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World Journal of Cardiology (*World J Cardiol*, *WJC*, online ISSN 1949-8462, DOI: 10.4330) is a peer-reviewed open access journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

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TOPIC HIGHLIGHT

WJC 6th Anniversary Special Issues (1): Hypertension

Are there ways to attenuate arterial calcification and improve cardiovascular outcomes in chronic kidney disease?

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Abstract

The risk of cardiovascular mortality among patients with end-stage renal disease is several times higher than general population. Arterial calcification, a marker of atherosclerosis and a predictor of cardiovascular mortality, is common in chronic kidney disease (CKD). The presence of traditional cardiovascular risk factors such as diabetes, hypertension, hyperlipidemia, and advanced age cannot fully explain the high prevalence of atherosclerosis and arterial calcification. Other factors specific to CKD such as hyperphosphatemia, excess of calcium, high dose active vitamin D and prolonged dialysis vintage play important roles in the development of arterial calcification. Due to the significant health risk, it is prudent to attempt to lower arterial calcification burden in CKD. Treatment of hyperlipidemia with statin has failed to lower atherosclerotic and arterial calcification burden. Data on diabetes and blood pressure controls as well as smoking cessation on cardiovascular outcomes in CKD population are limited. Currently available treatment options include non-calcium containing phosphate binders, low dose active vitamin D, calcimimetic agent and perhaps bisphosphonates, vitamin K and sodium thiosulfate. Preliminary data on bisphosphonates, vitamin K and sodium thiosulfate are encouraging but larger studies on efficacy and outcomes are needed.

Key words: Vascular calcification; Coronary calcification; Hemodialysis; Dialysis; Chronic kidney disease

Core tip: Arterial calcification is common in chronic kidney disease (CKD). Factors specific to CKD such as hyperphosphatemia, excess of calcium and high dose vitamin D therapy play important roles in the development of arterial calcification. Statin is ineffective in lowering the calcification burden. Data on diabetes and blood pressure controls and smoking cessation on cardiovascular outcomes in CKD population are limited. Available treatment strategies include non-calcium containing phosphate binders, low dose active vitamin D and calcimimetic agent. Preliminary data on bisphosphonates, vitamin K and sodium thiosulfate are encouraging but larger studies on efficacy and outcomes are needed.

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INTRODUCTION

Cardiovascular disease is the leading cause of death in

chronic kidney disease (CKD) population. The risk of cardiovascular mortality among those with end-stage renal disease is several times higher than general population[1]. Arterial calcification, a marker of atherosclerosis and a predictor of cardiovascular mortality, is common in CKD[2]. The presence of arterial calcification leads to an increase in arterial stiffness and a decrease in coronary perfusion resulting in cardiac hypertrophy and myocardial ischemia. Young adults who have been on hemodialysis for a long period of time have the prevalence of coronary artery calcification (CAC) that is at least ten times higher than those of the same age whose kidney function are normal[3]. Moreover, an inverse relationship between the estimated glomerular filtration rate and the degree of CAC was observed[4]. The presence of traditional cardiovascular risk factors such as diabetes, hypertension, hyperlipidemia, and advanced age cannot fully explain the high prevalence of atherosclerosis and arterial calcification in CKD[5]. Along with common cardiovascular risk factors, other factors specific to CKD population such as hyperphosphatemia, excess of calcium from calcium-containing phosphate binders and high calcium concentration in dialysis solution, high dose active vitamin D used in the treatment of hyperparathyroidism and prolonged dialysis vintage have been shown to positively influence the development of arterial calcification[3,6].

MINERAL METABOLISM IN CKD

In early CKD, the kidney's ability to excrete phosphate load is impaired resulting in a release of fibroblast growth factor 23 (FGF-23) whose action is to stimulate renal phosphate excretion in order to maintain neutral phosphate balance[7]. FGF-23, in the presence of its obligatory co-receptor klotho, binds to FGF receptors causing a decrease in phosphate reabsorption in the proximal tubules and a suppression of 1,25 dihydroxyvitamin D (1,25-OH_2-D) synthesis[8]. Continued phosphate retention and decreased 1,25-OH-D levels later on lead to an increase in parathyroid hormone (PTH) secretion. The accumulation of FGF-23 together with PTH work in concert to enhance renal phosphate excretion. As CKD advances, these compensatory mechanisms fail and phosphate retention ensues evidenced by the development of hyperphosphatemia[9]. Accumulation of PTH also enhances bone resorption giving rise to an increase in circulating calcium, bone loss and fracture. On the other hand, elevated FGF-23 has been linked to cardiac hypertrophy, vascular calcification, congestive heart failure and increased mortality[10-13].

PATHOGENESIS OF ARTERIAL CALCIFICATION

Pathogenesis of arterial calcification is no longer believed to be the passive precipitation of calcium and phosphate crystals but involves a tightly regulated process of cellular transformation of vascular smooth muscle cells (VSMCs) into osteoblast-like cells. These calcified VSMCs, instead of retaining smooth muscle cell markers, express specific osteoblast markers as well as several bone matrix proteins[14,15]. The process of calcification also has features that resemble bone matrix mineralization. For example, the formation and nucleation of mineral crystals require the presence of matrix vesicles. Dying VSMCs form apoptotic bodies which have the ability to concentrate calcium and phosphate in the same fashion as matrix vesicles[16]. Several factors related to CKD including high calcium and phosphate environment and high dose active vitamin D have been shown to promote VSMC transformation followed by matrix vesicle-mediated mineralization[17,18]. Moreover, the reduction and the alteration of function of naturally occurring calcification inhibitors such as fetuin A, matrix gla-protein, osteopontin and osteoprotegerin are also important in the development of arterial calcification in CKD[19,20]. Klotho deficiency has been observed in kidneys, parathyroid glands and other organs during the course of CKD[21,22]. In arterial wall, decreased klotho expression potentiates the development of arterial calcification[23,24]. The role of FGF-23 in arterial calcification is complex. Few studies have identified FGF receptor and its signaling pathway in the arterial wall whereas others have not[12,23,25]. Kidney transplantation can markedly improve both renal function and mineral metabolism in the long term. Several studies have demonstrated stabilization or decline in the rate of progression of arterial calcification in patients who received a kidney transplant as compared to those who remained on dialysis especially during the first 1-2 years[26-28]. However, with longer follow-up period up to 3-4 years post-transplantation, the progression becomes more evident. Overall the rate of CAC progression was estimated to be around 10% per year[29,30]. The severity of baseline calcification and the presence of hyperlipidemia were identified as independent predictors of progression in these studies. It appears that once calcification develops it probably cannot be reversed. Despite the significant improvement in kidney function and mineral metabolism, arterial calcification tends to become more severe as time passes probably triggering by the presence of common cardiovascular risk factors in kidney transplant recipients including aging, diabetes, hypertension and hyperlipidemia.

Due to the significant health risk of atherosclerosis and arterial calcification, it is prudent to attempt to lower calcification burden in CKD patients. Cardiovascular risk modification through the use of statin for hyperlipidemia has not been proved fruitful in attenuating CAC progression[31,32]. Studies on diabetes and blood pressure controls as well as smoking cessation on cardiovascular outcomes in CKD population are limited. The following review focuses on therapies that can modify CKD-related risk factors for arterial calcification which may have favorable impact on cardiovascular outcomes.

PHOSPHATE BINDERS

The purpose of phosphate binder is to bind phosphate in the ingested food and increase its elimination in the

stool. Calcium-containing phosphate binders such as calcium carbonate and calcium acetate are commonly used as phosphate binding agents since early 1980s as alternatives to aluminum hydroxide due to the high prevalence of aluminum toxicity. The use of calcium-containing phosphate binders is often limited by the development of hypercalcemia. Furthermore, over the past decade, increasing evidence have linked the amount of calcium intake derived from calcium-containing phosphate binders to the severity of vascular calcification[3,6,33]. Newer phosphate binding agents including sevelamer, lanthanum, calcium-magnesium combination and iron-based phosphate binders have been developed to overcome these limitations.

Sevelamer carbonate

Sevelamer is an ion-exchange resin that is commonly used as an alternative to calcium for phosphate binding. In addition to binding to phosphate, sevelamer has been shown to lower cholesterol, FGF-23, inflammatory markers, c-reactive protein and hemoglobin A1C and may improve endothelial function[34,35]. In hemodialysis patients, sevelamer attenuates the progression of CAC and aortic calcification compared to calcium[36-38] (Table 1). In two randomized controlled trials in incident hemodialysis patients, those who were treated with calcium had a greater risk of death compared to sevelamer[2,39]. However, a randomized study in prevalent hemodialysis patients did not show survival benefit associated with sevelamer use[40]. In non-dialysis CKD population, patients who were treated with sevelamer in order to keep serum phosphate within the normal range had better survival compared to those treated with calcium[41]. In another small randomized study in moderate CKD patients that compared calcium, sevelamer and lanthanum versus placebo revealed an increase in arterial calcification in all groups, however, the degree was highest in the calcium group[42]. It has been theorized that the use of phosphate binders in non-dialysis CKD may result in an increase in the availability of free calcium in the intestine. Similarly, when rosuvastatin, sevelamer and no drug were compared in a small randomized study in moderate CKD patients, a significant increase in CAC scores was observed in all three groups[43]. Despite the possible survival benefit, the use of phosphate binders may not be beneficial in reducing calcification burden in moderate CKD population. In order to justify the use of phosphate binders in non-dialysis CKD patients, more studies are required to confirm the beneficial or harmful effects.

Lanthanum carbonate

Lanthanum is a rare earth element that is as effective as aluminum and better than sevelamer in binding phosphate[44]. Long-term use of lanthanum in renal failure can result in an accumulation in various organs but without any obvious harmful effects[45,46]. Similar to sevelamer, the use of lanthanum in moderate CKD can lower FGF-23 levels[47]. In both uremic rats and dialysis patients, lanthanum attenuated the development of vascular calcification[48,49]. The data on patient-level outcomes are limited. A follow-up data on dialysis patients who were enrolled in the phase 3 study did not show survival benefit associated with lanthanum treatment. However, in a subgroup of patients > 65 years of age, those who received lanthanum carbonate appeared to have better survival compared to standard therapy[50]. The efficacy of lanthanum depends largely on the pills being chewed thoroughly prior to swallowing. Recently the company has developed the oral powder form that may work better in patients with problems with mastication.

