28 and increased susceptibility to CVD 27. Most patients with type II hyperlipoproteinemia are homozygous for the E2 isoform that binds with reduced affinity to cellular receptors. 29,30 Population-based studies reveal that the E2 isoform is associated with decreased levels of cholesterol and beta lipoprotein. 23 In the present study we aimed to find out the association of apo E genotypes with HTN by comparing hypertensives with normotensives, familial hypertensives and also apo E polymorphism in relation to lipid levels.

Methods

Patients: The study group consisted of patients who were admitted to the cardiology unit of CARE hospital as well as outpatients who had previously been diagnosed to have essential hypertension (EH). The diagnosis was based on the complete physical and clinical examination of patients by the cardiologist followed by appropriate investigations. Hypertension was considered to be present if an individual had a history of HTN or was using antihypertensive agents or if the systolic blood pressure (SBP) exceeded 140 mmHg or the diastolic blood pressure (DBP) exceeded 90 mmHg.

Controls: Random subjects who were free of HTN were included in the study as controls.

Inclusion and exclusion criteria: For the present study, only patients with EH were included while patients with secondary HTN and those associated with renal, pulmonary and Alzheimer's disease etc. were excluded.

Potential confounders: In the present study, evaluation of the contribution of confounding risk factors to the development of EH was based on the individual's personal history, physical examination and laboratory findings. Control subjects were also similarly evaluated. The confounding risk factors included smoking and alcohol consumption, body mass index (BMI), dyslipidemia, and family history of HTN.

Collection of samples: 5 ml of 12 hours fasting venous blood samples were collected from 185 hypertensive patients and 200 controls. Serum from the samples was used to estimate lipid levels.

Biochemical markers: TC, high-density lipoprotein (HDL)-cholesterol (HDL-c) and triglyceride concentrations were determined enzymatically using commercially available kits and auto analyzer available at the CARE Hospital. LDL-c was estimated using Friedewald's formula.

Apo E genotyping: Leucocyte DNA was extracted following standard protocols. DNA was amplified by PCR in a DNA thermal cycler (Perkin Elmer Cetus) using oligonucleotide primers (Hysel India Ltd), forward (5'ACAGAATTCCGCCCCTGCTGACAC-3') and reverse (5'-TAAGCCTGGGACGCTGTGCAAGGA-3') as described by Hixson et al. Electrophoresis of amplified products (244 bp) was performed on 14% polyacrylamide gel, after digesting with the Hha I restriction enzyme (10 µl PCR product + 5 units of enzyme incubated at 37°C overnight). After electrophoresis, the gel was stained with ethidium bromide (0.2 mg/L) for 10 min and the digested fragments were visualized under UV illumination. Sizes of Hha1 fragments (91 bp, 83 bp, 72 bp, 48 bp, 38 bp and 35 bp) were determined by comparison with known size marker (50 bp) and the genotypes were determined (Fig. 1).

Statistical analysis: Standard statistical procedures from the SPSS package were used for the analysis of the data. For major risk factors and potential confounders, differences between hypertensive cases and control groups were tested by using appropriate tests of significance (χ2, t test). As the distribution of triglycerides was skewed, the log-transformed values were used for the analysis. Allele frequencies were calculated as per gene frequency formula and following Hardy-Weinberg law and the frequencies were tested for Hardy-Weinberg equilibrium. The allelic association was further tested by Wolf's test.

To evaluate the association of apo E polymorphism with HTN, multiple logistic regression was used with maximum likelihood estimation of the regression coefficients and their standard errors. All the potential confounders were systematically tested in the regression model. Multivariate-adjusted odds ratios were calculated for apo ε2 allele (ε2/4 and ε3/4) and ε4 allele (ε2/2, ε2/3 and ε2/4) taking ε3 allele (ε3/3) as reference. For each odds ratio we calculated two-tailed p values and 95% confidence intervals (CI). Significance levels were set at 0.05 in all cases.
Results

Characteristics of the study sample: In the present study 185 patients with EH were compared with 200 normotensive controls for the association with different epidemiological factors as specified in Table 1. The patients were in the age group of 35-65 years with a mean age of 51.0 ± 0.64 years, while the controls were in age range from 35-60 years with a mean age of 45.6 ± 0.53 years. Significant differences were observed between the mean ages of patients and controls (χ²=5.495, p<0.05). SBP (150.4 ± 1.58 mmHg, p<0.05) and DBP (92.4 ± 0.57 mmHg, p<0.05) were found to be significantly higher in patients than in controls (119.9 ± 0.50 mmHg and 80.0 ± 0.24 mmHg, respectively).

Statistically significant sex differences were observed between patients and controls (χ²=5.495, p<0.05) with a significant preponderance of males in the patient group (87%) as compared to controls (52%); 42% of the patients had a positive family history for EH, compared to 18% positive family history among the controls (χ²=26.91, p<0.05, Table 1). Cigarette smoking did not show any statistically significant difference between patients and controls. However, alcohol consumption in patients was highly significant (30%, χ²=19.49, p<0.05) as compared to controls (12%). Though the BMI in patients (26.1 ± 0.28 kg/m²) showed slight elevation, the value did not differ significantly from the BMI of controls (25.5 ± 0.29 kg/m²).

Frequencies of alleles and genotypes: The distribution of apo E genotypes in hypertensives differed significantly from controls (χ²=10.87, p<0.05, Table 2). Similar observation was made when the distribution in males versus females and familial versus non-familial cases were considered (χ²=8.45, p<0.1, and χ²=26.93, p<0.05, respectively).

The prevalence of genotypes and the allele frequencies among patients and controls are shown in Table 3. It was observed that prevalence of ε3/4 genotypes was 1.5-fold high in patients when compared to controls (14.5% v. 10.0%, p<0.05) while prevalence of ε2/3 genotypes was high in controls than in patients (6.5% v. 4.3%). Statistically significant difference was not found between patients and controls with respect to ε3 and ε4 allele frequencies, while ε2 allele frequency was found to be much more prevalent in patients (12.16%) than in controls (5.75%, χ²=10.87, p<0.05). Further, the allelic distribution was found to be deviated significantly from Hardy-Weinberg equilibrium in patients but not in controls (χ²=14.4, p<0.05, Table 3). When tested by Wolf’s method, this allelic association showed higher relative incidence of ε2 allele (RI= 2.24, χ²= 9.13, p<0.05) as compared to other alleles and also in cases with family history of hypertension (RI= 2.407, χ²= 6.79, p<0.05, Table 4).

We divided individuals according to sex, age groups and either ε2 allele carriers (ε2ε2) or ε3 allele non-carriers (ε4ε4) in order to find out the relationship between age, sex and the occurrence of hypertension. We found that, ε2ε2/ε4ε4 groups had more male patients in the 45-55 years age group than in controls (χ²=6.78, p<0.05).

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Table 1. General characteristics observed in hypertensive patients and controls

<table>
<thead>
<tr>
<th>General characteristics</th>
<th>Patients (n=185)</th>
<th>Controls (n=200)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>51.0 ± 0.64*</td>
<td>45.6 ± 0.53</td>
</tr>
<tr>
<td>Male ratio (%)</td>
<td>87*</td>
<td>52</td>
</tr>
<tr>
<td>Family history of EH (%)</td>
<td>42*</td>
<td>18</td>
</tr>
<tr>
<td>DM (%)</td>
<td>50*</td>
<td>8</td>
</tr>
<tr>
<td>CAD (%)</td>
<td>27*</td>
<td>2</td>
</tr>
<tr>
<td>Stroke (%)</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>24</td>
<td>18</td>
</tr>
<tr>
<td>Alcohol consumption (%)</td>
<td>30*</td>
<td>12</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>150.4 ± 1.58*</td>
<td>119.9 ± 0.50</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>92.4 ± 0.57*</td>
<td>80.0 ± 0.24</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.1 ± 0.28*</td>
<td>25.5 ± 0.29</td>
</tr>
</tbody>
</table>

* p<0.05 difference between patients and controls (χ² or t test)
Apo E genotypes and lipids: Table 5 shows mean distribution of lipid levels in patients and controls. In general, TC, LDL-c and triglyceride levels were found to be significantly elevated in patients (200.0±2.83 mg/dl, 195.85±3.6 mg/dl and 195.61±8.91 mg/dl respectively, p<0.05) than in controls (180.78±1.9 mg/dl, 139.22±1.11 mg/dl and 122.4±1.86 mg/dl, respectively).

In general it was observed that TC, LDL-c and TG levels were significantly elevated in \( \varepsilon_4 \) allele non-carriers than in \( \varepsilon_3 \) allele non-carriers (p<0.05), while HDL-c levels were reduced in \( \varepsilon_4 \) allele non-carriers (p<0.05). These observations clearly indicate the correlation between the alleles at apo E locus and the lipid metabolism.

### Discussion

Studies conducted in different parts of the globe reveal that the gene frequencies at apo E locus are highly heterogeneous between the populations. The \( \varepsilon_3 \) is the most common form of the gene in most of the populations followed by \( \varepsilon_4 \) and \( \varepsilon_2 \) alleles. In a population-based study Venkataramana et al. reported the allele frequencies in Indian population as 85-92% for \( \varepsilon_3 \) allele, 3-9% for \( \varepsilon_4 \) allele and 3-5% for \( \varepsilon_2 \) allele. In the present study, apo E allele frequencies in the control group are comparable with the study of Venkataramana et al. It is well known that the \( \varepsilon_4 \) allele of Apo E is associated with the increased prevalence of atherosclerosis and CAD. However, there are controversial results
concerning the association between apo E genotype and some cardiovascular risk factors. Some studies have suggested that high blood pressure may be associated with the presence of the ε4 allele, while others have found its association with ε2 allele. However, no association was found in few studies. Apo E may interfere with smooth muscle cell proliferation and participate in smooth muscle cell hypertrophy in the arterial wall. These mechanisms may explain the association found in young and middle-aged populations that were mainly included in the previous studies. However, other mechanisms such as increased rigidity and decreased elasticity of the aorta and other large vessels may contribute to the development of high blood pressure, and thus explain the lack of association in the elderly subjects.

Recent investigations on the effect of age and sex on the apo E genotypes suggest that in middle aged men (<51 years), ε4 allele carriers are at a greater risk for the development of the disease. Context-dependency of apo E polymorphism and associated effects have been demonstrated suggesting other genetic components to be equally important, and lifestyle including dietary habits are now recognized factors that can either mask or expose an effect of a specific apo E genotype.

The lower plasma total cholesterol levels observed in subjects carrying the ε4 allele generally correlate with reduced coronary and peripheral artery atherosclerosis, and the higher cholesterol level seen in ε2 carriers is associated with a higher prevalence of cardiovascular disease. However, the effect of apo E variation on clinical atherosclerosis is not completely explained by its impact on risk factor levels, as recent studies have demonstrated association between carotid atherosclerosis and coronary artery calcification in asymptomatic adults.

There is convincing evidence that the relationship between apo E genotype and plasma lipoprotein-lipid levels is context-dependent, being significantly influenced by age and sex. Recent evidence also indicates that the

**Table 6. Mean distribution of lipid levels in hypertensive patients and controls with respect to apo ε alleles**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients (n=37)</th>
<th>Controls (n=178)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>198.2 (5.75)*</td>
<td>200.2 (7.67)</td>
</tr>
<tr>
<td>HDL-c</td>
<td>39.2 (1.31)*</td>
<td>40.2 (0.65)</td>
</tr>
<tr>
<td>LDL-c</td>
<td>135.2 (4.83)*</td>
<td>130.5 (3.40)</td>
</tr>
<tr>
<td>TG</td>
<td>150.6 (16.35)*</td>
<td>162.7 (7.99)</td>
</tr>
</tbody>
</table>

TC: total cholesterol; HDL-c: High-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; TG: triglyceride

Concerning the association between apo E genotype and some cardiovascular risk factors, some studies have suggested that high blood pressure may be associated with the presence of the ε4 allele, while others have found its association with ε2 allele. However, no association was found in few studies. Apo E may interfere with smooth muscle cell proliferation and participate in smooth muscle cell hypertrophy in the arterial wall. These mechanisms may explain the association found in young and middle-aged populations that were mainly included in the previous studies. However, other mechanisms such as increased rigidity and decreased elasticity of the aorta and other large vessels may contribute to the development of high blood pressure, and thus explain the lack of association in the elderly subjects.