Combined calcium acetate-magnesium carbonate

Both intracellular and extracellular magnesium are vital in preventing inflammation and oxidative stress. Decreased magnesium concentration is associated with impaired endothelial function, vasospasm and atherogenesis[51]. Increased severity of vascular calcification has been observed in hemodialysis and peritoneal dialysis patients with low normal magnesium levels[52]. *In vitro* studies and *in vivo* study in rodents demonstrated that increasing magnesium concentrations were protective against vascular calcification through upregulation of anti-calcification proteins[53-55]. In a study of 204 hemodialysis patients over a 24-wk follow-up, the European formulation of combined calcium-magnesium phosphate binder (calcium acetate 435 mg/magnesium carbonate 235 mg) was as efficacious as sevelamer in reducing serum phosphate without the side effect of increased serum ionized calcium. A small but significant increase in serum magnesium was observed. All patients were dialyzed against 0.5 mm magnesium dialysate and experienced no serious adverse events[56]. FGF-23 levels also decreased in the magnesium group[57]. Furthermore, a small observational study in 7 hemodialysis patients showed stabilization of CAC score after 18 mo of being on calcium-magnesium phosphate binder[58].

Iron-based phosphate binders

Recently food and drug administration (FDA) approved iron-based phosphate binder in the United States is sucroferric oxyhydroxide. Another preparation of iron-based phosphate binder, ferric citrate, is currently under review by the FDA. These drugs are as efficacious as sevelamer in lowering serum phosphate[59,60]. The use of iron-based phosphate binder is associated with an increase in serum ferritin and percent transferrin saturation leading to lesser requirement of intravenous iron and erythropoiesis-stimulating agents in dialysis patients[61]. Iron deficiency can increase FGF-23 levels and therefore iron-based phosphate binders can lower FGF-23[62]. In uremic rats, sucroferric oxyhydroxide prevented the development of vascular calcification[63]. More information regarding iron-based phosphate binders should become available within the next year.

ACTIVE VITAMIN D

Active vitamin D are primarily used for the treatment of

Table 1 Studies related to therapies that may influence arterial calcification and patient outcomes

Ref.	Subjects	*n*	Study type	Intervention	Follow-up (mo)	Results
Braun *et al*[38]	HD	114	RCT	Sevalamer *vs* calcium	12	↓ CAC and AC
Chertow *et al*[36]	HD	200	RCT	Sevelamer *vs* calcium	12	↓ CAC
Kakuta *et al*[37]	HD	183	RCT	Sevelamer *vs* calcium	12	↓ CAC
Suki *et al*[40]	HD	2103	RCT	Sevelamer *vs* calcium	19	↔ mortality
Block *et al*[2]	Incident HD	127	RCT	Sevelamer *vs* calcium	44	↓ mortality
Di Iorio *et al*[39]	Incident HD	466	RCT	Sevelamer *vs* calcium	24	↓ mortality
Block *et al*[42]	Non-dialysis CKD	148	RCT	Sevelamer, lanthanum, calcium *vs* placebo	9	↑ CAC and AC
Di Iorio *et al*[41]	Non-dialysis CKD	212	RCT	Sevelamer *vs* calcium	24	↓ mortality
Lemos *et al*[43]	Non-dialysis CKD	38	RCT	Rosuvastatin, sevelamer *vs* no drug	24	↔ CAC
Toussaint *et al*[49]	HD	45	RCT	Lanthanum *vs* calcium	18	↓ AC
Wilson *et al*[50]	HD	1354	RCT	Lanthanum *vs* calcium	27	↔ mortality
Spiegel *et al*[58]	HD	7	Observational	Combined magnesium-calcium	18	↔ CAC
Kalanter-Zadeh *et al*[111]	HD	58058	Retrospective	Paricalcitol *vs* no drug	24	↓ mortality
Naves-Diaz *et al*[112]	HD	16004	Retrospective	Alfacalcidol or calcitriol *vs* no drug	16	↓ mortality
Shoji *et al*[113]	HD	242	Prospective	Alfacalcidol *vs* no drug	61	↓ CVD mortality
Tentori *et al*[114]	HD	38066	Retrospective	Active vitamin D *vs* no drug	60	↓ mortality
Melamed *et al*[115]	Incident HD and PD	1007	Prospective	Calcitriol *vs* no drug	30	↓ mortality
Teng *et al*[116]	Incident HD	51037	Retrospective	Active D *vs* no drug	24	↓ mortality
Tentori *et al*[117]	Incident HD	14967	Retrospective	Calcitriol *vs* paricalcitol *vs* doxercalciferol *vs* no drug	37	↓ mortality in all active D groups compared to no drug
Kovesdy *et al*[118]	Non-dialysis CKD	520	Retrospective	Calcitriol *vs* no drug	24	↓ mortality
Shoben *et al*[119]	Non-dialysis CKD	1418	Retrospective	Calcitriol *vs* no drug	24	↓ mortality
Sugiura *et al*[120]	Non-dialysis CKD	665	Retrospective	Alfacalcidol *vs* no drug	55	↓ CVD events and mortality
Thadhani *et al*[75]	Non-dialysis CKD	227	RCT	Paricalcitol *vs* placebo	48	↔ left ventricular mass index
Tamez *et al*[76]	Non-dialysis CKD	196	RCT	Paricalcitol *vs* placebo	48	↓ left atrial volume index
Raggi *et al*[80]	HD	360	RCT	Cinacalcet + active D *vs* active D	12	↓ CAC and aortic valve calcification
Chertow *et al*[83]	HD	3883	RCT	Cinacalcet *vs* placebo	21	↔ CVD events or mortality
Hashiba *et al*[88]	HD	18	RCT	Etidronate *vs* no drug	6	↓ AC
Nitta *et al*[87]	HD	35	Observational	Etidronate	12	↓ CAC
Kawahara *et al*[91]	GP	108	RCT	Atorvastatin *vs* etidronate *vs* both	12	↓ thoracic and abdominal aortic plaques in combined therapy
Adirekkiat *et al*[33]	HD	32	Prospective	STS *vs* no drug	9	↓ CAC
Mathews *et al*[98]	HD	22	Observational	STS	5	↓ CAC

n: Number of patients; HD: Hemodialysis; PD: Peritoneal dialysis; CKD: Chronic kidney disease; RCT: Randomized controlled trial; CAC: Coronary calcification; AC: Aortic calcification; CVD: Cardiovascular disease; STS: Sodium thiosulfate.

hyperparathyroidism in CKD. In addition to lowering PTH, active vitamin D also stimulates calcium and phosphate absorption in the gastrointestinal tract and therefore can result in worsening hypercalcemia and hyperphosphatemia. Active vitamin D also reduces proteinuria, augments the response to erythropoietin and suppresses renin-angiotensin system[64-66]. The parent drug of active vitamin D is calcitriol or 1, 25-dihydroxyvitamin D3. The closely related analogs to calcitriol are alfacalcidol (1-alpha hydroxyvitamin D3) and doxercalciferol (1-alpha hydroxyvitamin D2). Both require 25-hydroxylation process in the liver prior to becoming active forms. Similar to the parent compound, alfacalcidol and doxercalciferol can precipitate hypercalcemia and hyperphosphatemia especially if given in high doses. Paricalcitol or 19-Nor-1-25-dihydroxyvitamin D2 was developed specifically for the treatment of hyperparathyroidism in CKD. Paricalcitol appears to act preferentially in the parathyroid glands and less so in the gastrointestinal tract[67]. In rodents with uremia, administration of calcitriol and doxercalciferol resulted in an increase in aortic calcification, whereas paricalcitol did not[68]. However when testing different doses of calcitriol and paricalcitol, both active vitamin D in high doses induced a similar degree of aortic calcification. Interestingly, in this study, lower doses of both calcitriol and paricalcitol seemed to be protective against vascular calcification[69]. The calcemic and phosphatemic effects of all forms of active vitamin D have been confirmed in a recent randomized crossover trial in hemodialysis patients that showed similar incidences of hyperphosphatemia and hypercalcemia among patients who received alfacalcidol or paricalcitol[70]. The increase in calcium and

phosphate load as a result of active vitamin D induced calcium and phosphate absorption is likely responsible for the development of vascular calcification. On the other hand, the direct effect of vitamin D on vascular wall appears to be positive. Active vitamin D can stimulate klotho and osteopontin expression in the arterial wall. Both of which help prevent vascular calcification[71]. This finding can probably explain the protective effect of low dose active vitamin D on vascular calcification. The development of vascular calcification associated with the use of active vitamin D is the result of systemic accumulation of calcium and phosphate rather than the local effect on arterial wall[72]. Therefore, low doses of active vitamin D that do not augment calcium and phosphate load may actually be protective against vascular calcification[23,69].

As for the beneficial effect of vitamin D on renin-angiotensin system, a study in rats with renal failure demonstrated that active vitamin D treatment could prevent left ventricular hypertrophy and myocardial fibrosis[73]. In an observational study in hemodialysis patients, treatment of hyperparathyroidism with intravenous calcitriol led to a decline in renin, angiotensin II and atrial natriuretic peptide levels associated with a decrease in left ventricular hypertrophy[74]. However, a recent randomized study in moderate CKD patients revealed only a non-significant trend toward a decrease in left ventricular mass index in the group of patients that received paricalcitol[75]. Nevertheless, subsequent analysis did demonstrate a significant decrease in left atrial volume index[76]. The lack of clear benefit of active vitamin D on cardiac hypertrophy may be related to the increase in FGF-23 in response to active vitamin D treatment. As for survival benefit of active vitamin D therapy in hemodialysis patients, several retrospective and observational studies have revealed a decrease in all-cause and cardiovascular mortality among patients who received active vitamin D regardless of PTH levels[77]. Details of these studies can be found in Table 1. The benefit seemed to be more pronounced in the low-dose range and among patients who received paricalcitol. At the present time, there is no published prospective randomized study that evaluates the effect of active vitamin D on survival in CKD population.

CALCIMIMETIC

Calcimimetic allosterically activates calcium-sensing receptors, thus can suppress PTH secretion without elevating serum calcium. Calcimimetic is used as an add-on to active vitamin D and phosphate binder in the treatment of hyperparathyroidism in CKD[78]. Currently, cinacalcet is the only calcimimetic drug available for this purpose. In nephrectomized rats, adding cinacalcet to active vitamin D helped decrease the severity of vascular calcification associated with high dose vitamin D treatment[79]. In a randomized study in 360 hemodialysis patients, the rate of progression of CAC and aortic valve calcification was reduced when cinacalcet was added to low dose active vitamin D compared to larger and varying doses of active vitamin D therapy alone[80,81]. Cinacalcet therapy also decreases FGF-23 levels[82]. However, significant benefits in terms of overall survival or cardiovascular events were not observed in a large randomized controlled trial in 3883 hemodialysis patients after 5 years of follow-up[83].

BISPHOSPHONATES

Bisphosphonates are synthetic analogs of inorganic pyrophosphate that have the ability to suppress bone resorption and therefore are commonly used in the treatment and prevention of osteoporosis in general population. The other important property of inorganic pyrophosphate is inhibition of calcium and phosphate crystal deposition in the bone matrix. Oral etidronate and intravenous pamidronate have been utilized in the treatment of calcific uremic arteriolopathy (CUA), a condition of wide spread small-vessel calcification that results in progressive cutaneous ulcer due to ischemia[84,85]. In uremic rats, daily pamidronate or etidronate therapies prevented aortic calcification[86]. In hemodialysis patients, oral and parenteral etidronate have been shown to delay the progression of CAC and aortic calcification[87,88]. However, this anti-calcification effect was not observed with the newer generation bisphosphonates including alendronate and ibandronate[89,90]. A recent randomized study in general population with hypercholesterolemia revealed the combined regimen of daily atorvastatin and etidronate reduced atherosclerotic plaque burden in thoracic and abdominal aorta[91]. It was suggested that etidronate was responsible for the regression of calcified plaques in abdominal aorta while atorvastatin attenuating the non-calcified plaques in thoracic aorta. Worsening adynamic bone disease with the use of bisphosphonates in the setting of CKD is of concern, thus recent Kidney Disease Improving Global Outcomes recommendation advised against prescribing bisphosphonates in patients with an eGFR < 30 mL/min per 1.73 m^2[92].

SODIUM THIOSULFATE

Sodium thiosulfate (STS) is a reducing, chelating and anti-oxidant agent that is useful as an antidote in cyanide poisoning. STS also has the ability to chelate calcium in precipitated minerals forming calcium thiosulfate that is more soluble than calcium oxalate and calcium phosphate. Thus its use has been expanded in conditions with increased calcification burden such has nephrolithiasis, metastatic calcification, tumoral calcinosis and CUA[93-96]. In a large observational study in 172 hemodialysis patients with CUA, intravenous STS therapy resulted in clinical improvement in most patients[96]. In uremic rats, parenteral administration of STS has been shown to prevent the development of vascular calcification[97]. Twice weekly intravenous STS therapy in hemodialysis patients was able to delay the rate of progression of CAC after six months compared to the non-treatment group but with a significant decline in hip bone mineral density in one study[33,98]. Long-term intravenous or intraperitoneal

STS therapy in dialysis patients are well tolerated with minimal side effects[33,96,99]. The mechanism by which STS reduces calcification burden is poorly understood. It has been suggested that mechanisms other than calcium chelation are responsible for the decreased calcification burden[94,100].

VITAMIN K

There are two types of naturally occurring vitamin K: vitamin K1 (phylloquinone) found mostly in green leafy vegetables and vegetable oils and vitamin K2 (menaquinone) found in animals, bacteria, and fermented food such as cheese and natto. Five to twenty five percent of ingested vitamin K1 can be converted to vitamin K2 in the body. Colonic bacteria can synthesize vitamin K2 and antibiotics that interfere with the growth of these colonic flora impair vitamin K2 production[101]. Vitamin K is required as a co-factor in the process of gamma-carboxylation of several extracellular matrix proteins turning inactive uncarboxylated proteins into active carboxylated forms. Prothrombin, coagulation factors 7, 9 and 10 require vitamin K1 for their carboxylation processes; whereas, osteocalcin and matrix gla-protein require vitamin K2[102]. Osteocalcin is important in bone mineralization; therefore, menaquinone is used in the treatment of osteoporosis. Matrix gla-protein is a calcification inhibitor that plays important role in the prevention of arterial calcification. Warfarin, an antagonist to vitamin K, not only inhibits coagulation but long-term use can also promote arterial calcification[103]. Vitamin K deficiency is common in end-stage renal disease patients and the accumulation of inactive form of matrix gla-protein is associated with an increase in the severity of arterial calcification and mortality[104,105]. High menaquinone intake is also associated with reduced CAC and coronary heart disease in general population[106,107]. Vitamin K1 or K2 supplementation especially in high doses can significantly decrease the amount of inactive matrix gla-protein in hemodialysis patients[108]. In CKD rats treated with warfarin, high dietary vitamin K1 can blunt the development of vascular calcification[109]. The favorable impact of vitamin K1 on vascular calcification is likely depending on the conversion of vitamin K1 to vitamin K2 in the body. A prospective randomized controlled trial to evaluate the effect vitamin K1 supplementation on the progression of CAC (VitaVasK trial) in hemodialysis patients is currently ongoing[110].

In conclusion, the currently available treatment options for arterial calcification in CKD include non-calcium containing phosphate binders, low doses of active vitamin D, calcimimetic agent and perhaps bisphosphonates, vitamin K and STS. Preliminary data on bisphosphonates, vitamin K and STS are encouraging but larger studies on efficacy and outcomes are needed.

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TOPIC HIGHLIGHT

WJC 6th Anniversary Special Issues (1): Hypertension

Management of hypertension in primary aldosteronism

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Abstract

Hypertension causes significant morbidity and mortality worldwide, owing to its deleterious effects on the cardiovascular and renal systems. Primary hyperaldosteronism (PA) is the most common cause of reversible hypertension, affecting 5%-18% of adults with hypertension. PA is estimated to result from bilateral adrenal hyperplasia in two-thirds of patients, and from unilateral aldosterone-secreting adenoma in approximately one-third. Suspected cases are initially screened by measurement of the plasma aldosterone-renin-ratio, and may be confirmed by additional noninvasive tests. Localization of aldostosterone hypersecretion is then determined by computed tomography imaging, and in selective cases with adrenal vein sampling. Solitary adenomas are managed by laparoscopic or robotic resection, while bilateral hyperplasia is treated with mineralocorticoid antagonists. Biochemical cure following adrenalectomy occurs in 99% of patients, and hemodynamic improvement is seen in over 90%, prompting a reduction in quantity of anti-hypertensive medications in most patients. End-organ damage secondary to hypertension and excess aldosterone is significantly improved by both surgical and medical treatment, as manifested by decreased left ventricular hypertrophy, arterial stiffness, and proteinuria, highlighting the importance of proper diagnosis and treatment of primary hyperaldosteronism. Although numerous independent predictors of resolution of hypertension after adrenalectomy for unilateral adenomas have been described, the Aldosteronoma Resolution Score is a validated multifactorial model convenient for use in daily clinical practice.

Key words: Primary hyperaldosteronism; Hypertension; Adrenalectomy; Aldosteronoma; Treatment

Core tip: Primary hyperaldosteronism is the most common reversible form of secondary hypertension. After appropriate diagnosis and localization studies, adrenalectomy is the procedure of choice for unilateral aldosterone-secreting adenomas, while medical therapy is best for bilateral adrenal hyperplasia. Surgical resection improves or cures biochemical and hemodynamic perturbations in most patients, and halts or reverses many of the deleterious effects of hyperaldosteronism. Predicting which patients will benefit most from adrenalectomy is aided by the Aldosteronoma Resolution Score.

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INTRODUCTION

Hypertension is one of the most prominent risk factors for morbidity and mortality worldwide, accounting for 45% of deaths due to heart disease and 51% due to stroke[1,2]. In the United States alone, 69 million adults

(29%) have hypertension, in whom it is significantly associated with myocardial infarction, cerebrovascular accidents, heart failure and renal disease[3,4]. Given the large impact on global health, controlling hypertension is of utmost importance. Significant efforts have been made to characterize potentially curable, or secondary, types of hypertension such as renovascular hypertension, pheochromocytoma, Cushing's syndrome and primary hyperaldosteronism.

Primary hyperaldosteronism (PA) is the leading cause of secondary hypertension, and can be identified in 5% to 18% of hypertensive patients[5,6]. First described by Conn in 1955 in a patient presenting with resistant hypertension and hypokalemia who was found to have an aldosterone-secreting adrenal adenoma[7], PA can present in a myriad of clinical scenarios. Most recent epidemiologic studies have shown that approximately 60% of patients are found to have bilateral idiopathic hyperplasia, also known as idiopathic hyperaldosteronism (IHA), while 30% present with unilateral aldosterone-producing adenomas (APA)[8]. One to two percent of patients present with primary or unilateral adrenal hyperplasia (UAH), 1% with aldosterone-secreting adrenocortical carcinoma, 1% with familial hyperaldosteronism, and 1% with ectopic aldosterone-producing adenoma or carcinoma[6,9,10].

Classically, excessive aldosterone secretion not only results in difficult to manage hypertension in the majority of patients, but also produces biochemical effects of hypokalemia in 10%-30% of patients[11]. More recent data, however, suggest that most patients with PA are actually normokalemic[6,11,12]. In addition, aldosterone hypersecretion has been linked to significant and potentially reversible end-organ damage, particularly in the cardiovascular and renal systems[13]. For instance, Tanabe *et al*[14] demonstrated that patients with PA have more pronounced cardiac hypertrophy compared to patients with essential or other secondary causes of hypertension. Fortunately, timely correction of aldosterone levels can prevent or reverse some of these effects[15]. This review will describe the current methods of diagnosis and management of primary hyperaldosteronism, with a particular focus on the systemic effects of adrenalectomy as well as the predictors of resolution of hypertension after surgery.