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**Table 7. Multiple logistic regression analysis showing the contribution of risk factors to hypertension**

<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>SE</th>
<th>df</th>
<th>p value</th>
<th>Odds ratio</th>
<th>95% CI for odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo E4</td>
<td>0.771</td>
<td>0.291</td>
<td>1</td>
<td>0.008*</td>
<td>2.160</td>
<td>1.22 – 3.83</td>
</tr>
<tr>
<td>Apo E2</td>
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<td>0.514</td>
<td>1.153</td>
<td>0.75 – 1.77</td>
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<td>0.000*</td>
<td>5.317</td>
<td>2.87 – 9.84</td>
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<tr>
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<td>0.351</td>
<td>1</td>
<td>0.000*</td>
<td>4.338</td>
<td>2.18 – 8.63</td>
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<tr>
<td>TC (mg/dl)</td>
<td>2.215</td>
<td>0.331</td>
<td>1</td>
<td>0.000*</td>
<td>9.161</td>
<td>4.49 – 17.52</td>
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<td>6.217</td>
<td>3.19 – 12.13</td>
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<tr>
<td>Smoking</td>
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<td>0.403</td>
<td>1</td>
<td>0.000*</td>
<td>5.028</td>
<td>2.29 – 11.07</td>
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<tr>
<td>Sex</td>
<td>0.723</td>
<td>0.295</td>
<td>1</td>
<td>0.014*</td>
<td>2.060</td>
<td>1.16 – 3.67</td>
</tr>
</tbody>
</table>

a E4/4 and E3/4 vs. E2/3 as reference
b E2/4 and E2/3 vs. E3/3 as reference
c Non-alcoholic as reference
d Absence of family history as reference
e Non-smokers as reference
f Female sex as reference

Acknowledgements

We extend our gratitude to K Vishweshwar Rao (formerly statistician at National Institute of Nutrition) for his valuable guidance in the statistical analysis.

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Brief Report

M-Shaped Doppler Signal Across Ventricular Septal Defect: Potential Implications for Estimation of Right Ventricular Systolic Pressure

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An 8-year-old child suffering from ventricular septal defect and severe valvular pulmonary stenosis was evaluated by echo-Doppler technique and cardiac catheterization. A peak instantaneous transventricular systolic gradient of 64 mmHg was recorded across the ventricular septal defect with an interesting M-shaped spectral pattern. However, cardiac catheterization revealed a peak-to-peak non-simultaneous gradient between the right and the left ventricles of only 14 mmHg. This discrepancy along with its implications are discussed in this report.

Key Words: Ventricular septal defect, Echocardiography, Congenital heart disease

Case Report

An 8-year-old asymptomatic boy underwent Doppler-echocardiography for a systolic murmur detected at routine examination. He denied history of effort intolerance. Physical examination revealed a healthy child with heart rate of 78 beats per min (bpm), supine systolic blood pressure of 90/60 mmHg and no evidence of heart failure. Precordial examination revealed imperceptible apical impulse, systolic thrill along left sternal border, normal first heart sound, widely split second sound with muffled pulmonary component and a grade IV/VI pansystolic murmur along the left parasternal border radiating on both sides. A 12-lead electrocardiogram (ECG) showed sinus rhythm and complete right bundle branch block (RBBB). Plain chest skiagram had prominent hilar pulmonary shadow with normal cardiac size. Two-dimensional (2D) echocardiography showed situs solitus, levocardia, normal-sized LV and normal left and right atria, RV hypertrophy, and a small (4 mm) perimembranous VSD (Fig. 1) with an aneurysm of the membranous septum and domed restricted pulmonary valve. Color flow mapping revealed left-to-right shunt across the VSD, mild tricuspid regurgitation and turbulent systolic flow in the RV outflow tract.

Continuous-wave Doppler examination showed a peak systolic gradient of 71 mmHg across the RV outflow tract (Fig.2), an M-shaped Doppler spectrum across the VSD (Fig.3) and an incomplete Doppler signal of tricuspid regurgitation. The VSD Doppler signal had an early peak systolic velocity of 4.0 m/s (gradient : 64 mmHg ).
velocity of 3.5 m/s (gradient: 49 mmHg) and the mid-systolic dip at 1.8 m/s (gradient: 13 mmHg). Cardiac catheterization performed within two hours of the echocardiographic examination under light sedation revealed a peak LV systolic pressure of 96 mmHg (aortic pressure 96/60 mmHg) and the non-simultaneous peak RV systolic pressure of 82 mmHg with a pullback pressure gradient of 66 mmHg across the RV outflow tract. Mean right atrial pressure (4 mmHg) and the pulmonary artery pressure (17/8 mmHg) were normal. The patient was recommended for percutaneous balloon pulmonary valvuloplasty.

Discussion

Echo-Doppler is a robust and validated technique to assess intracardiac pressures by deploying modified Bernoulli equation despite minor discrepancies.\(^1\) Transventricular pressure gradient across the VSD provides an accurate assessment of the RV pressures.\(^{2,3}\) Plateau appearance of the transventricular Doppler signal is necessary for such an estimation. This is to make sure that both the ventricular systolic pressures peak almost simultaneously. Delay in peaking of ventricular pressure curves may introduce inaccuracy in assessment of RV pressure by use of transventricular peak gradient. Physiologically, RV systolic pressure peaks later than the LV but this delay is minor and can be ignored. However, delayed peaking of the RV has been described as the cause of sloped VSD signals leading to erroneous pressure calculations.\(^5\) This delayed peaking of the RV systolic pressure may occur due to right bundle branch block (RBBB) and/or due to significant RV outflow tract obstruction.\(^5,6\) Sloped Doppler signal peaks in early systole and tapers off in mid and late systole. It has been suggested that mid-systolic or late systolic pressure gradient provides an accurate assessment of RV pressure in such cases.

M-shaped VSD Doppler signal is rare and only one such case has been reported so far.\(^6\) This occurred in a 4-year-old child who was operated for VSD, pulmonary atresia and multiple branch pulmonary stenoses. By simultaneous ventricular pressure recordings with transducer-tipped catheters, the authors demonstrated larger early systolic and a smaller late systolic pressure gradients corresponding with the M-shaped pattern of the Doppler signal although mean systolic pressure difference between the two ventricles was negligible. The RV systolic pressure curve peaked later but reached its peak earlier than the LV. The authors hypothesized that the RBBB was the cause of initial delayed increase in RV pressure but did not have satisfactory
explanation for the prolonged LV pressure curve that accounted for the terminal peak of the M-shaped signal. Our patient had a classic M-shaped VSD Doppler signal with a nadir velocity of 1.8 m/s (peak gradient: 13 mmHg) which was very close to the peak-to-peak gradient (14 mmHg) obtained at cardiac catheterization. Estimating RV systolic pressure from early or late systolic velocity gradients could result in significant underestimation which may impact the management of such patients.

It is easy to explain early systolic gradient in our patient (first limb of M) because of the delay in RV pressure rise due to the RBBB and the RV outflow tract obstruction. We do not have a cogent explanation for the prolonged LV pressure curve responsible for the late peak velocity gradient (second limb of M). However, it is possible that instead of prolonged LV pressure decay, it is the rapid right ventricular pressure decay during late systole and isovolumic relaxation which is responsible for this phenomenon. Such an explanation needs further validation in these patients.

Conclusions: This is a rare case report of a patient with an M-shaped Doppler signal across a VSD that shows the potential for underestimation of RV pressure. In this case, maximum instantaneous Doppler gradient erroneously overestimates peak-to-peak systolic gradient between the RV and the LV. Velocity at the valley of the Doppler signal provides a true estimate of peak-to-peak transventricular gradient, and should be used.

References

Transcatheter Closure of Aortopulmonary Window

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Aortopulmonary window is an uncommon congenital cardiac defect. Most infants presenting with aortopulmonary window will require conventional surgical repair. Experience with transcatheter closure of aortopulmonary window is limited. We report the case of a 9-year-old girl with aortopulmonary window, in whom transcatheter closure was performed successfully using Amplatzer ductal occluder device. (Indian Heart J 2005; 57: 161-163)

Key Words: Aortopulmonary window, Amplatzer ductal occluder, Congenital heart disease

A n aorto-pulmonary window (APW) is a communication between the pulmonary artery and the ascending aorta in the presence of two separate semilunar valves. The window is usually a large oval defect but in about 10% of cases it is small. APW accounts for approximately 0.1% of all congenital cardiac anomalies. Associated anomalies occur in 50% of cases and include interrupted aortic arch, right pulmonary artery from aorta, coarctation of aorta, coronary artery from main pulmonary artery, tetralogy of Fallot, or even pulmonary atresia or aortic atresia. Mori et al. classified APW as Type I (proximal defect between ascending aorta and main pulmonary artery), Type II (distal defect between ascending aorta and right pulmonary artery), and Type III (all the defects inclusive, Type I+II). In most cases, surgical repair is undertaken during infancy using different techniques like ligation without cardiopulmonary bypass (CPB), division and over-sewing between clamps on CPB, or transaortic patch closure. The experience with transcatheter closure of APW is limited. We report a case of successful closure of APW using Amplatzer ductal occluder device.

Case Report

A 9-year-old girl presented to us with history of recurrent episodes of respiratory tract infection and congestive heart failure during infancy and childhood, which was managed medically. On examination, she had a high volume collapsing pulse with a rate of 80 per min. Her blood pressure was 100/60 mmHg. Apex beat was felt in the left 6th intercostal space 1 cm outside the mid-clavicular line. Grade V/VI continuous murmur was present which was best heard over the left 2nd and 3rd intercostal space. Hemoglobin was 12 gm/dl and baseline biochemical parameters were normal. Echocardiogram showed turbulent flow across APW and holo-diastolic run off in descending aorta. However, the size of APW was not clear on echocardiography. Left atrium and ventricle were dilated. There was no patent ductus arteriosus or coarctation of aorta. The pulmonary valve was thick, doming with a gradient of 40 mmHg. Cardiac catheterization and selective aortogram were performed. A 26% step-up in oxygen saturation was noted in the main pulmonary artery. Pulmonary artery pressure was 22/12 mmHg (mean 16 mmHg). Pulmonary to systemic flow ratio (Qp/Qs) was 6.5 (Table 1). Selective ascending aortogram in lateral view showed the presence of APW measuring 6 mm in diameter (Fig. 1). It was round in shape, and was located in the left lateral wall of the ascending aorta, 8 mm away from the origin of the left main coronary artery (LMCA). It communicated with the main pulmonary artery on its right wall with adequate margin from the pulmonary valve and the bifurcation of the pulmonary trunk. The APW was crossed from the venous side via the right femoral vein, inferior vena cava, right atrium, right ventricle and pulmonary trunk with a 5 F Picard catheter and 0.035” Terumo wire. The Picard catheter was then advanced over the wire and its tip positioned in the descending aorta. The Terumo wire was exchanged with an extra stiff Amplatzer J-tipped 0.035” wire leaving its tip in the descending aorta. A long 7 F Cook’s sheath and dilator were advanced over the wire and its tip positioned across the APW into the descending aorta. A 10×8 mm Amplatzer ductal occluder device was deployed across the APW. A check aortogram...
was done to ensure complete occlusion of the defect without impinging upon LMCA. The device was delivered and the sheath was withdrawn to the inferior vena cava. An aortic root angiogram performed after 10 min showed the device occluding the APW completely (Fig. 2). There was no impingement of left coronary artery or aortic or pulmonary regurgitation. The continuous murmur disappeared and the follow-up echocardiography done at 3 months showed no residual shunt and valvular pulmonary stenosis was mild (28 mmHg).

Table 1. Pressure and oximetry data

<table>
<thead>
<tr>
<th>Pressure data (mmHg)</th>
<th>Oximetry (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA wedge (mean)</td>
<td>12</td>
</tr>
<tr>
<td>MPA</td>
<td>22/12 (16)</td>
</tr>
<tr>
<td>RV (systolic)</td>
<td>58</td>
</tr>
<tr>
<td>RA (mean)</td>
<td>6</td>
</tr>
<tr>
<td>LV (systolic)</td>
<td>108</td>
</tr>
<tr>
<td>Aorta</td>
<td>108/45 (70)</td>
</tr>
</tbody>
</table>

RV: pulmonary artery; MPA: mean pulmonary artery; RV: right ventricle; RA: right atrium; LV: left ventricle; SVC: superior vena cava

Subsequently, Rashkind double umbrella occluder systems were successfully used in a child with post-operative APW and an infant with native APW. A buttoned device was used to close post-operative APW in an adult. In our patient, we used an Amplatzer duct occluder device. Literature review revealed three case reports of closure of APW using Amplatzer device; one in a child with post-operative APW using a custom-made Amplatzer device and one in native APW. In the third report, a septal occluder device and a ductal occluder device was used in two patients. Our patient also had an associated mild valvular pulmonary stenosis which did not require balloon dilation. Most infants presenting with APW will require conventional surgical repair. The type of APW most suitable for device closure is small APW located in the middle of the two great arteries, away from the origin of the left coronary artery and the right and left main pulmonary arteries, with no associated congenital anomalies. Significantly less discomfort, avoidance of CPB and surgical scar, and shorter duration of hospital stay make device closure the treatment of choice in such selected cases of APW.

Discussion

This case report demonstrates the feasibility of non-surgical closure of APW using Amplatzer duct occluder device in carefully selected cases. Transcatheter closure of APW should be considered when anatomy is favorable in terms of location and size of the defect, in the absence of associated anomalies. Initial report of transcatheter closure of APW describing the use of umbrella occluder system in a child resulted in incomplete closure.

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Device Closure of Residual Ventricular Septal Defect after Repair of Tetralogy of Fallot Using the Amplatzer Duct Occluder

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Residual ventricular septal defect after surgical repair for tetralogy of Fallot can occasionally be hemodynamically important requiring re-intervention. Transcatheter closure using ventricular septal defect devices is an attractive option for such defects. We describe two such cases where the Amplatzer duct occluder was used as an innovative, less costly alternative for closure of residual membranous ventricular septal defects. Complete occlusion of the residual ventricular septal defect with significant symptomatic improvement could be accomplished in both patients. (Indian Heart J 2005; 57: 164–166)

Key Words: Ventricular septal defect, Tetralogy of Fallot, Amplatzer duct occluder

Case Reports

Case 1: A 17-year-old girl who had undergone intracardiac repair for TOF at the age of 3 years presented with gradually worsening dyspnea and palpitation on effort. Clinical evaluation suggested a hemodynamically significant VSD with no evidence of congestive heart failure. Chest X-ray revealed mild cardiomegaly (cardiothoracic ratio 55%) with increased pulmonary blood flow. Transthoracic echocardiogram (TTE) revealed a 7.8 mm residual VSD at the inferior margin of the VSD patch with left-to-right shunt (Fig. 1A). There was free pulmonary regurgitation with no pulmonary arterial hypertension. The left ventricular (LV) function was normal. At cardiac catheterization, the mean right atrial and pulmonary arterial pressures were 10 mmHg and 19 mmHg, respectively. Oximetry revealed a Qp : Qs ratio of 2.1. The LV end-diastolic pressure was 14 mmHg. The basal indexed pulmonary vascular resistance was 0.5 Wood units/m². The LV angiogram profiled the VSD patch projecting into the right ventricle as a diverticulum with a 7 mm residual VSD at its inferior margin (Fig. 2A). The remaining procedure was carried out under general anesthesia for the purpose of transesophageal echocardiographic (TEE) guidance.

The defect was crossed with a no-torque right coronary catheter (Bard USCI Division; CR Bard Inc). An arteriovenous railroad was created with an exchange length (360 cm) 0.038" Terumo glide wire (Terumo Inc., Japan) using the standard technique. A 9 F long delivery sheath was introduced from the venous side over the guidewire and was placed in the ascending aorta. After removing the guidewire, a 10-8 ADO was taken into the sheath and deployed. The aortic retention disc of ADO could open only above the aortic valve and hence full deployment was not possible. Attempts to pull the sheath below the aortic valve resulted in slippage of the entire assembly into the right ventricle. The arteriovenous railroad was formed again; the
sheath was placed across the VSD just below the aortic valve. A 6 F internal mammary catheter was taken parallel to the guidewire through the sheath through which a 0.038" Teflon wire was directed toward the LV cavity (Fig. 3). The guidewire in the aorta was removed and the delivery sheath was tracked over the catheter into the left ventricle. The same 10-8 ADO could then be deployed across the residual VSD successfully. LV angiogram (Fig. 2B) and TEE showed the device to be well away from the aortic valve with no residual flow across the VSD. Aortic root angiogram showed no aortic regurgitation. The fluoroscopy time was 35 min. The patient was extubated immediately after the procedure and was discharged from the hospital on the next day on anti-platelet doses of aspirin. TTE at discharge showed stable device position with no residual flow and no impingement on aortic valve (Fig. 1B). She was asymptomatic when reviewed 1 month later.

**Case 2:** An 11-year-old girl with TOF with relatively small-sized branch pulmonary arteries (right pulmonary artery 3.5 mm at origin; 6.5 mm at hilum; left pulmonary artery 6.7 mm at hilum) underwent intracardiac repair with an 8 mm fenestration in the VSD patch. Post-operatively, the patient developed severe and refractory congestive heart failure with generalized edema and ascites. Attempts to stabilize the patient with high doses of diuretics and inotropes were unsuccessful. Echocardiogram revealed significant left-to-right shunt across the fenestration in the VSD patch. The patient was taken up for cardiac catheterization to quantify the left-to-right shunt and to determine whether closure of the fenestration would improve the hemodynamic parameters.

Oximetry showed a 12% step up yielding a Qp-Qs ratio of 1.8. The right atrial pressures were markedly elevated (a 34 mmHg, v 27 mmHg, mean 25 mmHg). The right ventricular pressures were high (systolic 60 mmHg, end-diastolic 24 mmHg). The pulmonary artery pressure was 45/20 mmHg (mean 28 mmHg). The fenestration was temporarily balloon-occluded with a 7 F Swan-Ganz catheter that was passed over an arteriovenous railroad between right femoral artery and vein. This resulted in reduction of the right atrial mean pressure to 15 mmHg with normalization of pulmonary arterial pressure (21/16 mmHg; mean 20 mmHg). Hence, we proceeded to close the VSD using the same technique as described for the first
patient. A 12-10 ADO was deployed successfully in the first attempt through a 9 F long delivery sheath that completely sealed the fenestration. TEE showed no residual shunt without any impingement onto the aortic valve by the device. The fluoroscopy time was 28.4 min. Central venous pressure decreased and peripheral edema started improving on the same day. She could gradually be weaned off inotropes and mechanical ventilation over the next 3 days and was discharged from the hospital one week after the procedure. The patient was symptom-free at one-month and at 3 months follow-up.

Discussion

Hemodynamically significant residual VSD following repair for TOF imposes significant morbidity, mortality and economic burden on the family of the patient. This is specially relevant in developing countries like India. Though closure of such defects using AMVSO has been proved to be a safer alternative, it costs more than the repeat surgery. The two cases described in this report have shown that ADO is safe and effective to close residual post-operative VSD in perimembranous area and thus a cheaper alternative to AMVSO. Both patients had significant symptomatic improvement and perhaps it was life-saving in the second patient who had refractory congestive cardiac failure. To our knowledge, this is the first report of using ADO for closure of a post-operative residual VSD.

In both the patients, the residual defects were away by a safe distance from the aortic valve. Hence one could have chosen Amplatzer muscular VSD device. Even this device costs 1.5 times the cost of ADO and hence we did not opt for it. While there are two retention discs at either end in the muscular VSD device, the ADO has retention disc only at one end. The concern regarding the use of ADO for closing VSD arises because the device can potentially dislodge and embolize into the LV cavity if there is elevation in the right ventricular pressure. Our patients either had normal or only mildly elevated pulmonary artery pressure. Moreover, we chose slightly larger-sized devices that resulted in the formation of a waist at the level of the septum, splaying the right ventricular end (Fig. 2B). Hence no instability of the device was noted.

Because of the close proximity of the VSD to the aortic valve it becomes important not to impinge on the aortic valve while deploying the device. Placement of the delivery sheath well into the body of the left ventricle before the formation of the retention disc is a key step in the procedure. Using an additional arterial catheter, Pedra et al. have described a method where they allowed the same wire to be used for forming arteriovenous railroad to loop into the left ventricle over which the delivery sheath was maneuvered into the left ventricle. We adopted a different technique by passing an internal mammary artery catheter through the delivery sheath parallel to the guidewire to direct the delivery sheath toward the LV cavity (Fig. 3). TEE plays a vital role in safe deployment of the device. Complete closure could be achieved in both our cases with no procedure-related morbidity.

Conclusions: The present report illustrates the innovative use of the ADO for the transcatheter closure of residual VSD in the perimembranous region after surgical repair of tetralogy of Fallot. The ADO is an effective alternative for the more expensive AMVSO and is a particularly attractive option in a developing country where cost constraint is a major factor.

References

Infective Endocarditis Following Implantation of Amplatzer Atrial Septal Occluder

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Infective endocarditis is a rare but serious complication following device closure of atrial septal defect. Surgical removal of the device is mandatory in such cases. We report a rare case of polymicrobial endocarditis following implantation of Amplatzer septal occluder in an eight-year-old child. (Indian Heart J 2005; 57: 167-169)

Key Words: Amplatzer septal occluder, Atrial septal defect, Infective endocarditis

Device closure of atrial septal defect is now an accepted modality of treatment. Of the various devices used for the closure of atrial septal defect (ASD), the preferred device is the Amplatzer septal occluder (AGA Medical Corporation, Minnesota, USA). Complete closure is observed in 95.5% of patients with this device. Reported complications with the use of Amplatzer device include device embolization, arrhythmias, device malposition, thromboembolism, access site complications, cardiac tamponade and infective endocarditis. We present a case of endocarditis on an Amplatzer atrial septal occluder in an 8-year-old boy.

Case Report

An 8-year-old male child underwent implantation of 24 mm Amplatzer atrial septal occluder in December 2003 at a secondary level hospital. Three months after the device implantation, the patient developed prolonged fever for which he empirically received linezolid, cefotaxime and amikacin for 4 weeks. The fever responded initially but recurred in 2 weeks and the child was referred to our institution for further management.

At presentation to our institution the patient was febrile, had a pulse rate of 110 beats per min (bpm) and a blood pressure (BP) of 100/70 mmHg. His spleen was palpable 2 cm below the left costal margin and examination of cardiovascular and other systems was unremarkable. The blood cultures grew Klebiella pneumoniae and Acinetobacter, both of which were sensitive to cefaperazone-sulbactam combination, piperacillin-tozabactam combination and meropenam. A transthoracic echocardiographic (TTE) examination revealed the device in place and there was no residual ASD flow. There was no evidence of any vegetations on the device or any valve. In view of the high suspicion of infective endocarditis, a transesophageal echocardiographic (TEE) examination was done under short general anesthesia, which revealed vegetations on the left atrial side of the device (Fig. 1). A contrast-enhanced multislice computerized tomographic (CT) angiography of head did not show any infarct or mycotic aneurysm. After 10 days of appropriate antibiotic therapy with cefaperazone-sulbactam combination along with vancomycin and rifampicin, the child underwent surgery for device removal and ASD closure.

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Fig. 1. Transeosophageal echocardiogram showing vegetation (arrow) attached to the left side of the Amplatzer atrial septal occluder.
LA: left atrium; Ao: aorta
The patient was operated through a mid sternotomy approach using mildly hypothermic cardiopulmonary bypass (CPB) and cold, hyperkalemic cardioplegic arrest. On opening the right atrium, the device was found to be completely endothelialized and there were numerous small (2 mm - 5 mm), rounded, smooth, mushroom-like vegetations at the junction of the device and the septal rim in the superior part. The septum was incised for about 1 cm inferiorly and a plane was developed between the device and septal margin. Gradually the device was separated from the septal margin. On the left side also, there were similar vegetations along the rim with a small necrotic pocket superiorly. All the vegetations and the necrotic material were removed and cardiac chambers washed thoroughly with antibiotic-mixed saline. The resultant defect was closed with a free patch of right atrial wall keeping the endocardial surface on the left atrial side.