DIAGNOSIS

Patients with hypertension and hypokalemia, regardless of suspected cause (diuretics, incidentaloma), and patients with medically-resistant hypertension, should be considered for screening for primary hyperaldosteronism[16]. Initial evaluation of patients involves biochemical testing with plasma aldosterone (ng/dL) to renin (ng/mL per hour) ratio (ARR). This test identifies excessive aldosterone secretion with simultaneous suppression of plasma renin activity. Although ARR is regarded as the ideal screening tool for PA, there exists some controversy regarding the clinical conditions under which the ARR is obtained, as well as the test's diagnostic accuracy. Certain drugs, including beta-blockers, angiotensin-converting enzyme inhibitors (ACE- I), selective-serotonin reuptake inhibitors and oral contraceptives, have been shown to affect the results of the test[17,18]. Ideal testing conditions involve discontinuation of such medications two weeks prior [10,17,18]. However, in a recent study, Fisher *et al*[19] showed that doing so is impractical, and most patients are unable to be taken off their anti-hypertensive medications without the need for substitution by other agents to adequately control blood pressure or serious side effects such as hospitalization. Others suggest that only use of spironolactone will absolutely interfere with the interpretation of this ratio[16]. In addition, there is some disagreement regarding the requirement of a minimum plasma aldosterone level and the critical ARR cutoff for diagnosis. Most authors recommend an ARR of 20-40, and researchers found that ARR of at least 35 has 100% sensitivity and 92.3% specificity in diagnosing primary hyperaldosteronism[17,20,21]. Furthermore, biochemical testing should be done in the morning, in a seated position after an initial two-hour ambulatory period[18]. False negative and positive results can occur, as affected by age, smoking, medications, posture, and renal function, so it is generally advisable to repeat biochemical testing in patients with high pretest probability of PA, typically four weeks later[18].

Patients with suspected primary aldosteronism identified by screening ARR may undergo confirmatory testing or go on to localization studies. Confirmatory testing includes: the oral sodium loading test, the saline infusion test, the fludrocortisone suppression test, and the captopril challenge test[22]. Time, cost, patient compliance, and certain physiologic parameters need to be considered in choosing the specific confirmatory test. For instance, in patients with severe hypertension, cardiac or renal insufficiency, clinicians should avoid the oral sodium loading test and the saline infusion tests. In general, such additional testing often proves burdensome and in 30%-50% of cases does not prove to be abnormal in patients with high ARR suggestive of PA[10,22,23]. Currently, there is lack of evidence encouraging the use of any one of these tests as a gold standard and many physicians, including those in our own practice, no longer recommend confirmatory testing.

LOCALIZATION

The etiology of aldosterone hypersecrecretion is established by imaging and adrenal vein sampling (AVS). The distinction between unilateral APA from bilateral hyperplasia is a key factor in determining the appropriate management. APAs are best managed by surgical resection, whereas the treatment for IHA is medical therapy. Current high-resolution computed tomography (CT) imaging has enhanced the classification of subtypes of hyperaldosteronism and the ability to identify APAs. The sensitivity and specificity of adrenal imaging with 1.25-3 mm cuts for APA is 78% and 75%, respectively[22,24]. Findings on adrenal CT include normal-appearing adrenals, unilateral macroadenomas (greater than 1 cm), unilateral microadenomas (less than 1 cm), bilateral micro- or macroadeno-

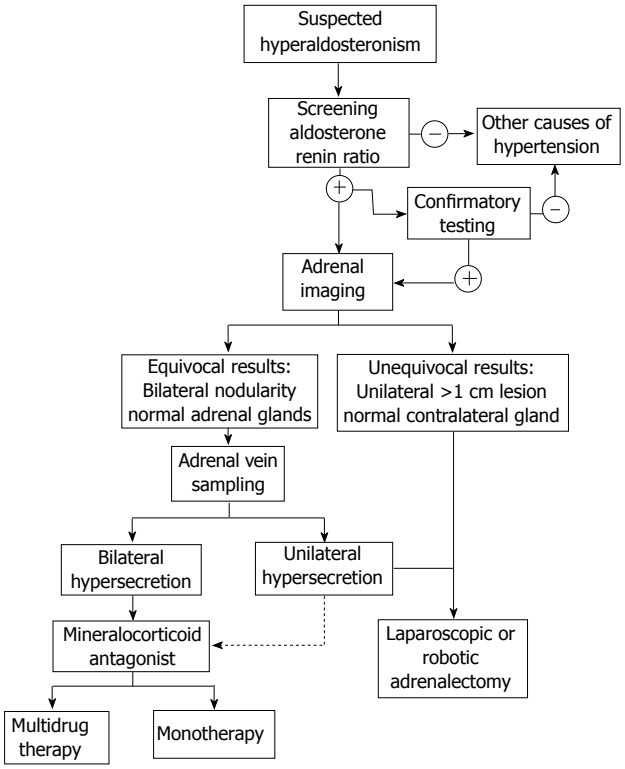

Figure 1 Treatment algorithm.

mas, and minimal unilateral adrenal limb thickening[22]. Imaging in IHA can reveal normal-appearing adrenal glands or show nodular changes. As a result, radiologists can misread APAs as IHA, whereas microadenomas can be incorrectly labeled as areas of hyperplasia[22]. Several studies have shown that CT alone may lead to misdiagnosis in PA. In a systematic review, Kempers *et al*[25] found that 37.8% of patients who showed lateralization on CT/magnetic resonance imaging (MRI) had conflicting results on AVS. If imaging alone was used for localization, 14.6% of patients would have undergone inappropriate adrenalectomy, while 19.1% would have been inappropriately excluded from surgery. Furthermore, in 3.9% of patients, CT/MRI lateralized to the opposite side. These considerations have prompted many to regard AVS as a gold standard for lateralization. However, mandatory use remains a contentious topic. The United States Endocrine Society and Japan Endocrine Society guidelines recommend that AVS be performed in all patients who have diagnosed PA and are considering surgical resection[22,25,26]. However, the Adrenal Vein Sampling International Study showed that AVS is utilized routinely in only a few centers worldwide[27]. AVS requires highly skilled radiologists for successful cannulation of both adrenal veins and the procedure is not without complications. AVS is unsuccessful in up to 20% due to failure to cannulate the right adrenal vein, and even in experienced centers, the complication rate averages 0.5%-2.5%[24,25,28,29].

Despite recommendations from the endocrine societies, several groups continue to advocate for selective use. Zarnegar *et al*[30] and Tan *et al*[31] both demonstrated the effectiveness of AVS in cases of equivocal findings on initial imaging studies. Specifically, Zarnegar *et al*[30] compared outcomes after adrenalectomy for patients with > 1 cm adenomas with normal contralateral adrenal glands on CT to those who required AVS and CT (< 1 cm). They found similar outcomes in both groups as measured by biochemical and hemodynamic resolution, advocating for selective use of AVS for patients with smaller tumors or indeterminate imaging findings. A recently-issued consensus statement recommends certain patients with PA do not necessarily require AVS, including: patients who are < 40 years old with marked PA and clear unilateral adrenal adenoma and normal contralateral gland on imaging; patients who are not surgical candidates due to unacceptably high operative risk; patients with suspected adrenocortical carcinoma; or patients who have proven familial hyperaldosteronism[32].

MANAGEMENT

Treatment of PA is aimed at prevention of morbidity and mortality associated with hypertension, hypokalemia and direct aldosterone-associated organ damage. Once the cause of hyperaldosteronism is established, the proper management strategy can be instituted (Figure 1). Adrenalectomy is the procedure of choice for documented unilateral secretion of aldosterone (APA or UAH), while medical therapy is warranted for bilateral aldosterone hypersecretion as with IHA and bilateral APA, or for patients who refuse surgery or are poor surgical candidates.

Medical management involves antagonism of the mineralocorticoid (MR) receptor with spironolactone or eplerenone. Spironolactone has been utilized for over four decades as a first-line agent at doses ranging 25-400 mg/d[22,33]. Hypokalemia typically resolves immediately, but blood pressure reduction may take several months to occur[6]. Anti-androgen side effects such as gynecomastia and dysmenorrhea can result from spironolactone due to cross-antagonism of the sex-steroid receptors, usually in a dose-dependent fashion[34,35]. Eplerenone is more specific for the aldosterone receptor and therefore causes fewer undesired side effects. It is, however, less potent[36]. A recent randomized trial comparing the two therapies showed that spironolactone from 75 to 225 mg/d was more efficacious than eplerenone at 100-300 mg/d for hypertension control[36]. In addition, since spironolactone is cheaper and more widely available, clinicians should weigh these factors when recommending the appropriate agent for medical management of PA[10,36]. It is noteworthy that hypervolemia can be prohibitive in using MR antagonists as sole agents for PA, and in approximately 50% of patients, a second agent such as a low-dose thiazide diuretic can help achieve adequate blood pressure control[37]. Other agents including sodium channel blockers (amiloride, triamterene), calcium channel blockers, ACE- I , and angiotensin-receptor blockers (ARB) have also been employed as secondary agents in PA, with variable effects on blood pressure and plasma aldosterone levels[37,38].

Adrenalectomy is the preferred treatment strategy for

patients with demonstrable unilateral hypersecretion of aldosterone. The standard approach employed by most centers is lateral transperitoneal laparoscopic adrenalectomy as first described in 1992 by Gagner *et al*[39]. However, some surgeons prefer a posterior retroperitoneoscopic approach or robotic-assisted surgery. Proponents of the retroperitoneoscopic approach recommend this technique for smaller tumors (< 6 cm), prior abdominal surgery and lower body mass index (BMI)[40-42]. Several recent meta-analyses comparing transabdominal to retroperitoneal laparoscopic adrenalectomy found no significant differences between the two approaches[43,44]. Additionally, Brandao *et al*[45] systematically reviewed robotic-assisted adrenalectomy and found that it is equally safe and may even result in less blood loss and shorter hospital stay, compared to laparoscopic approaches.