The vegetations removed during the surgery showed inflammatory infiltrates in the form of pus cells and necrotic debris. Gram stain and culture of the vegetations did not yield any organisms. The patient was continued on the same antibiotics for a further three weeks. He developed allergic reaction to the combination of antibiotics with fever and eosinophilia. The antibiotics were changed to meropenam and teicoplanin that were continued for a further two weeks. He remained afebrile subsequently and repeat blood cultures after stopping antibiotics were sterile. However, he developed multiple discharging sinuses from the sternal suture sites and superficial infection of the sternal wound needing debridement and regular antibiotic dressings for over a month, even after the antibiotic treatment for endocarditis was over.

Discussion

Atrial septal defect occurs in one child per 1500 livebirths.1 With the early attempts in the seventies by Mills and King,4 device closure of ASDs gained widespread acceptance after the introduction of the Amplatzer septal occluder. Results have been comparable to surgical closure and the procedure is associated with less morbidity and more patient comfort.5,6

Of the various complications described following ASD device closure, the least common is the infection of the device. Only four such cases are available in literature including two cases involving ASDOS device, one case involving Amplatzer septal occluder and another involving CardioSEAL device for patent foramen ovale.7,8 The case involving the Amplatzer septal occluder was in a 10-month-old infant with ASD and pulmonary stenosis. The procedure (ASD device closure with pulmonary valve balloon dilation) was carried out in this infant who was suffering from recurrent lower respiratory tract infection by Staphylococcus aureus. The case involving a CardioSEAL device for the closure of a patent foramen ovale was in an adult patient. This patient presented with fever following device implantation with large vegetation on the left atrial side of the device. His blood cultures grew Bacillus pumilus. The vegetation as such was sterile and gram staining did not yield any microorganism.

In adult patients, TEE is more sensitive and specific for the diagnosis of endocarditis. TEE is also indispensable in the diagnosis of vegetations in the presence of prosthetic material. In our patient, despite good transthoracic windows, the vegetations could not be picked up by TTE probably because of acoustic shadowing on the left atrial side of the device, whereas TEE directly profiles that aspect of the device. This reinforces the assumption that TEE is superior to TTE for detection of vegetations in patients with prosthetic material and high suspicion of infective endocarditis.

Device infection can occur in two ways: one due to introduction of microbes during the procedure of implantation, other due to seeding of microorganisms after the procedure. After device implantation, it takes 6 months for complete neoendothelialization of the device. Till such time the device is prone to development of thrombus formation on the surface, and hence, also predisposed to non-bacterial thrombotic endocarditis. Any transient bacteremia during this time can cause seeding of the device material with the microorganisms.9 The organisms grown in our patient (Klebsiella and Acinetobacter) are classical of a nosocomial infection and hence probably indicate that the seeding occurred during the procedure of device implantation. This also stresses the importance of prompt treatment of infection and need for infective endocarditis prophylaxis in these patients for at least 6 months after device implantation.

This report emphasizes the significance of aseptic precautions during cardiac catheterization and the interventional procedures.

References


Successful Closure of Coronary Artery Perforation Using Non-Conventional Embolic Material

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Coronary perforation during percutaneous coronary interventions is a rare but dreadful complication. While coronary perforation involving large vessels are managed successfully by covered stents, small distal vessel perforation is usually managed by prolonged balloon inflation or embolization of gel foam/thrombogenic metallic coils. We describe a case, where perforation of a small ventricular branch of the right coronary artery was successfully occluded by packing it with pieces of thrombogenic floppy tips of used coronary angioplasty guidewires instead of conventional metallic coils. (Indian Heart J 2005; 57: 170–171)

Key Words: Coronary angioplasty, Coil embolization, Coronary artery disease

Coronary perforation during percutaneous transluminal coronary angioplasty (PTCA) is a rare but potentially serious complication. The therapeutic approach to these perforations is determined by various factors like size of the vessel, size of the perforation, its location (near a bifurcation, within a stented segment, distal to a chronic occlusion), whether patient has received glycoprotein IIb/IIIa receptor antagonist and hemodynamic stability of the patient. Small distal vessel perforation in hemodynamically stable patients is usually managed by prolonged balloon inflation and reversal of anticoagulation. Recent reports have also demonstrated successful use of gel foam and thrombogenic metallic coil embolization.

We describe a case, where rupture of a small ventricular branch of the right coronary artery (RCA) due to dilation with an oversized balloon was successfully sealed by placing cut floppy tips of used PTCA wires instead of conventional metallic coils in the leaking vessel.

Case Report

A 54-year-old hypertensive male patient on regular medical therapy for chronic stable angina for last two years, presented with crescendo angina not being controlled with maximal antianginal drugs of one month's duration. He was on a combination of aspirin and clopidogrel prior to coronary angiography which was undertaken with plan for angioplasty, if required. Coronary angiogram revealed 99% stenosis in mid left anterior descending artery (LAD) with TIMI-II flow and total occlusion of proximal RCA with distal RCA filling through homocoronary collaterals. The patient was taken up for angioplasty of LAD and RCA after receiving a bolus of 5000 units of heparin. The LAD was successfully stented with a 3×28 mm Pura Vario stent (Devon, Germany) after serial dilations with 1.5×20 mm and 2.5×20 mm balloons (Maverick, Boston Scientific/Scimed, France) resulting in TIMI-III flow. Following this, the RCA was intubated with a 7 F left Amplatz 1 guiding catheter (Medtronic Vascular, Ireland) after failing to intubate it with a Judkin's right coronary catheter. Then the lesion was crossed with a Crosswire (Terumo Corporation, Japan). As no balloon could be passed over it, so a 0.014" floppy Rotawire was passed across the lesion and rotational ablation was done using a 1.25 mm burr. Following rotational ablation, TIMI-II flow was established with multiple lesions seen in mid and distal RCA. Hence, dilation was started from distal RCA. However, following dilation of the distal RCA with a 2.5 mm balloon, it was realized that the balloon which was passed over the guidewire, presuming it to be lying in distal RCA, was actually lying in a small branch of the RCA. Following dilation with a 2.5 mm balloon, the artery perforated, with contrast leaking freely into the pericardium (Fig.1).

Activated clotting time (ACT) at this point of time was > 300 s. After reversing anticoagulation by giving protamine at rate of 1 mg/100 units of heparin, we tried to seal the perforation by prolonged balloon inflation with a 2x9 mm balloon (Maverick, Boston Scientific/Scimed, France).
However, since there was persistent leak, we decided to seal the perforation by coil embolization. Instead of using conventional metallic coils, we thought of occluding the artery with pieces of thrombogenic floppy tips of used PTCA guidewires sterilized in ethylene oxide. An over-the-wire Ranger balloon 2.5×10 mm (Boston Scientific, USA) was passed on the wire lying in the leaking artery. Then the guidewire was withdrawn and through the central lumen of the balloon, two pieces of floppy tips of used PTCA wires (each about 1 cm long) were pushed into the target artery using the non-floppy end of an exchange-length PTCA wire. After placing two pieces of wire in the artery, check angiogram after 15 min showed complete occlusion of the leaking artery (Fig. 2). Following this, successful angioplasty and stenting of the RCA was done establishing TIMI-III flow in the artery.

Fig. 2. Arrow showing floppy tips of used angioplasty wires sealing the perforation.

reversal of anticoagulation, embolization of gel foam or thrombogenic metallic (stainless steel/platinum) coils into the leaking vessels. But delivery of metallic coils not only requires an additional system for delivering the coils, it also adds to the cost of the procedure. Our method of using cut floppy tips of used PTCA guidewires can be considered as an alternative to metallic coils for sealing perforations arising from small vessels. The advantages of our procedure are (i) easy availability of the material (used PTCA guidewires) needed for sealing the perforation in the catheterization laboratory (ii) no requirement of a special delivery system (iii) cost effectiveness and (iv) simplicity.

Fig. 1. Arrow showing site of perforation through small ventricular branch of right coronary artery.

Discussion

Coronary perforation following PTCA is a dreadful complication. The therapeutic approach to these perforations includes initial stabilization of the patient and addressing any compromise from cardiac tamponade. This is followed by determination of the vessel size at the site of the perforation. The choice of therapy for larger vessels has traditionally been emergency surgery with suture ligation of the perforation and usually coronary artery bypass surgery. Polytetrafluoroethylene-coated stent and autologous vein-covered stents have also been reported to be efficacious for large proximal vessels provided they can be delivered. The different methods described for small distal vessel perforation include prolonged balloon inflation and

References

**Calcified Ductal Aneurysm with Severe Aortic Regurgitation**

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A rare case of calcified aneurysm of the ductus arteriosus with severe aortic regurgitation is presented. We believe this is the first report of such a case in the English literature. (Indian Heart J 2005; 57: 172–174)

**Key Words:** Aortic regurgitation, Ductal aneurysm, Congenital heart disease

Ductus arteriosus aneurysm is a rare condition that can be associated with serious complications including thromboembolism, rupture and death. To the best of our knowledge the combination of severe aortic regurgitation (AR) and patent ductus arteriosus (PDA) aneurysm has not been reported. We report such a case in an adult who was successfully treated at our institution.

**Case Report**

A 27-year-old man presented with history of palpitations for 20 years, and dyspnea on exertion (New York Heart Association class II) of 2 years duration. He had no history of chest pain, fatigue or syncope.

On examination he was well-built, with no pallor, cyanosis or pedal edema. The jugular venous pressure was within normal limits. Blood pressure was 130/40 mmHg supine. An early diastolic decrescendo murmur was audible in the left parasternal area.

A plain chest X-ray revealed an enlarged heart, prominent aorta and dilated main pulmonary artery (PA) with increased hilar vascularity. A calcified shadow was observed in the region of the ductus (Fig. 1). A trans-thoracic echocardiogram (TTE) showed a dilated and hypertrophied left ventricle (LV) with severe AR. Mobile vegetations were seen on the aortic valve. There was a PDA with a large aneurysm at its aortic end. The branch pulmonary arteries were normal. The estimated PA systolic pressure was 40 mmHg.

A multi-slice computerized tomographic (CT) angiogram revealed a large patent ductus with a calcified aneurysm measuring 5 x 4.3 x 3.6 cm causing splaying and compression of the right and left pulmonary arteries. No thrombus was seen in the aneurysm (Fig. 2).

Cardiac catheterization and angiography was deferred in view of mobile vegetations on the aortic valve and the clarity of the anatomy obtained on CT angiogram. One stage repair of both lesions through mid-sternotomy was planned.

**Fig. 1.** Plain chest radiograph, left lateral view showing calcification in the middle mediastinum, below the aortic arch in the region of the ductus.

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Operative technique: At surgery, the findings included a mildly dilated aortic root and a tense dilated PA which was pushed anteriorly by the calcified duct aneurysm. The entire wall of the ductus was calcified except for a rim of 5 mm at the PA end. The branch pulmonary arteries were normal in caliber, but stretched across the aneurysm. The ductus was dissected and looped around the pulmonary end. The patient was placed on cardiopulmonary bypass with ascending aortic and two-stage venous cannulation. The ductus was then carefully snugged and core cooling to 18°C was commenced. At 32°C the aorta was clamped, a transverse aortotomy was done and coronary ostial cardioplegia administered. The aortic valve was tricuspid and there were healed mobile vegetations on its right and non-coronary cusps. Aortic valve replacement was done using 21M St Jude Regent valve. The aortotomy was closed and root cardioplegia administered as core temperature reached 18°C. Circulatory arrest was established. The ductus was then transsected at the pulmonary end and the pulmonary artery bifurcation was dissected free from the anterior wall of the aneurysm. The aneurysm was then split open anteriorly to expose the aortic opening from within. Calcium-free margins of the aortic end were identified and these were closed with an oval piece of Meadox vascular graft (Boston Scientific, Meadox Medicals Inc., Oakland, NJ) measuring 6 x 4 cm using running 3/0 prolene suture. Before tightening the suture line, blood was returned to the patient through the arterial line and the aorta was de-aired. Cardiopulmonary bypass was resumed and re-warming started. The pulmonary artery was reconstructed using a pericardial patch, while re-warming was completed. The patient was successfully weaned off bypass on minimal inotropic support. Cardiopulmonary bypass time was 153 min, aortic cross clamp time was 76 min and circulatory arrest time was 25 min.

A post-operative CT angiogram revealed satisfactory exclusion of the aneurysm (Fig. 3).

Discussion

Surgical experience with aneurysm of the ductus arteriosus in adult is scant. Yoshitaka et al. reported the case of a giant aneurysm of the ductus arteriosus with severe mitral regurgitation in a 58-year-old adult, which was repaired in two stages utilizing circulatory arrest.
Aneurysm of the diverticulum of the ductus has been reported more frequently, with surgical closure being relatively more straightforward as only the narrow mouth of the diverticulum has to be tackled. Mitchell et al. reported successful management of 5 cases of aneurysm of diverticulum of the ductus in adult patients.

A calcified aneurysm of the ductus is a difficult entity to manage. The risks of thromboembolism and rupture are real although rare, and merit early intervention. The challenge in this case was that both lesions had to be managed simultaneously. The AR was significant enough to preclude prior management of the ductus aneurysm through a thoracotomy. The mid- sternotomy approach necessitated control of duct flow upon initiation of cardiopulmonary bypass. We were lucky to find a small area of non-calcified ductus at the pulmonary artery end which could be occluded at the onset of cardiopulmonary bypass.

The pre-operative CT angiogram clearly defined the lesion and helped us formulate a plan for management which was efficiently executed on the operating table. It defined the areas which were free of calcium, enabling us to get control of the ductus, and suture the patch. We believe this modality has certainly enhanced the accuracy of diagnosis and should play an increasing role in the management of such difficult patients in the future.

References
Leukotrienes and Atherosclerosis

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Developing drugs to treat atherosclerosis is a daunting task. Understanding the role of mediators involved in atherosclerosis could be of great value in managing as well as developing new strategies for the treatment of atherosclerosis. Recent studies of advanced human atherosclerotic lesions have yielded new information on potential mechanism of inflammation and immune response. Atherosclerosis is well recognized as an inflammatory disorder and proinflammatory role of leukotrienes has been postulated in its pathogenesis and progression. Lipid peroxidation, monocyte attraction, smooth muscle proliferation and foam cell formation are the various steps involved in atherogenesis. In this review we summarize our current understanding of the role of leukotrienes and their antagonists in the genesis of atherosclerosis.

Coronary Atherosclerosis

Coronary atherosclerosis is a multifactorial disease. It is now viewed as the outcome of hypercholesterolemia with inflammation of the vessel wall. The most likely sequence of events involved in atherosclerosis is vascular dysfunction or injury, monocyte recruitment and macrophage formation, lipid deposition and synthesis of extracellular matrix. Complex interactions among the resident endothelial cells, smooth muscle cells, the infiltrating monocytes and T lymphocytes determine the progression of fatty streaks into vascular lesions. Recent studies have shown that leukotrienes could influence the activities of all the cell types involved in this process. Leukotriene antagonists have also been tested to see their effect on progression of atherosclerosis in appropriate animal models. These studies suggest a previously unrecognized role of leukotrienes and their antagonists in the genesis of atherosclerosis.

Mammalian lipoxygenase have two principal functions, one is to modify membranes by peroxidation reactions by 12/15 lipoxygenase (12/15-LO), the other is to produce signaling lipid mediators which exert effects via G-protein coupled plasma membrane-bound receptors by way of 5-lipoxygenase (5-LO) and its product, the leukotrienes. Accordingly, lipoxygenases may in principle contribute to pathophysiology of atherosclerosis by oxidation of low-density lipoprotein (LDL) and also by production of proinflammatory leukotrienes.

Biosynthesis and Metabolism of Leukotrienes

Leukotrienes are a class of biologically active lipids, synthesized and released from leukocytes and have a variety of proinflammatory effects (Fig.1). The synthetic pathway for leukotrienes is initiated by the release of arachidonic acid from the cell membrane by phospholipase A2. This free arachidonic acid is sequestrated at the nuclear envelope and brought into contact with 5-LO by FLAP (5LO-activating protein). This free arachidonic acid is sequestrated at the nuclear envelope and brought into contact with 5-LO by FLAP. The enzyme LTA₄ hydrolase can convert the unstable compound LTA₄ to biologically active leukotriene LTB₄ or conjugated with reduced glutathione by action of LTC₄ synthase to LTC₄. Whereas LTD₄ is produced by removal of glutamic acid by an enzyme γ-glutamyl transpeptidase and LTE₄ results from subsequent cleavage of glycine by dipeptidase. LTC₄ and its metabolites are collectively known as cysteinyl leukotrienes.

Cellular sources of leukotrienes: In contrast to prostaglandins, which are generated by a wide variety of cells and tissues in response to injury and other noxious stimuli, leukotrienes are generated by restricted range of cell types. Leukotrienes are produced mainly by leukocytes and endothelial cells through transcellular synthesis. Phagocytic leukocytes, including neutrophils, monocytes and/or macrophages and eosinophils were thought to be the major effector cells for LTB₄, and smooth muscle cells for cysteinyl leukotrienes. However, recent discovery of additional receptors and careful expression analysis have resulted in considerable expression of known cellular...
targets for leukotrienes. Various human cells produce specific leukotrienes, the predominant leukotrienes synthesized by human cells are given in Table 1.

**Table 1. Production of leukotrienes from different human cells**

<table>
<thead>
<tr>
<th>Human cells</th>
<th>Leukotrienes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>LTB₄</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>LTC₄, LTC₅</td>
</tr>
<tr>
<td>Monocytes</td>
<td>LTB₄, LTB₅</td>
</tr>
<tr>
<td>Macrophage</td>
<td>LTC₄, LTC₅</td>
</tr>
<tr>
<td>Mast cell</td>
<td>LTC₄</td>
</tr>
<tr>
<td>Basophils</td>
<td>LTC₄</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>LTC₄, LTC₅, LTD₄, LTE₄</td>
</tr>
<tr>
<td>Smooth muscle cells</td>
<td>LTC₄, LTC₅, LTD₄, LTE₄</td>
</tr>
</tbody>
</table>

**Leukotriene receptors:** Receptors have been identified for both LTB₄ and cysteinyl leukotrienes. Pharmacological characterization identified at least two subtypes of cysteinyl leukotrienes (Cyst) receptors based on the agonist and antagonist potency for biological responses. Molecular identification of the human and mouse Cyst LT₁ and Cyst LT₂ receptor has confirmed their structure as putative 7-membrane domain G-protein coupled receptors. Currently known LTB₄ receptors are G-protein coupled receptors. BLT1, BLT2 and the peroxisome proliferator activator receptor α (PPARα) are the receptors for LTB₄. While BLT1 and BLT2 likely mediate the pro-inflammatory response of LTB₄, PPARα-a transcription factor-might serve as a mediator with the anti-inflammatory effects of LTB₄. The rank potency of leukotriene receptors for activation of agonist and sites of expression are shown in Table 2.

**Table 2. Sites of expression and rank potency for activation of leukotriene receptors by agonists**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Agonist</th>
<th>Site of expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyst LT₁</td>
<td>LTD₄ &gt; LTC₅ &gt;LTE₄</td>
<td>Spleen, peripheral blood leukocytes, eosinophils, lung smooth muscle cells and interstitial lung macrophages³ fifteen</td>
</tr>
<tr>
<td>Cyst LT₂</td>
<td>LTC₄ =LTD₄&gt;LTE₄</td>
<td>Heart, adrenal medulla, placenta and peripheral blood leukocytes, smooth muscle cells and macrophages⁹,10</td>
</tr>
<tr>
<td>BLT1</td>
<td>LTB₄</td>
<td>Neutrophils, spleen and thymus⁹</td>
</tr>
<tr>
<td>BLT2</td>
<td>LTB₄</td>
<td>Spleen, leukocytes, liver, ovary, pancreas, heart, testis, small intestine, kidney, lung, thymus, colon and placenta⁹</td>
</tr>
</tbody>
</table>

**Role of Leukotrienes in Atherosclerosis**

Before understanding the role of leukotrienes in the pathogenesis of atherosclerosis we must look briefly into the various studies conducted to establish the role of leukotrienes in atherosclerosis, summary of which is given in Table 3.

Many studies conducted on preclinical models of atherosclerosis have shown reduction in progression by varied mechanism. Aiello et al. used inbred strain of LDLR⁻/⁻ (LDL knockout) mice, MCP-1⁻/⁻ (monocyte chemoattractant protein-1 null) mice and apoE⁻/⁻ (apolipoprotein E deficient) mice. These mice were treated with a specific LTB₄ receptor antagonist, CP-105, 696, for 35 days. When compared with age-matched controls, lipid accumulation and monocyte infiltration were significantly reduced in apoE⁻/⁻ mice at all time points tested. Lesion area reduction was also demonstrated in LDLR⁻/⁻ mice maintained on high-fat diet. LTB₄ antagonism had no significant effect on lesion size in mice possessing the null alleles for another chemotactic agent MCP-1 (MCP-1⁻/⁻ X apoE⁻/⁻), suggesting MCP-1 and LTB₄ may either interact or exert their effect by a common mechanism. Another study on murine sepsis model by Matsukava et al. showed a decreased MCP-1 production upon treatment with LTB₄ receptor antagonist and decreased LTB₄ production in mice treated with a MCP-1 blocking antibody. It is further reported that MCP-1 is the prototype of the CC-chemokine β subfamily and exhibits the most potent chemotactic activity for monocytes. MCP-1 is highly expressed in human atheromatous plaque and its overexpression contributes to the development of atherosclerosis in mouse models. Similar results were found in a study conducted by Porreca et al. on rat vascular smooth muscle cell (VSMC) which provides direct evidence that exogenously added 5-LO metabolite i.e. LTD₄ enhanced IL-1β production in rat VSMC and MK-571, a specific receptor antagonist of LTD₄, antagonizes this effect and strongly inhibits mRNA expression. An experimental drug MK-886 binds to FLAP and blocks production of leukotrienes. De Code team showed that almost 30% of 779 heart attack patients in Iceland and 15% of 753 British heart attack patients had alterations in a gene called ALOX5AP that cause their arteries to produce unusually high levels of the bad leukotrienes. A gene nucleotide polymorphism (SNP) haplotype in this locus spanning the gene ALOX5AP encoding 5-lipoxygenase activating protein (FLAP - an integral membrane protein essential for leukotriene production) is associated with a two times greater risk of myocardial infarction in Iceland. This haplotype also confers almost two times greater risk of stroke. Another ALOX5AP haplotype is associated with myocardial infarction in individuals from the UK. Stimulated neutrophils from individuals with myocardial infarction produce more leukotriene B₄, a key product in the 5-lipoxygenase pathways, than do neutrophils from...
controls, and this difference is largely attributed to cells from males who carry the at-risk haplotype. This suggested that variants of ALOX5AP are involved in the pathogenesis of both myocardial infarction and stroke by increasing leukotriene production and inflammation in the arterial wall predisposing to myocardial infarction.26 De Code mapped the gene to a locus on chromosome 13q12-13. GSK 480848 gene-based drug hits inflammation-producing enzyme in arteries, Lp-PLA2.

These results demonstrate that in preclinical model of atherosclerosis, leukotriene receptor blockade reduces lesion progression and further suggest a previously unrecognized role for leukotrienes or other oxidized lipids in the pathogenesis of this disease.

From these independent studies we have outlined the function of leukotrienes in the development and progression of atherosclerosis. Mammalian lipoxygenases have two principal functions: producing signaling lipid mediators by 5-lipoxygenase, and modification of membranes by 12/15 lipoxygenase through peroxidation reactions which is involved in the step of oxidation of LDL.72

The role of these two lipoxygenase enzymes is so interlinked with each other that it is difficult to demarcate their separate role in atherosclerosis. ALOX5AP gene located on to a locus on chromosome 13q12-13, a four-marker single-nucleotide polymorphism (SNP) haplotype encoding 5-lipoxygenase activating protein (FLAP), is involved in the pathogenesis of atherosclerosis by increasing leukotriene production and inflammation in the arterial wall predisposing to myocardial infarction.