OUTCOMES

Aldosterone hypersecretion causes hypertension and biochemical abnormalities with potassium hemostasis by activation of the renin-angiotensin-aldosterone-system (RAAS). It has been shown that abnormal activation of the RAAS correlates directly with end-organ damage in the cardiovascular and renal systems and it is well-documented that blockade of the angiotensin-Ⅱ arm by ACE-Ⅰ or ARB provides significant cardiovascular protection[13]. Pathophysiologically, aldosterone works to increase sodium absorption in the kidneys, leading to increased intravascular volume and thereby increased blood pressure. Cardiovascular damage occurs from increased left ventricular mass and hypertrophy as well as aldosterone-driven fibrosis and collagen production in the interventricular septum. Furthermore, perivascular inflammation, vascular remodeling in the heart and kidney, and direct damage to the nephron anatomy and physiology, are thought to contribute to sustained deleterious end-organ effects from aldosterone excess that may occur independent of hypertension[46-48]. In fact, compared to patients with essential hypertension, patients with primary hyperaldosteronism are at increased risk for these adverse effects, which are significantly reduced by surgical or medical management[49-51]. Milliez *et al*[52] demonstrated in a retrospective study a markedly increased incidence of stroke (12.9% *vs* 3.4%), non-fatal MI (4.0% *vs* 0.6%), and atrial fibrillation (7.3% *vs* 0.6%) in patients with PA compared to those with essential hypertension. There was no difference in the PA subtype. Additionally, Ribstein *et al*[53] reported significant decrease in proteinuria in patients with PA with treatment of aldosterone excess by adrenalectomy or spironolactone compared to control essential hypertension patients.

The treatment of aldosterone hypersecretion either by medical or surgical means is very effective. Nearly 100% of patients will experience a biochemical cure with normalization of hypokalemia and aldosterone levels[54,55]. These effects follow surgery relatively quickly. It is recommended that potassium supplements and MR antagonists should be discontinued on post-operative day 1, and antihypertensive medications reduced simultaneously. Patients are also instructed to eat a diet generous in salt for the first month after surgery to account for a suppressed contralateral adrenal gland[56]. Interestingly, a minority of people can develop prolonged zona glomerulosa insufficiency causing hyperkalemia after adrenalectomy. Reported by Fischer *et al*[57], this outcome had an incidence of 5% of adrenalectomized PA patients in their cohort and required long-term fludrocortisone treatment post-operatively.

Resolution of hypertension in primary hyperaldosteronism is etiology-specific. For cases not appropriate for surgical resection, blood pressure control is best achieved by mineralocorticoid antagonists, as previously discussed. Conversely, for localized APAs adrenalectomy results in improvement in blood pressure control in over 90% of patients, and complete resolution, as defined by BP < 140/90 mmHg without the need for antihypertensive medications, in 30%-60%[6,58]. Patients that are not cured generally experience lower mean blood pressures and take fewer antihypertensive medications after surgery[59]. Persistent hypertension after adrenalectomy may result from misdiagnosis of unilateral aldosterone hypersecretion, or more likely, coexistent essential hypertension with underlying end organ damage. Chronic aldosterone excess has been shown to increase arterial stiffness, and may contribute to enduring hypertension in these patients[60]. Blood pressure typically normalizes or shows maximal improvement in one to six months after adrenalectomy, though it can continue to decrease for up to one year following surgery[56].

Multiple studies have looked at outcomes of adrenalectomy for APA to characterize predictive factors for resolution of hypertension. Factors that have been correlated with favorable results include younger age, female sex, lower BMI, fewer pre-operative antihypertensive medications, shorter duration of hypertension preoperatively, fewer first-degree family members with hypertension, better renal function as evidenced by higher glomerular filtration rate, lower creatinine and less proteinuria, lower serum aldosterone and higher urine aldosterone, histopathologic features, and smaller tumor size[58,61-64]. Recently, in a large series, Zhang *et al*[65] showed by multivariate regression that shorter duration of hypertension and lower serum aldosterone level were predictive of resolution of hypertension after adrenalectomy. Furthermore, several studies have linked the *TT* genotype of *CYP11B2* gene encoding aldosterone synthase to successful outcomes after adrenalectomy for PA[66-68].

To better predict which of these features result in resolution of hypertension after adrenalectomy in patients with APA, Zarnegar *et al*[55] proposed the Aldosteronoma Resolution Score (ARS) which takes into account four readily available pre-operative clinical parameters including BMI $\leqslant$ 25 kg/m^2, female sex, duration of preoperative hypertension $\leqslant$ 6 years, and number of preoperative antihypertensive medications $\leqslant$ 2. Each parameter receives a score of 1, with the exception of number of preoperative medications, which is scored by 2 points due to its relative significance in the prediction model. A score

of 0-1 predicts a low likelihood of resolution, while patients with ARS 4-5 have a high likelihood of resolution of hypertension after adrenalectomy. In the study, 27.6% of patients with ARS 0-1 were cured, whereas 75% with ARS 4-5 had complete resolution of hypertension. Using an external cohort, the authors also demonstrated external validity of the model. Utsumi *et al*[61] further validated the accuracy of the ARS model using a Japanese population, confirming the utility of the ARS as a clinical tool for counseling patients on expected surgical outcomes.

While surgery abolishes the source of excess aldosterone secretion and significantly improves or resolves biochemical disturbances and blood pressure control, the long-lasting effects of exposure on the vasculature, heart, brain and kidney have yet to be completely delineated[63]. Nonetheless, several studies have shown that the progression of at least some of these effects are slowed or even reversed by adrenalectomy. Strauch *et al*[60] showed that resection of APA reduced arterial stiffness parameters compared to medical management. Rossi *et al*[15] showed regression of left ventricular hypertrophy in patients with primary hyperaldosteronism after appropriate medical or surgical intervention compared to optimally treated patients with primary hypertension, while Lin *et al*[69] showed adrenalectomy reversed myocardial fibrosis in these patients. Renal function has also been shown to improve after resection with resolution of microalbuminuria in APA patients compared to those with essential hypertension owing to the resolution of relative glomerular hyperfiltration in PA from the volume-expanding and hypertensive effects of the hormone[50,70].

CONCLUSION

Primary hyperaldosteronism is a common and treatable cause of secondary hypertension. Aldosterone excess has been linked to systemic disturbances in the cardiovascular, renal, and vascular systems, in addition to causing hypokalemia and hypertension. Multiple studies have shown worse morbidity with higher rates of myocardial infarction, stroke and renal dysfunction compared to patients with essential hypertension. Depending on the subtype, medical or surgical treatment is effective at halting or even reversing some, if not all, of these effects. Diagnosis and subtype differentiation relies on ARR, possible confirmatory testing, and localization studies with CT and adrenal venous sampling. Unilateral adrenalectomy for patients with APA successfully reverses biochemical disturbances, resolves or significantly improves hypertension, and halts progression of systemic perturbations. Though a variety of parameters have been found to be associated with resolution of hypertension after resection of APA, the ARS is currently the most accurate prediction model for resolution. Adrenalectomy for APA is a safe procedure that should be performed for appropriate candidates to improve long-term outcomes.

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TOPIC HIGHLIGHT

WJC 6th Anniversary Special Issues (1): Hypertension

Anti-hypertensive drugs in children and adolescents

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Abstract

Worldwide the prevalence of essential hypertension in children and adolescents continues to increase. Traditionally providers have used "off-label" drugs to treat pediatric hypertension, meaning that rigorous clinical trials of these drugs have not been specifically performed in pediatric patient populations. Consequently providers have extrapolated dosing, safety and efficacy from trials in adults. This practice is sub-optimal as children demonstrate unique differences in drug metabolism and response. Use of unstudied or understudied drugs increases risk of adverse events and/or can lead to sub-optimal efficacy. Recognizing these concerns, regulatory agencies have created financial incentives for industry to conduct pediatric clinical trials. These incentives, coupled with the emerging pediatric hypertension epidemic, have spurred over 30 clinical trials of anti-hypertensive drugs over the past 15 years and have resulted in labeling of 10 new drugs by the United States Food and Drug Administration for treatment of hypertension in children and adolescents. Unfortunately the financial incentive structures focus on newer drugs and drug classes. Consequently there is now a relative dearth of trial data for older but sometimes commonly prescribed pediatric antihypertensive drugs. This article reviews recent pediatric antihypertensive drug trials with a focus on trial design and endpoints, drug dosing, safety, efficacy and specific drug indications. We also review the available data and experience for some of the more commonly prescribed, but less well studied "older" pediatric antihypertensive drugs.

Key words: Hypertension; Children; Clinical trials; Dosing; Safety

Core tip: This review focuses on the major clinical trials of anti-hypertensive drugs that have been completed over the past 15 years in response to regulatory initiatives by the United States Food and Drug Administration and the European Medicines Agency. These trials have changed the landscape of anti-hypertensive drug management in children.

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INTRODUCTION

Nations throughout the developed world are facing an emerging epidemic of pediatric hypertension that has paralleled an increasing prevalence of childhood obesity[1-5]. In recent cross-sectional studies, greater than one out of every seven United States children and adolescents demonstrate prehypertension with over 3% meeting diagnostic criteria for hypertension[6]. Prevalence trends are similar in population-based assessments in numerous other nations[7-11]. Elevated blood pressure during childhood and adolescence is associated with end organ damage[12,13], most commonly left ventricular hypertrophy, and

is predictive of hypertension in early adulthood[5,14,15].

With increasing prevalence of pediatric hypertension, there is a need for data supporting safety and efficacy of antihypertensive drugs. While a wide variety of antihypertensive drugs have been studied in clinical trials in adults, traditionally there has been a paucity of evidence to support safety and efficacy of antihypertensive drugs in children and adolescents. Consequently, providers were forced to use drugs "off-label", extrapolating dosing and efficacy from adult data[16]. This practice is sub-optimal as children demonstrate unique physiology and pathology, and off-label drug use risks inadequate disease treatment and/or safety events. Furthermore most drugs designed for use in adults do not have pediatric specific tablets or formulations, which can complicate dosing. Recognizing these concerns, regulatory agencies in both the United States and Europe have passed recent regulatory initiatives aimed at stimulating pediatric clinical trials[17,18]. These initiatives have been very successful and over the preceding 15 years, more than 20 clinical trials of antihypertensive agents have been completed in children leading to approval of 10 drugs by the United States food drug administration (FDA) for treatment of hypertension in children and/or adolescents (Figure 1).

This review summarizes the available data and experience supporting the use of antihypertensive drugs in children and adolescents diagnosed with essential hypertension with a particular focus on recent pediatric clinical trials. Angiotensin converting enzyme (ACE) inhibitors, angiotensin Ⅱ receptor blockers, calcium channel blockers, beta blockers and diuretics will be covered with a critical appraisal of available clinical trial data supporting dosing, efficacy, safety, and treatment in specific patient populations. Approval of drugs for pediatric use by the United States FDA will be used as a meaningful benchmark of adequate drug study, reflecting the stringent standards required for FDA approval.