### Table 3. Summary of the studies conducted to establish role of leukotrienes in atherosclerosis

<table>
<thead>
<tr>
<th>Material and Method</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mehrbanian et al.16 5-LO-/-/LDLR-/- double knockout mice were developed, aortic atherosclerotic lesion were analyzed in mice fed on high fat/cholesterol diet.</td>
<td>These mice showed a dramatic decrease in lesion development. Transplant of bone marrow from 5-LO-/- mice LDLR-/- led to significant reduction in atherogenesis suggesting the role of 5-LO in atherosclerosis.</td>
</tr>
<tr>
<td>Spanbroek et al.17 Study was conducted on coronary, carotid arteries and aorta (obtained from 50 patients), The number of 5-LO+ macrophage foam cells and 5-LO+DC foam cells were counted.</td>
<td>The number was found to be high in samples from advanced coronary heart disease, presence of 5LO-activating protein FLAP, LTA4 hydrolase, and LTC4 synthase in human atherosclerotic vessel wall was seen, confirming the concept that 5-LO cascade generates circuits of inflammation during critical stages of atherogenesis.</td>
</tr>
<tr>
<td>Dwyer et al.18 Degree of atherosclerosis was judged by estimating carotid intima media thickness bilaterally in a randomly sampled cohort of 573 women and men. Dietary arachidonic acid and marine n-3 fatty acid were measured with the use of 6, 24-hour recalls of food intake.</td>
<td>Variant 5-LO genotype showed large increase in intima-media thickness with intake of arachidonic acid while significantly inverse association was seen with marine n-3 fatty acids. Arachidonic acid is primary substrate for 5-LO which enhances production of leukotrienes, while marine n-3 fatty blunts this effect by competing with eicosapentanoic acid.</td>
</tr>
<tr>
<td>Harats et al.19 LDLR-/- and 15-LO overexpressing crossed mice, fed high cholesterol/fat diet, quantification of fatty streaks was performed at aortic sinus.</td>
<td>LDLR-/-15-LO mice developed large lesions than LDLR-/- mice supporting the hypothesis that 15-LO over expression in the vessel wall is associated with enhanced atherogenesis.</td>
</tr>
<tr>
<td>Subbarao et al.12 Cryosections from aortic tree were analyzed for atherosclerotic lesion in BLT1 null mice crossed with apo E-/- mice, also LTB4+ induced gene expressions were determined in a rat basophilic leukemia cell line.</td>
<td>Deletion of BLT1 significantly reduced lesion formation in apoE-/- mice, LTB4+ induced up regulation of 17 genes with well established association in atherosclerosis.</td>
</tr>
<tr>
<td>Allen et al.20 Response of non atherosclerotic and atherosclerotic coronary artery ring segment to LTC4 and LTD4 was seen in organ bath.</td>
<td>Non-atherosclerotic segment were unresponsive to LTC4 and LTD4, atherosclerotic segment showed concentration-dependent contractions suggesting that atherosclerosis augments contractions to cysteinyl leukotrienes and contributes to hyperreactivity of vessels.</td>
</tr>
</tbody>
</table>

5-LO-/-: 5-lipoxygenase null; LDLR-/-: low-density lipoprotein receptor knockout; DC: dendritic cell; FLAP: 5-LO-activating protein; apo E-/-: apo-E deficient; LT: leukotriene
in the vessel wall, increase vascular permeability or both. These mechanisms may create a vicious circle in which inflammatory cells, by producing these lipid mediators, cause local vascular inflammation, perpetuating the recruitment of inflammatory cells and the production of mediators. This has been further substantiated by use of LTD₄ antagonist in pre-clinical models, which inhibited the progression of atherosclerosis.²²,²⁸ LTA₄ is the first product of the 5-LO pathway; this unstable compound sits at the crossroads of this pathway.⁸ It is released from macrophages/DCs/foam cells/mast cells and the subsequent uptake and conversion to other leukotrienes by neighboring cell may provide mechanism for augmentation of transcellular leukotriene synthesis.¹⁷ The LO enzymes and its products may influence all the major steps involved in process of atherosclerosis (Fig. 1).

**Oxidation of LDL:** 12,15-lipoxygenase has unique ability to oxidize fatty acid esterified to membranes and low-density lipoprotein (LDL) without requiring phosphorylase.²¹ This lipid peroxidation in turn produces isoleukotrienes. The oxidized LDL has been shown to increase 5-lipoxygenase activity.²⁹ Further reduction in atheroma lesion was seen in mice fed on high fat diet when treated with LTB₄ antagonist.²²

**Uptake of LDL by monocytes:** Formation of foam cells is mediated by a gene which is upregulated by LTB₄, fatty acid translocase/CD36, a transmembrane protein and a specific receptor for oxidized LDL that transports cholesterol into monocytes thereby converting them to foam cells.³⁰ When treated with LTB₄ antagonist apoE⁻/⁻ mice were demonstrated to accumulate less fat in monocytes.²²

**Chemoattraction of monocytes:** LTB₄ promotes atherosclerosis via two receptors - BLT1 and BLT2 mediate chemotaxis, leukocyte adhesion by upregulating gene CCL2 which presumably is produced at the site of vascular lesion and attracts monocyte to the area.³⁰ LTB₄ also interacts with MCP-1 by increasing expression of MCP-1 mRNA which is antagonized by LTB₄ antagonist.²²

Other upregulated genes are urokinase plasminogen activator (uPA), colony stimulating factor (CSF-1) and osteopontin, which are essential for development of atherosclerosis. CD44, sarc-like adaptor protein (SLAP) and protein tyrosine phosphatase (SHP-1) are among the other genes, which are also upregulated; they may have important function in leukocyte recruitment and signal transduction.²⁰

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**Fig. 1.** The key enzymatic and biochemical steps involved in leukotrienes synthesis pathway and important steps mediated by different leukotrienes in atherosclerosis.

PLA₂: phospholipase A₂; 5-LO: 5-lipoxygenase; LT: leukotriene; FLAP: 5-lipoxygenase activating protein; 5-HPETE: 5S-hydroxyperoxy-6, 8-trans-11, 14-cis-eicosatetraenoic acid; VSMC: vascular smooth muscle cell; LDL: low-density lipoprotein; IL: interleukine; PAF: platelet-activating factor
The concept of fighting heart disease with asthma drugs is still in its infancy. It will take several years to confirm the various gene findings and to test whether leukotriene-blockers work on human heart disease. Currently marketed drugs may not work because they do not stop all types of leukotrienes; it may be necessary to use broader-acting leukotriene-blockers to help heart patients. It is of interest to consider the physiological role and pharmacological strategies to develop the anti-leukotriene drugs in prevention of atherogenesis.

Acknowledgement

The authors thank Monika Agarwal for rendering her help in preparation of this manuscript.

References

Multiple Coronary and Aortic Aneurysms in a Young Female: A Diagnostic Dilemma

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An 18-year-old female presented with classical angina pectoris and breathlessness on exertion of four months duration. On examination she was afebrile, pulse rate was 82 beats per min, regular, and all peripheral pulsations were equally well felt, with no bruit. Blood pressure (BP) in right upper limb was 120/70 mmHg, with no significant difference in the BP in the other limbs. There was no skin lesion, cervical lymphadenopathy, xanthomas or xanthelasma. Cardiovascular, and other system examinations were normal. Complete blood count was normal, and ESR was 5 mm at the end of first hour. ASLO, CRP, ANA, RA factor, and VDRL were negative. Liver and kidney function tests, lipid profile, electrocardiogram (ECG)
at rest, chest X-ray and fundus were normal. Two-
dimensional echocardiography revealed aneurysmal left
main coronary artery (LMCA) and grade 1 aortic
regurgitation. Cardiac stress test (CST) was positive at
moderate workload of 6 METs with 3 mm ST depression in
anterior leads, which persisted for 5 min into recovery.
Angiographic evaluation revealed an aneurysm of the
LMCA and 90% stenosis at the origin of the left anterior
descending artery (LAD) (Fig. 1). The left circumflex (LCx)
artery was normal. There was a large aneurysm involving
the right coronary sinus with a 90% ostial stenosis of the
right coronary artery (RCA). Aortic root angiogram
revealed grade 1 aortic regurgitation (Fig. 2). Arch
aortogram revealed the origin of all great vessels to be
normal. The descending aorta showed two saccular
aneurysms while there was a fusiform aneurysm in the
suprarenal aorta (Figs 3 and 4). Pulmonary angiogram was
normal. The patient was advised coronary artery bypass
graft surgery.

Most discrete coronary artery aneurysms are
atherosclerotic in origin; other causes include Kawasaki
disease (KD), Takayasu’s arteritis [non-specific aortoarteritis
(NSAA)], polyarteritis nodosa, and systemic lupus
erythematosus. The age of presentation of this patient was in
favor of NSAA. Non-involvement of the subclavian vessels
ruled out type 1 and type 3 NSAA but combined type 2
(thoracoabdominal type) and type 5 (coronary) NSAA was a
strong possibility. Stenotic lesions most commonly involve
the aorta and its branches. Pure aneurysmal form of the
disease is rare and occurs in only 8.6% cases. Coronary
involvement occurs in 20% cases of NSAA in India. Stenotic
lesions are commoner and usually involve the ostia and
proximal 2-3 cm of the artery. Coronary aneurysms are rare
and only few isolated case reports are available.

Coronary involvement occurs in 15-25% cases of KD. Aneurysms change dynamically with time with over 65%
showing resolution in 1-2 years. Rarely, aneurysms persist
or stenosis develops (due to myointimal proliferation).
Aneurysms outside the coronary system occur in up to 2%
of cases and involve the axillary, subclavian, common iliac,
internal iliac, femoral, pancreatic, paraovarian, paratesticular and splenic arteries. They usually occur in
patients with coronary aneurysms and likewise show regression in majority of cases. However, these may
progress to stenotic lesions although ischemic symptoms
are rare. However, involvement of the aorta was strongly
against the diagnosis of KD, as large-sized arteries are not
affected in KD.

Intravascular ultrasound (IVUS) may be helpful to
differentiate NSAA and KD. IVUS in KD with regressed
aneurysm typically shows marked intimal thickening
and lumen size similar to that at sites near the regressed
aneurysm that has an angiographically normal coronary
artery (due to extensive medial smooth cell proliferation).
At sites of persistent aneurysms IVUS reveals marked
intimal thickening with calcification, but lacks increased
echogenicity in the wall or lumen (helps in differentiation
from atherosclerotic disease where, apart from intimal
thickening, increased echogenicity due to plaques or
calcification may be seen). Some intimal thickening may
occur in the vicinity of the healed aneurysm, which appears
normal on angiography. However, sites in angiographically
normal artery away from the coronary aneurysm
(persistent or regressed) demonstrate normal artery
structure. On the contrary, Suzuki et al. reported that sites
distant from the aneurysm site (persistent or healed) might
also reveal intimal involvement. Both the groups showed
that such sites show impaired vasodilatory responses to
vasodilators. Thus KD not only causes structural but also
stenotic lesions which may later complicate functional
changes at these sites. IVUS in NSAA typically shows
thickening and altered echogenicity of media, adventitia
and periarterial tissues and loss of aortic wall pliability in
stenosed segments. The most striking feature, however, is
that though angiogram reveals skip lesions, IVUS shows
that these areas are also affected by disease process. No
functional abnormalities, as seen in KD, occur in NSAA.

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Infra-Hisian Mobitz Type I/Wenckebach Block

A 65-year-old lady presented with several episodes of syncope. Clinical examination revealed occasionally missing pulse and blood pressure was 150/80 mmHg. There was no murmur, gallop or evidence of heart failure. Routine hematological and biochemical examinations were within normal limits. The electrocardiogram (ECG) showed presence of right bundle branch block with left anterior hemiblock. In addition, the PR interval was prolonged with varying Wenckebach block and 3:2 and 4:3 conduction ratios. The heart size on chest X-ray was normal, so was the echocardiographic examination. The patient was subjected to electrophysiological studies to ascertain the level of block. In the baseline study, surface ECG showed 4:3 Wenckebach block, the PR interval in the first beat was 240 ms, 340 ms in second beat and 380 ms in the third beat. The fourth P wave was not followed by QRS complex. Simultaneous His bundle recording showed AH interval of 120 ms (normal 54-130 ms), H wave of 20 ms whereas the HV interval (normal 31-55 ms) showed typical Wenckebach periodicity; 100 ms in the first beat, 200 ms in second beat and 240 ms in the third beat. The fourth H wave was not followed by a V wave (Fig. 1). Thus, the block was found to be at the level below the His bundle. Provocative maneuvers were not undertaken because the baseline measurements showed markedly prolonged HV intervals. Pacing from the right ventricle apex did not show retrograde ventriculoatrial conduction. The patient was advised to undergo permanent pacemaker implantation and a VVI pacemaker was implanted later on.