IDENTIFICATION OF CLINICAL TRIAL DATA

To identify anti-hypertensive drug trials in children and adolescents, we used four principle sources: the United States FDA website (http://www.accessdata.fda.gov/), the FDA approved drug label, the European Medicines Agency (EMA) website (http://www.ema.europa.eu/) and PubMed. The FDA website and drug label include detailed information summarizing clinical trials completed in response to an FDA issued written request (a requirement for trials completed for drug labeling) including trial design, drug dosing, efficacy and safety data. Similarly the EMA publishes the results of reviews conducted for EMA pediatric drug approval. We also reviewed publications cited on PubMed for relevant clinical trials. Publications were identified following a PubMed search restricted to children and adolescents ≤ 18 years and using MeSH terms "Hypertension" and "clinical trial".

ANGIOTENSIN CONVERTING ENZYME INHIBITORS

ACE inhibitors target the renin-angiotensin-aldosterone-system (RAAS). ACE converts angiotensin I to angiotensin Ⅱ (Ang Ⅱ), a peptide that causes vasoconstriction and stimulates aldosterone production, itself a potent vasoconstrictor. ACE inhibitors lower blood pressure by decreasing Ang Ⅱ and mitigating its downstream effects. In adults, ACE inhibitors are commonly used antihypertensives and have the additional benefit of reducing cardiovascular and renal events[19]. In pediatric populations, ACE inhibitors are the most commonly prescribed antihypertensive for both primary and secondary hypertension[20,21]. ACE inhibitors have anti-proteinuric effects and are particularly beneficial in children with chronic kidney disease[22-24] (Table 1). However, similar to adult trials, pediatric trials provide evidence that some ACE inhibitors may be less efficacious in blacks[25-27]. In adult anti-hypertensive trials, side effects associated with use of ACE inhibitors include hyperkalemia, chronic cough and angioedema. In pediatric trials there have been no reports of angioedema and there are fewer reports of cough in pediatric compared to adult trials. However, many of the pediatric trials have been of shorter duration[28]. ACE inhibitors are teratogenic and should be discontinued as soon as pregnancy is detected. ACE inhibitors approved for treatment of pediatric hypertension by the FDA include enalapril, fosinopril, benazepril and lisinopril. Table 2 summarizes the FDA label dosing recommendations that resulted from review of the various pediatric clinical trials of these ACE inhibitors.

Enalapril[29]

Enalapril was the first ACE inhibitor approved by the United States FDA for pediatric hypertension following completion of the required clinical trials in 2002 (Figure 1). Compared to placebo, children treated with moderate or high doses (2.5 or 20 mg for children < 50 kg and 5 mg or 40 mg for children > 50 kg) demonstrated significantly lowered diastolic blood pressure (DBP) and systolic blood pressure (SBP). However, the low dose group (0.625 mg/1.25 mg) did not demonstrate lowering of DBP or SBP. There was no significant difference in antihypertensive effects across race, age, sex or Tanner stage. Enalapril was well tolerated and safe in the four-week trial. The most common side effects were dizziness (3.6%) and headache (1.8%), and there was only one drug discontinuation (< 1%) due to adverse events. The enalapril FDA label is unique in that the drug has a pediatric indication for all young children with the only exception being neonates.

Fosinopril[25,30]

Fosinopril was approved for treatment of pediatric hypertension by the United States FDA after the trials (including a 52-wk open label safety assessment) were completed in 2003 (Figure 1). In the clinical trials, all three

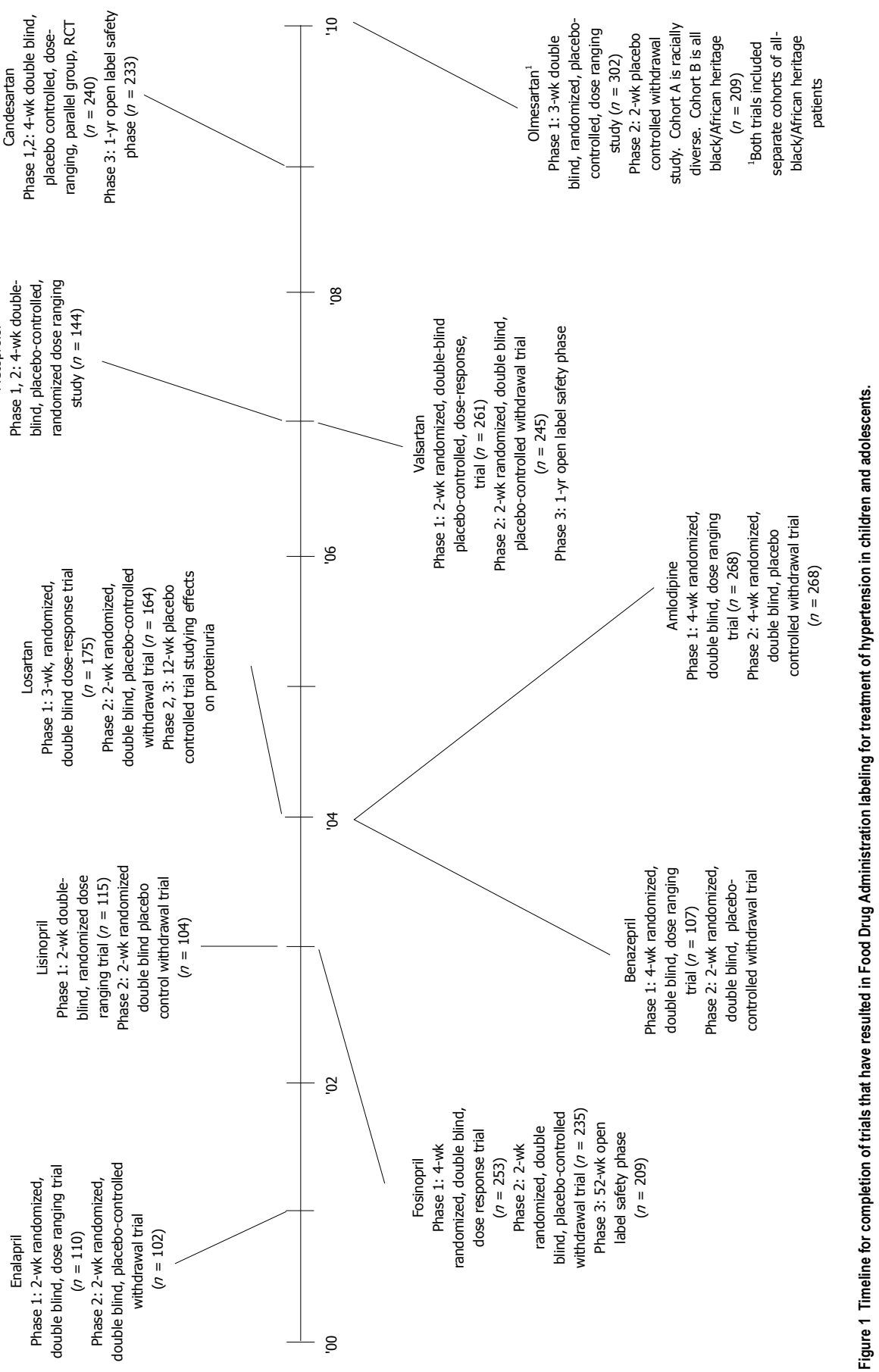

Figure 1 Timeline for completion of trials that have resulted in Food Drug Administration labeling for treatment of hypertension in children and adolescents.

Table 1 Anti-hypertensive class effects

Drug class	Special indications	Precautions	Contraindications	Common adverse events
Angiotensin converting enzymeinhibitors	Proteinuria Chronic kidney disease	Less efficacious in blacks Risk of angioedema, increase risk of hyperkalemia Decreased glomerular filtration rate	Prior history of angioedema with use of ACE inhibitor Discontinue if pregnant: Pregnancy class C in 1st trimester, pregnancy class D in 2nd and 3rd trimester	Headache Dizziness Abdominal pain Nausea Cough
Angiotensin receptor blockers	Proteinuria	Less efficacious in blacks Increase risk of hyperkalemia Decreased GFR	Discontinue if pregnant: Pregnancy class C in 1st trimester, pregnancy class D in 2nd and 3rd trimester	Headache Dizziness Cough
Calcium channel blockers	None	Drug interactions with compounds that change cytochrome P450s metabolism (*i.e.*: Azole antifungals, grapefruit juice, anti-seizure medications)	Pregnancy class C	Headache Peripheral edema Fatigue Dizziness Abdominal pain Epistaxis
Beta blockers	None	Increased risk of bronchoconstriction in asthma	Severe bradycardia Heart block greater than first degree Cardiogenic shock Decompensated cardiac failure	Headache Cough Nasopharyngitis Fatigue Diarrhea Dizziness

Pregnancy class C: Animal reproduction studies have shown an adverse effect on the fetus and there are no adequate and well-controlled studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks; Pregnancy class D: There is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience or studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks; ACE: Angiotensin converting enzyme.