Mobitz Type I or Wenckebach AV block was first described by Wenckebach long before the invention of the ECG. He described it as a form of conduction disturbance characterized by a progressive lengthening of the interval between the ‘a’ and the ‘c’ waves of the jugular pulse, terminating in a dropped ‘c’ wave. After this, the ECG features were characterized by Mobitz as progressive prolongation of the PR interval until there was a dropped ventricular beat. The phenomenon is usually repetitive. Wenckebach and Winterberg further characterized this phenomenon by describing other characteristics, including: (i) Progressive lengthening of the PR interval, (ii) The increment between the first and the second conducted beats being the largest, (iii) Progressive decrease in the R-R intervals because of decrease in the PR increment, and (iv) A short ventricular pause produced by the non-conducted P wave that is equal to the difference between the last PR interval and the first PR interval subtracted from twice the PP interval (during an electrophysiologic study, this translates into an increase in the AH interval, which parallels the PR prolongation).

The term ‘typical Wenckebach periodicity’ has been applied to second-degree block that shows all of these characteristics. When P wave activity is not clearly discernible on the surface ECG, Wenckebach periodicity can be recognized because of one of its after-effects, resultant ‘group beating’. The regular appearance of grouped QRS complexes has been called ‘the footprints of Wenckebach’. However, it is now generally recognized that in clinical situations, all of the features are found in perhaps fewer than 50% of cases. Mobitz Type I or Wenckebach block usually occurs at the atrioventricular node. Infra-Hisian Wenckebach block is rare and there are only anecdotal case reports. When the phenomenon occurs with narrow QRS complexes in surface ECG, the site is more likely to be above the His bundle. With wide QRS complexes, the site is more likely to be infra-Hisian. Signal-averaged ECG is another technique, which can distinguish between the two types. Otherwise, invasive electrophysiologic study is the method to differentiate the two varieties.

The present case further strengthens the concept that Wenckebach type block in surface ECG in presence of fascicular block is likely due to block in infra-Hisian location.

Fig. 1. The upper panel shows surface electrocardiogram. There is 4:3 Wenckebach block; the PR interval being 240 ms in 1st beat, 340 ms in 2nd and 380 ms in the 3rd beat. A QRS complex does not follow the fourth P wave. The lower two panels show His bundle recording; the AH interval is 120 ms and the H wave is 20 ms. The HV interval is 100 ms in 1st beat, 200 ms in 2nd and 240 ms in the 3rd beat. The fourth H wave is not followed by a V-wave. The recording speed is 50 mm/s.
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Is it Possible to Prevent Rheumatic Fever

I must congratulate R Tandon for such an important topic and also giving practical advice. It is always good to hear from such senior people with vast experience about such a common diseases as rheumatic fever (RF) and rheumatic heart disease (RHD).

I would like to mention briefly my observation from my study of RF and RHD in school children aged 5-15 years in Alleppey district of Kerala. We covered 28,359 students in 23 schools. This study was started in 1966.

Primordial prevention is a difficult task and good result depends upon educating teachers of the school and also educating the parents. Improvement in socioeconomic status plays a major role in reducing the incidence of RF and RHD.

Primary prevention of identifying Group A beta-hemolytic streptococci (GABHS) and prompt and adequate treatment is also difficult. Most of the sore throats are not due to GABHS. The results I got in my series were good due to many reasons. Besides educating teachers and parents, the doctors (of Primary Health Centre and Taluk and District Hospitals) were also actively involved. Health care delivery system is much better in Kerala and 95% of children are school-going. Literacy rate is almost 100%. Besides, all patients receive treatment free in the hospital.

Secondary prevention is very important. Injection benzathine penicillin 1.2 mega units should be given once in 3 weeks. Oral penicillin is not as effective as injection and recurrence of RF is possible. All our patients received injections free in the hospital. The anaphylactic reaction is very rare. Lately, doctors are afraid to give injection penicillin. There are other reasons also like consumer awareness, legal problems etc. This attitude of medical practioners must change in favour of benzathine penicillin.

My advice in that injection benzathine penicillin 1.2 mega units should be given once in 3 weeks taking all precautions. We could bring down incidence of RF and RHD from 8/1000 to 2/1000 in 15 yearstime in Alleppey during our study (1966 to 1981). Prophylaxis should be continued in RHD throughout life. Exception may be made depending upon age and presence of risk factors. In RF, prophylaxis should be continued till 20 years.

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Reply

I would like to thank DV Nair for his comments since his letter emphasizes the main purpose of my write-up, "Is it possible to prevent rheumatic fever (RF)?"

Under active surveillance it is possible to reduce the burden of RF/rheumatic heart disease (RHD). However, it is not possible to eliminate the problem of RF/RHD. Nair points out that inspite of 100% literacy and 95% children going to school, even with active surveillance, after 15 years, the prevalence was still 2/1000 but not absent.

It is essential that three-weekly injectable penicillin is used. Oral penicillin may not be able to prevent RF.

As far as secondary prevention is concerned, it is equal to "closing the barn door, after the horse has escaped". It is ethically essential and mandatory to prevent recurrences and thus subsequent damage and worsening of RHD. Secondary prevention will not reduce the total burden of RHD.

Primary prevention will be feasible only with vaccine, when it becomes available.

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Thrombolytic Therapy for Acute Myocardial Infarction in Acute Lymphoblastic Leukemia

Acute lymphoblastic leukemia (ALL) is an uncommon malignant hematopoietic disorder in adults. Commonly used chemotherapeutic drugs to treat this leukemia consist of combination of anthracyclines (daunorubicin, doxorubicin), cytarabine, cyclophosphamide, vincristine and steroids. Anthracyclines are associated with acute toxicity in the form of atrial and ventricular arrhythmias, but these are rarely of clinical significance. In contrast, cumulative doses of doxorubicin are associated with a 10% increase in incidence of chronic cardiomyopathy.1 Another drug used in the treatment of ALL, L-asparaginase can predispose to thrombosis by causing clotting factor abnormalities. Though patients with ALL are not immune to have lifestyle-related diseases like diabetes, hypertension and coronary artery disease, the management issues are complex when a patient of ALL develops acute myocardial infarction (MI). Acute MI has been reported rarely among patients of ALL usually during therapy with L-asparaginase.2 The safety and efficacy of thrombolysis as a treatment modality for acute MI developing in such a setting is not known. We present a patient of ALL who did not receive L-asparaginase and who, in remission, developed acute MI and was successfully thrombolysed with streptokinase.

A 44-year-old man presented to us with epistaxis 4 years ago. On further investigations he was found to have pancytopenia and peripheral blood film was showing blast cells. Bone marrow examination confirmed the diagnosis of ALL. He was treated with modified German ALL protocol consisting of a combination of doxorubicin, vincristine, cytarabine and steroids.3 He was not given L-asparaginase as he developed allergic reaction to it. During intensive phase of chemotherapy lasting for 9 months, he received a total of 180 mg of daunorubicin and 160 mg of doxorubicin. He completed his maintenance chemotherapy and his last bone marrow examination was normal. The patient remained asymptomatic for next three months. Then, he presented to our emergency department with acute chest pain lasting for half an hour. Electrocardiogram (ECG) revealed changes in acute inferior wall MI. Although the patient was in remission for ALL, there was a doubt in the mind of treating clinicians regarding the safety of thrombolysis in such patients. The dilemma was further compounded due to paucity of literature on this issue. After discussion with relatives of patient, the patient was thrombolysed with injection streptokinase in the dosage of 1.5 million units over one hour. Post thrombolysis, his pulse and blood pressure remained stable. Repeat ECG was normal indicating successful thrombolysis. He was discharged from hospital on day 7. Echocardiography done before discharge showed an ejection fraction of 55%. A stress test done at 6 weeks was positive for inducible ischemia. A follow-up echocardiography done at 3 months post-MI showed a normal left ventricular ejection fraction and no regional wall motion abnormality. His hemogram did not show any evidence of relapse. In conclusion, it can be stated that if patient of ALL in remission develops acute MI, he can be treated on the same lines as non-leukemia patients and thrombolysis with streptokinase is a safe option in such patients.

References

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The Effect of Cardiac Resynchronization on Morbidity and Mortality in Heart Failure


Summary
The Cardiac Resynchronization Heart Failure (CARE - HF) study was a randomized multicenter trial undertaken with the aim to assess effects of cardiac resynchronization therapy (CRT) (without a defibrillator) on morbidity and mortality in patients with symptomatic heart failure who had objective evidence of dyssynchrony. Patients with NYHA class III or IV heart failure due to left ventricular (LV) systolic dysfunction and cardiac dyssynchrony who were receiving standard pharmacologic therapy were randomly assigned to receive medical therapy alone or with CRT. Inclusion criteria were age ≥18 years, heart failure for at least six weeks, and in NYHA class III or IV despite receiving standard pharmacologic therapy, with an LV ejection fraction (EF) < 35%, an LV end-diastolic dimension of at least 30 mm (indexed to height), and a QRS interval of at least 120 ms. Patients with heart failure requiring continuous intravenous therapy were excluded. Those who had had a recent major cardiovascular event, and those who had conventional indications for a pacemaker or an implantable defibrillator were also not included. Device used was Medtronic InSync or Insync III and operators were specifically asked to position the LV lead to pace the lateral or posterolateral LV wall transeptally. The primary end point was the composite of death from any cause or an unplanned hospitalization for a major cardiovascular event that included worsening heart failure, acute coronary syndrome, stroke or arrhythmias. The principal secondary end point was death from any cause and also included NYHA class, quality of life and echocardiographic parameters. A total of 813 patients were enrolled, 404 patients were assigned to receive medical therapy alone and 409 to receive medical therapy plus CRT and followed for a mean period of 29.4 months ranging from 18 to 44.7 months. The primary end point was reached by 159 patients in the CRT group, as compared with 224 patients in controls (39% v. 55%, hazard ratio, 0.63; p<0.001). There were 82 deaths in the CRT group, as compared with 120 in the medical-therapy group (20% v. 30%, hazard ratio 0.64; p<0.002). The mode of death was sudden in 32% of patients in the medical group and 35% in the CRT sub group died suddenly. As compared with medical therapy alone, CRT reduced the interventricular mechanical delay, the end-systolic volume index and the area of the mitral regurgitant jet; increased the LVEF; and improved symptoms and the quality of life (p<0.01 for all comparisons). The most common adverse device-related events in CRT group were lead displacement, coronary sinus dissection, pocket erosion, pneumothorax and infection.

Comments
Several studies in the recent past have consistently shown improvement in the LV function manifesting in decreased symptoms, improved quality of life and increased exercise capacity with CRT. However, there is no clear evidence about the effect of CRT on mortality. The recent COMPANIAN trial showed a reduction in the composite end point of death or hospitalization but there was no significant reduction in the risk of death. The present study showed that in patients with heart failure and cardiac dyssynchrony, CRT improves symptoms and the quality of life and reduces the risk of death. This is the first study that proves that CRT actually reduced the risk of death. Statistical analysis reveals that for every nine devices implanted, 1 death and 3 hospitalizations are avoided. These benefits are in addition to those afforded by standard pharmacologic therapy. The overall mortality rate in this study was relatively low compared to other studies in a similar cohort of heart failure patients. The authors attribute this to either the presence of relatively less severe heart failure or to the high standard of care and close follow-up. However, all patients were in at least NYHA class III. It makes one think that the risk may be modified to a greater extent among patients with less severe disease. Another interesting point is that the hazard ratio for death in those receiving CRT compared with medical therapy was similar to the ratio comparing the two groups in the COMPANIAN trial that evaluated CRT plus AICD. This prompted the authors to consider that CRT induced improved cardiac function, and may also reduce the risk of sudden death; however, till a trial compares directly the two arms it is not possible to prove the assumption. The fact remains that 29 patients died suddenly in the CRT arm in this study and a defibrillator would certainly have further reduced the risk of death. However, again only a trial specifically designed to address this issue would resolve it.
Effect of Glucose-Insulin-Potassium Infusion on Mortality in Patients with Acute ST-Segment Elevation Myocardial Infarction

The CREATE-ECLA Trial Group Investigators

JAMA 2005; 293: 437-446

Summary

The Clinical Trial of Reviparin and Metabolic Modulation in Acute Myocardial Infarction Treatment Evaluation (CREATE) group of investigators used a 2x2 partial factorial design comparing patients on reviparin versus placebo administered for 7 days (double-blind) and those on glucose-insulin-potassium (GIK) versus control (open) administered for 24 hours, in addition to usual care. The aim of the study was to find out if there is a therapeutic benefit of using high-dose GIK infusion for patients with ST-segment elevation myocardial infarction (STEMI). This randomized controlled trial was conducted in 470 centers worldwide among 20,201 patients with STEMI who presented within 12 hours of symptom onset. Patients were randomly assigned to receive either GIK (25% glucose, 50 U insulin/L, 80 meq/L of potassium, at a rate of 1.5 ml/kg per hour for 24 hours) plus usual care (n=10,091) or usual care alone (controls; n=10,110). Individuals with contraindications for GIK infusion i.e. type 1 diabetes, renal impairment or hyperkalemia were excluded. Primary outcome measure was mortality while secondary outcome measures included cardiac arrest, cardiogenic shock, or reinfarction at 30 days after randomization. The mean age of patients was 58.6 years, and evidence-based therapies for STEMI were commonly used. At 30 days, 976 control patients (9.7%) and 1004 GIK infusion patients (10.0%) died. There were no significant differences in the rates of cardiac arrest (1.5% in controls and 1.4% in GIK infusion), cardiogenic shock (6.3% v. 6.6%) or reinfarction (2.4% v. 2.3%). The rates of heart failure at 7 days after randomization were also similar between the groups (16.9% v. 17.1%). There were no significant differences in brady or tachyarrhythmia between two groups. The lack of benefit of GIK infusion on mortality was consistent in pre-specified subgroups, including in those with diabetes (17.7%) and without diabetes (82.3%) in those presenting with and without heart failure, in those presenting early and later after symptom onset, and in those receiving thrombolysis (74.1%) and not receiving thrombolytic therapy or undergoing primary percutaneous coronary intervention (9.1%). In this large, international randomized trial, high-dose GIK infusion had a neutral effect on mortality, cardiac arrest, and cardiogenic shock in patients with acute STEMI.

Comments

Glucose-insulin-potassium (GIK) therapy has survived decades of clinical practice without a definitive test of its safety and effectiveness. GIK solution was initially advocated for the treatment of acute myocardial infarction (AMI) as a polarizing agent to promote electrical stability and later as an agent to provide metabolic support. Exogenous insulin suppresses circulating levels and myocardial uptake of free fatty acids, which are toxic to the ischemic myocardium. Provision of high-dose glucose can improve the efficiency of myocardial energy production during acute ischemia by becoming the preferred fuel for the heart. Because intracellular levels of potassium are depleted during ischemia, provision of exogenous potassium increases levels within the myocyte, thereby raising the threshold for ventricular arrhythmias. Unfortunately, regardless of its scientific rationale and the positive results of small studies, the present study of over 20,000 patients with STEMI demonstrated that there was no benefit (or harm) of GIK. DIGAMI trial demonstrated in a randomized controlled trial in 600 diabetics that initial intensive metabolic control by insulin-glucose infusion for at least 24 hours followed by 3 months of subcutaneous insulin improved long-term outcome. The GIPS and polish trial found no significant benefit of GIK therapy in AMI. Though GIK per se showed no benefit in CREATE trial, tight metabolic control using intravenous insulin/glucose might improve outcome. Hence it is worthwhile to test tight glycemic-control hypothesis in patients with STEMI. This definitive trial, combined with a previous overview that showed only a modest potential benefit, answers the question beyond reasonable doubt: there is no benefit of GIK therapy. Societal resources would be better spent on evaluating other approaches in clinical trials and using other therapies in practice.
Effects of Reviparin, a Low-Molecular-Weight Heparin, on Mortality, Reinfarction and Strokes in Patients with Acute Myocardial Infarction Presenting with ST-Segment Elevation

The Clinical Trial of Reviparin and Metabolic Modulation in Acute Myocardial Infarction Treatment Evaluation (CREATE) study was a randomized, double-blind, placebo-controlled study of 15,570 patients from 341 hospitals in India and China with ST-segment elevation myocardial infarction (STEMI) or new left bundle branch block (LBBB), presenting within 12 hours of symptom onset. The primary objective of this trial was to evaluate the effects of reviparin, a low-molecular-weight heparin (LMWH), when initiated early (prior to or within 15 min of thrombolytic therapy) and given for 7 days in addition to usual therapy on the primary composite outcome of death, myocardial reinfarction, or strokes at 7 and 30 days. The primary composite outcome was significantly reduced from 854 (11.0%) of 7790 patients in the placebo group to 745 (9.6%) of 7780 in the reviparin group [hazard ratio (HR), 0.87; p=0.005] at 7 days. These benefits persisted at 30 days [1056 (13.6%) v. 921 (11.8%); HR, 0.87; p=0.001] with significant reductions in 30-day mortality [877 (11.3%) v. 766 (9.8%); p=0.005] and reinfarction [199 (2.6%) v. 154 (2.0%); p=0.01], and no significant differences in strokes [64 (0.8%) v. 80 (1.0%); p=0.19]. Reviparin treatment was significantly better at 7 days when it was initiated very early after symptom onset (<2 hours: 30/1000 events prevented; 2 to <4 hours: 21/1000 events prevented; 4 to <8 hours: 16/1000 events prevented; and ≥8 hours p=0.58; p=0.04 for trend). An increase occurred in life-threatening bleeding at 7 days with reviparin compared to placebo (0.2% v. 0.1%, respectively; p=0.07). The benefits of reviparin were independent of the type of reperfusion therapy (streptokinase, tissue plasminogen activator or primary angioplasty and was also beneficial in those not thrombolyzed). Thus, in patients with acute STEMI or new LBBB, reviparin reduces mortality and reinfarction, without a substantive increase in overall stroke rates and with a small absolute excess of life-threatening bleeding.

Antithrombotic therapy has become the mainstay of treatment of acute coronary syndromes (ACS), but the accrual of definitive evidence has been hampered by the fact that unfractionated heparin (UFH) became standard before the era of definitive clinical outcome trials. Trials with unfractionated heparin (ISIS-3, GISSI-2) in patients receiving streptokinase or tissue plasminogen activator have not demonstrated a reduction in major vascular events at 1 month. This lack of benefit may have been because of the inherent limitations of unfractionated heparin or delays in therapy after a thrombolytic agent. Trials with LMWH, enoxaparin (v. placebo or unfractionated heparin) have shown some reduction in reinfarctions but no impact on mortality. There are concerns of substantial increases in strokes when enoxaparin and some fibrin-specific agents such as tenecteplase are used in elderly patients (ASSENT-3 study and ASSENT-Plus study). However, unlike these trials, the CREATE trial not only observed reductions in recurrent MI and recurrent ischemic events but also observed significant reductions in mortality. The anti-Xa/IIa ratio for reviparin used in the present trial is about 3.3, which is similar to enoxaparin (3.3) and nadroparin (3.0), but higher than that of dalteparin (2.0) and tinzaparin (1.8). The rates of intracranial hemorrhage in CREATE trial in the reviparin group at 7 days were lower than previous trials of enoxaparin used in conjunction with fibrin-specific thrombolytic agents, tenecteplase (0.3% v. 0.88% in the ASSENT-3 study). Overall, there were 17 fewer major events (death, reinfarction, strokes or life-threatening bleeding) per 1000 patients receiving reviparin. Therefore, given the clearly lower mortality and reinfarction rates with no excess in overall stroke rates, the combination of reviparin with less expensive, non-specific thrombolytic therapy is a reasonable option in patients with acute MI receiving other effective therapies. CREATE advances the field in the management of ACS by demonstrating that the recommendation for antithrombotic therapy can now be made with confidence. The strength of the trial lies in the fact that it is one of the largest trials with LMWH and since it was conducted in our country, the results are more applicable to us.
Effectiveness and Safety of Sirolimus-Eluting Stents in the Treatment of Restenosis after Coronary Stent Placement (TROPICAL study)

Franz-Josef Neumann et al. Circulation 2005; 111: 2107-2111

Summary

Restenosis continues to be a significant limitation of placement of bare metal stents for native coronary artery lesions occurring at a rate of 15% to 50%, depending on various factors including lesion and patient characteristics. Treatment of in-stent restenosis (ISR) by repeat angioplasty is confounded by a recurrence in almost half of those treated, with repeat intervention required in about two-thirds. From the several catheter-based treatment modalities for ISR tested, only brachytherapy has been effective to prevent recurrence. Drug-eluting stents (DESs) inhibit neointimal proliferation which is the key mechanism of restenosis after primary stent placement. However, there is no large experience with the DES in the treatment of ISR. This (TROPICAL) multicenter study was undertaken to assess the effectiveness and safety of the sirolimus-eluting stent in patients with ISR in a native coronary artery lesion. This study was a prospective multicenter registry that included 162 patients with ISR. Eligibility criteria included a clinical indication for repeat percutaneous catheter intervention for ISR, beyond 4 weeks after the initial percutaneous catheter intervention. Patients with percent diameter stenosis ≥ 60%; vessel size between 2.5 and 3.0 mm and lesion length of ≤ 45 mm were included. Exclusion criteria were: total occlusion at the site of ISR, previous brachytherapy, lesion in the unprotected left main, myocardial infarction (MI) within the preceding 14 days, contraindication to aspirin, clopidogrel, or heparin. All lesions were stented with 1 or 2 sirolimus-eluting stents (CYPHER, Cordis) after balloon pre-dilation. The primary end point was in-lesion late loss at follow-up. Secondary end points were angiographic restenosis and the rate of target lesion revascularization. Follow-up angiography at 6 months was performed in 155 patients. There was an in-lesion late loss of 0.08±0.49 mm and a binary restenosis rate of 9.7% (15/155), which needed reintervention in 7.4% (12/162) at 9 months. The 9-month mortality rate was 1.2% (2/162) and the rate of non-fatal MI was 1.2% (2/162). In the subanalysis, there was no specific predictor of restenosis like diabetes or lesion length. However there was a trend toward higher in-lesion late loss with longer lesions.

Comments

Aggressive neointimal proliferation is the underlying process for the poor results achieved with conventional treatment of ISR. Previous studies have revealed an average late loss of around 1 mm with routine catheter-based treatment. The most promising modality prior to the advent of DES was brachytherapy. But even with this therapy, there was a late loss that ranged from 0.22±0.84 to 0.64±0.69 mm depending upon the lesion characteristics and technical factors. This is the first large multicenter experience with sirolimus-eluting stents in the treatment of ISR. The results of the TROPICAL study have proven the efficacy of sirolimus-eluting stents in the treatment of ISR. In fact the late loss (0.08±0.49 mm) measured in this study was of the same magnitude as seen in primary stent placement using DES. This study showed much lower in-lesion late loss than previous studies. The ISAR-DESIRE study, reported an in-lesion late lumen loss of 0.45 mm in the 100 patients treated with sirolimus-eluting stents for ISR. Similar in-stent late lumen loss was reported from the Rotterdam Cardiology Hospital (RESEARCH) Registry. In the TROPICAL study it was lower, probably due to the exclusion of total occlusion lesions from the study. Moreover, special care was taken to minimize balloon trauma during pre-dilation. Care was taken to dilate only the stenosis with minimal length balloon and to cover 5 mm safety margins at both ends. So, endothelial injury was minimized and the injured area was adequately covered. Although brachytherapy for ISR was superior to conventional treatment in various studies, it is logistically more demanding and limited by risk of edge restenosis and late stent thrombosis. The late loss of 0.08±0.49 mm reported in TROPICAL study is remarkable. Brachytherapy has been the current standard of care in the treatment of ISR. However, between these two (brachytherapy or sirolimus eluting stent) the best treatment option for ISR is still not answered. The ongoing Sirulimus for Instent ReStenosis (SIRS) trial is addressing this very issue.
Calendar of Conferences/CSI Executive Committee

December 1-4, 2005, 57th Annual Conference of Cardiological Society of India and 15th Asian Pacific Congress of Cardiology, Mumbai, India
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