Table 2 Anti-hypertensive drugs that have been studied in pediatric clinical trials for Food Drug Administration labeling

Drug class	Drug	Starting dose	Max dose	Frequency	Suspension formulation	Pediatric indication
Angiotensin converting enzyme inhibitor	Enalapril	0.08 mg/kg (up to 5 mg)	0.58 mg/kg or 40 mg	Daily	Yes	All except neonates
	Fosinopril	0.1 mg/kg (5-10 mg)	0.6 mg/kg or 40 mg	Daily	No	Children > 50 kg
	Lisinopril	0.07 mg/kg (up to 5 mg)	0.6 mg/kg or 40 mg	Daily	Yes	> 6 yr
	Benazepril	0.2 mg/kg (up to 10 mg)	0.6 mg/kg or 40 mg	Daily	Yes	>6 yr
Angiotensin receptor blocker	Losartan	0.7 mg/kg (up to 50 mg)	1.4 mg/kg or 100 mg	Daily	Yes	> 6 yr
	Valsartan	1.3 mg/kg (up to 40 mg)	2.7 mg/kg or 160 mg	Daily	Yes	> 6 yr
	Candesartan	1-6 yr: 0.2 mg/kg 6-17 yr, < 50 kg: 4 mg 6-17 yr, > 50 kg 8 mg	1-6 yr: 0.4 mg/kg 6-17 yr, < 50 kg: 16 mg 6-17 yr, > 50 kg 32 mg	Daily or divided dose	Yes	> 1 yr
	Olmesartan	20 to < 35 kg: 10 mg ⩾ 35 kg: 20 mg	20 to < 35 kg: 20 mg ⩾ 35 kg: 40 mg	Daily	Yes	> 6 yr
	Irbesartan	No FDA pediatric indication (efficacy not demonstrated)				
Beta blocker	Metoprolol XL	1.0 mg/kg (< 50 mg)	2 mg/kg up to 200 mg	Daily	No	> 6 yr
	Bisoprolol	No FDA pediatric indication (efficacy not demonstrated)				
Calcium channel blocker	Amlodipine	2.5 mg	0.3 mg/kg or 10 mg	Daily	No	> 6 yr
	Felodipine	No FDA pediatric indication (efficacy not demonstrated)				
Diuretic	Eplerenone	No FDA pediatric indication (efficacy not demonstrated)				

FDA: Food Drug Administration.

dose levels (0.1, 0.3 and 0.6 mg/kg) of fosinopril were equally effective at reducing SBP and DBP with no dose response in the overall cohort. It remains unclear whether the lack of dose response was attributable to: (1) the dose levels being too high; (2) an overly narrow dose range; or (3) true absence of a dose response. Further analysis showed that fosinopril was effective at reducing SBP in a dose responsive manner in black children however, blacks required a higher dose per body weight to achieve adequate control[25]. Fosinopril was well tolerated with no serious adverse events in the 52-mo open label extension study. Discontinuation of fosinopril secondary to adverse events during the dose ranging and withdrawal phase was rare (1.6%). In the open label extension phase 83% successfully reached target BP with headache (20.1%), nasopharyngitis (9.6%), cough (9.1%), pharyngitis (8.6%), and abdominal pain (6.2%) being the most common adverse events.

Table 3 Other commonly used "off-label" antihypertensive drugs[1]

Drug class	Drug	Starting dose	Max dose	Frequency
Angiotensin converting enzyme inhibitor	Captopril	0.3-0.5 mg/kg per dose	6 mg/kg up to 450 mg/d	Two to three times daily
Beta blocker	Atenolol	0.5 mg/kg per day	2 mg/kg per day up to 100 mg	Once to twice daily
	Propranolol	1 mg/kg per day	16 mg/kg per day up to 640 mg	Two to four times daily
Calcium channel blocker	Extended release nifedipine	0.25 mg/kg per day	3 mg/kg per day up 120 mg/kg per day	Once to twice daily
Diuretic	Furosemide	0.5 mg/kg per dose	6 mg/kg per dose	Twice to three times daily
	Hydrochlorothiazide	0.5-1 mg/kg	3 mg/kg up to 50 mg	Daily

[1]These drugs have not been well studied in pediatric clinical trials and dosing/safety/efficacy are largely extrapolated from trials in adults.

Lisinopril[31]

Lisinopril was approved for pediatric hypertension by the United States FDA in 2003. In the pivotal trial (Figure 1), lisinopril demonstrated a dose response reduction in SBP and DBP that was consistent across age groups, tanner stages, and ethnicity. Lisinopril was safe and well tolerated in the four-wk trial with no serious adverse events and few discontinuations (< 1%). The most common adverse events were headache (3.5%), dizziness from hypotension (1.7%), and abdominal pain (1.7%).

Benazepril[32]

Pediatric trials for benazepril have not been published in the literature, but the United States FDA approved it for pediatric hypertension in 2004 and the trials are summarized on the FDA label (Figure 1). Benazepril significantly lowered SBP but did not exhibit a dose response. Benazepril was well tolerated. The FDA label does not report if any patients discontinued the trial due to drug related adverse events.

Captopril

Captopril is not approved for treatment of hypertension in children and adolescents, as it is an off-patent agent with no financial incentive for industry to sponsor clinical trials. Because captopril was one of the earliest ACE inhibitors approved for use in adults, there is a substantial body of clinical experience in children and adolescents and several trials have demonstrated clinical efficacy[33,34]. However, a major disadvantage of captopril is the need for frequent dosing (typically three times per day) (Table 3).

ANGIOTENSIN RECEPTOR BLOCKERS

Angiotensin receptor blockers (ARBs) target the Angiotensin Ⅱ type 1 receptors located on the heart, kidney, blood vessels, and adrenal glands. By blocking the final step of the RAAS, ARBs inhibit vasoconstriction and lower blood pressure[35]. Similar to ACE inhibitors, ARBs are particularly beneficial in reducing left ventricular hypertrophy in adults with heart failure. In adults and children, ARBs are effective at reducing proteinuria secondary to diabetes and may be particularly useful in patients with chronic kidney disease[36-38] (Table 1). However, ARBs are generally less efficacious in African Americans[26,39-42]. Adults who experience cough and cannot tolerate ACE inhibitors often take ARBs as an alternative[43]. ARBs approved for the treatment of pediatric hypertension include losartan, valsartan, candesartan, and olmesartan. Table 2 summarizes the FDA label dosing recommendations that resulted from review of the various pediatric clinical trials of these ARBs. Children tolerated ARBs well, and the side effects most frequently experienced were headache and dizziness.

Losartan[38,44]

Losartan was the first ARB approved for pediatric hypertension by the United States FDA in 2004 following completion of the required clinical trials (Figure 1). Losartan demonstrated a dose response reduction in SBP and DBP with efficacy demonstrated for the moderate and high dose groups (2.5 or 25 mg for children < 50 kg and 5.0 or 50 mg for children ⩾ 50 kg) but no significant difference in BP between the low dose Losartan or placebo group. There were too few non-white patients to evaluate race related differences in dose repose. Losartan was well tolerated with few discontinuations due to adverse events (< 1%).

Losartan was also studied in a clinical trial focused on reduction of proteinuria in hypertensive (n = 60) and normotensive (n = 246) children with chronic kidney disease[38]. Losartan reduced proteinuria by 35.9% (95%CI: 27.6%-43.1%) and was superior to both placebo (normotensive cohort) and amlodipine (hypertensive cohort). Additionally, Losartan reduced SBP and DBP in both cohorts and was superior to amlodipine, although authors postulated that a lack of change in BP in children on amlodipine was due to titration effect. There were no serious adverse events in this trial and 0.7% of subjects discontinued losartan due to adverse events.

Valsartan[45]

Valsartan was approved for pediatric use by the United States FDA in 2007. The Valsartan pediatric clinical trials are summarized in Figure 1. Valsartan demonstrated a dose response reduction in SBP and DBP but no statistically significant difference in blood pressure between the low or medium-dose groups (10, 20 mg for children < 35 kg and 20, 40 mg for children ⩾ 35 kg). Valsartan's antihypertensive effects were observed across all subgroups including sex, age, tanner stage and race (black and non-black). During the dose response and withdrawal phase

of the study, there were no serious adverse events and few subjects (1.6%) discontinued therapy due to adverse events. Headache (11.6%) and dizziness (2.7%) were the most commonly reported adverse events in the dose response phase. In the 52-wk open label trial, 3.6% of subjects discontinued valsartan due to adverse events. Gastroenteritis (< 1%) and hyperkalemia (< 1%) were the only adverse events considered to be drug-related.

Candesartan[46]

Candesartan was approved for pediatric use by the United States FDA in 2009. Pediatric clinical trials are summarized in Figure 1. In the dose ranging study, Candesartan demonstrated a significant decrease in SBP and DBP compared to placebo at all dose levels but not a dose response. The lack of dose response was attributed to a narrow dose range[46,47]. In the extension study, the 1-year response rate (SBP < 95%) was 52%. Black children had a lesser reduction in SBP and DBP and a lower response rate compared to white children (response rate in black *vs* white 43 *vs* 61%). Drug discontinuation due to adverse events was rare (1% in dose ranging study and 2.1% in open label study) and there were no serious adverse events.

Olmesartan[48]

Olmesartan was approved for pediatric hypertension by the United States FDA in 2010. In clinical trials (Figure 1) olmesartan demonstrated a dose response reduction in SBP and DBP, but the BP reduction was smaller in blacks. Olmesartan was well tolerated and drug discontinuation due to adverse events was rare (< 1%) with no serious adverse events. The most commonly experienced side effects in the 6-wk period were headache (1.7%) and dizziness (1.3%).

Irbesartan[49,50]

Irbesartan was not approved for pediatric hypertension due to lack of efficacy. The irbesartan pediatric trials (Figure 1) failed to demonstrate a dose response and although subjects demonstrated statistically significant increases in blood pressure following drug withdrawal, the effect size (+ 2.3 mg Hg increase in SBP) was small and was not felt to be clinically meaningful. Adverse events were more frequent than in other ARB trials and 2.5% discontinued study drug. There was also one case of erythema multiforme possibly related to irbesartan use.

CALCIUM CHANNEL BLOCKERS

Calcium channel blockers (CCBs) encompass a diverse group of agents with different targets and functions. Second and third generation dihydropyridine CCBs, such as felodipine and amlodipine, are highly selective for vascular smooth muscle and are commonly prescribed for pediatric hypertension[20,21,51]. They target L type (long acting) voltage sensitive calcium channels and inhibit further influx of calcium into already depolarized smooth muscle cells, thereby inhibiting actin-myosin activation and muscle contraction[51]. Unlike ACE inhibitors and ARBs, dihydropyridine CCBs do not demonstrate any anti-proteinuric effects in adults[52-54]; however, other studies have shown renoprotective effects in renal transplant patients[55].

Side effects associated with CCBs include gingival hyperplasia and lower extremity edema. Other side effects such as flushing and headache are more commonly associated with immediate release preparations used for acute hypertension. Dihydropyridine CCBs are metabolized/excreted by the liver and dosing can be affected by drugs or compounds that alter CYP metabolism (*e.g.*, Azole antifungals, grapefruit juice)[51]. Pediatric trials have been performed for the CCBs amlodipine and felodipine and FDA dosing recommendations from these trials are summarized in Table 2. Only amlodipine is approved for treatment of pediatric hypertension as felodipine did not demonstrate efficacy.

Amlodipine[56]

Amlodipine was approved for pediatric hypertension by the United States FDA in 2004. It is the most commonly prescribed CCB for pediatric hypertension[21]. In pediatric trials (Figure 1), amlodipine demonstrated a dose response reduction in SBP and DBP. SBP reduction was slightly greater in females compared to males; otherwise, SBP reduction across race, age, and etiology of HTN did not differ significantly. Amlodipine was generally well tolerated with few discontinuations due to adverse events (2.2%). Reasons for discontinuation included worsening hypertension (1.1%), facial edema (< 1%), edema of the fingers with rash (< 1%), and premature ventricular contractions (< 1%). Peripheral edema, an adverse event commonly seen in adults, was reported in 3.8% of children in dose ranging phase and 2.3% of children in placebo withdrawal phase.

Felodipine ER[57]

Felodipine is a long acting calcium channel blocker that has not been approved for pediatric HTN due to lack of efficacy. The felodipine pediatric trial included a three-wk dose response trial (n = 128) in children with primary hypertension and a 14-wk open label extension period to assess safety. Felodipine was well tolerated (0.8% discontinued due to adverse event) and there were no serious adverse events.

Nifedipine

Nifedipine is a calcium channel blocking agent that was previously frequently prescribed to children and adolescents but was off patent and did not qualify for financial incentives and therefore has not been specifically studied for FDA labeling. Data are lacking on efficacy of short acting nifedipine and concerns have been raised about the dosing formulations which can lead to significant blood pressure fluctuations[34,58]. Sustained release nifedipine perhaps has more utility but also has not been formally studied in children and adolescents and therefore must be used "off-label"[34] (Table 3).

BETA BLOCKERS

Beta blockers have been used for over 40 years and are recommended for hypertension treatment in adults with coronary artery disease, heart failure, post-myocardial infarction, and diabetes because of their beneficial cardiac effects[59]. Beta blockers lower blood pressure by antagonizing the beta 1 adrenergic receptor located on the myocardium to reduce heart rate and decrease contractility. However, beta blockers may also act on beta 2 adrenergic receptors on the smooth muscle of vasculature and the bronchi, increasing peripheral resistance and risk of bronchospasm[60]. Second generation beta blockers such as metoprolol, bisoprolol, and atenolol are relatively more selective for beta 1 receptors compared to first generation non-selective beta blockers, but at high doses, they may act on beta 2 receptors. Compared to other antihypertensives, first and second generation beta blockers are associated with a higher rate of insulin resistance and new onset diabetes[60-64]. The newest class of beta blockers including carvedilol and nebivolol are vasodilatory and do not appear to have negative effects on metabolic profile[60-63].

Bisoprolol and extended release (XR) metoprolol have been studied in pediatric populations for the treatment of hypertension and their FDA dosing recommendations are summarized in Table 2. In both trials, children with asthma were excluded because of the drugs' potential broncho-constrictive effects. Bisoprolol did not demonstrate efficacy and, as a result, extended release metoprolol is the only FDA approved beta blocker for pediatric hypertension. Carvedilol has also been studied in pediatric populations but for the treatment of heart failure[65,66]. Efficacy was not demonstrated and, although indicated for treatment of hypertension in adults, carvedilol has never been studied for this indication in children or adolescents. Nonetheless there are data to support dosing of a pediatric formulation[65]. In all pediatric trials of beta blockers drug-related serious adverse events were rare.

Metoprolol[67]

Metoprolol was approved for pediatric hypertension by the United States FDA in 2007.in clinical trials (Figure 1) metoprolol significantly reduced SBP compared to placebo, but with no dose-response effect. Only high doses of XR metoprolol (2 mg/kg) demonstrated significant reductions in DBP compared to placebo. Authors postulated that the lack of dose response reduction in SBP may have been due to a flattening of the dose response curve or a limitation of the study design. At the end of the dose ranging study, the response rate for metoprolol was 46% (95%CI: 37%-55%). Metoprolol's anti-hypertensive effects were independent of age, Tanner stage, and race. Authors note that overweight patients (BMI > 95%) tended to have less pronounced SBP reductions. Metoprolol was safe and well tolerated with a maximum decrease in heart rate of only 6.5 beats per minute. Drug discontinuation was rare in all trial phases (0.7% in the dose response phase and 5.9% in the open label trial). The most commonly reported adverse events were headache (30%), upper respiratory tract infection (20%), cough (19%), nasopharyngitis (13%), pharyngolarygeal pain (12%), fatigue (9%), diarrhea (7%), and dizziness (6%).

Bisoprolol fumarate/hydrochlorothiazide[68]

Bisoprolol fumarate/hydrochlorothiazide (HCT) (B/HT) is a combination hypertensive that failed to gain United States FDA approval for pediatric hypertension due to lack of efficacy. In a placebo controlled dose ranging pediatric trial (n = 94), the percentage of patients in the B/HT group that achieved blood pressure control (SBP and DBP < 90th%) was not significantly different from placebo (45% for B/HT, 34% for placebo). Discontinuation of B/HT due to adverse events was rare (1.6%) and overall fewer adverse events were reported for the B/HT group compared to placebo.

Propranolol and atenolol

As some of the oldest beta blockers, propranolol and atenolol fall into the category of off-patent drugs that have not qualified for financial incentives and no large pediatric trials have been performed. As a result, propranolol and atenolol are not labeled for treatment of hypertension in children and adolescents. Most pediatric studies of these beta blockers have been in small case series or for other non-hypertensive indications such as arrhythmias, syncope, hypertrophic cardiac cardiomyopathy, portal hypertension. In these studies, propranolol and atenolol have been effective with acceptable tolerability[34]. Due to the lack of pediatric data, dosing, safety, and efficacy have been extrapolated from adult trials (Table 3).

DIURETICS

Most diuretics were off-patent before the implementation in Europe and the United States of financial incentives to conduct pediatric trials. Because off-patent drugs do not qualify for the financial incentives, diuretics represent the class of anti-hypertensive drugs with the least available pediatric clinical trial data. The only diuretic to be tested in a pediatric trial is eplerenone, but it was not approved due to lack of efficacy. Because other diuretics are often used as first line treatment in adults, they will be discussed briefly. Table 3 summarizes generally recognized (albeit not well studied) dosing recommendations for diuretics and select other commonly used antihypertensive drugs that are off-patent and thus have not been studied in clinical trials for FDA or EMA labeling.

Overall, diuretics are a diverse class of drugs that contain some of the oldest and most commonly prescribed agents for adult hypertension[59,69,70]. They can be broadly divided into three categories, thiazide diuretics, loop diuretics, and potassium sparing diuretics. All three classes target different parts of the nephron to decrease sodium

and water reabsorption, thereby creating a natriuretic effect that decreases extracellular volume and reduces blood pressure.

Potassium sparing diuretics

Potassium sparing diuretics inhibit reabsorption of sodium in the collecting duct and can be further divided into two groups, pteridine analogs and aldosterone antagonists. Pteridine diuretics inhibit epithelial sodium channels (ENaC) and aldosterone antagonist down regulate the Na/K pump and (ENaC) on the collecting duct. Potassium sparing diuretics are often used in conjunction with other potassium losing diuretics to maintain serum potassium levels in a normal range[71,72]. Eplerenone is the only diuretic to be studied for FDA labeling but was not approved. In adults, eplerenone is sometimes preferred over spironolactone because it more selectively binds to aldosterone receptors and does not have unwanted progestational and anti-androgenic effects[72].

Eplerenone[73,74]

Eplerenone is a selective aldosterone antagonist that was not approved for pediatric hypertension by the United States FDA due to lack of efficacy. The pediatric trial consisted of a 6-wk dose ranging study (n = 304) and a 4-wk dose withdrawal study (n = 277). Children on concomitant therapy with a potent CYP3A4 inhibitor (clarithromycin, ketoconazole), potassium supplement, or potassium level > 5.5 mEq/L were excluded and eplerenone is considered contraindicated under such circumstances. In children ages 4 to 17 years old, eplerenone did not demonstrate a dose-response effect and reduced SBP was only seen for the high dose level (50 mg twice a day for children > 20 kg). There was no significant difference in DBP compared to the placebo group. Eplerenone was well tolerated with few serious adverse events (2.6%) or discontinuations in the ten-wk trial (< 1%).

Thiazide diuretics

Thiazide diuretics, such as hydrochlorothiazide and chlorthalidone, are first line agents for uncomplicated adult hypertension and are commonly combined with beta blockers, loop diuretics, and ACE inhibitors in multi-drug regimens and in fixed-dose combination formulations[59,75,76]. They are preferred because of their efficacy and superiority in preventing cardiovascular disease compared to other classes of antihypertensives[77]. Thiazides block sodium-chloride co-transporters on the distal convoluted tubule to decrease sodium reabsorption; however, these effects are acute. The exact mechanism by which thiazides reduce peripheral resistance and chronically lower blood pressure is unknown[71,78]. Thiazides are contraindicated in patients with sulfa allergies. Side effects in adults include hypokalemia, hypercalcemia, orthostatic hypotension, worsening of gout (due hyperuricemia), and a worsened metabolic profile (increased rates of new onset diabetes, increase in low density lipoprotein (LDL) cholesterol triglycerides, and glucose)[64,71,78].

Loop diuretics

Loop diuretics (furosemide, bumetanide, torsemide, ethacrynic acid) are most commonly prescribed in combination with thiazide diuretics for reducing fluid volume in edematous disorders or patients with renal failure[71,79]. There is no data supporting the efficacy of loop diuretics alone to reduce blood pressure. When prescribed alone, loop diuretics lower blood pressure acutely, but not chronically because the activated RAAS will compensate for the lost fluid volume. Loop diuretics inhibit the sodium/potassium/chloride transporter (Na-K-2Cl transporter) on the thick ascending loop of Henle to decrease the osmotic gradient producing a potent natriuretic effect. All loop diuretics, other than ethacrynic acid are contraindicated in patients with sulfa allergies. Side effects in adults associated with loop diuretics include hyponatremia, hypokalemia, hypocalcemia, hypomagnesemia, and worsening of metabolic profile (increased cholesterol, LDL, and triglycerides)[71,80].

CONCLUSION

Regulatory initiatives in the United States and Europe over the last one and a half decades have stimulated numerous clinical trials of antihypertensive agents in children. The result has been an increase in the number of United States FDA approved drugs for treatment of pediatric hypertension from zero in 2000 to 11 at present (including esmolol approved for intravenous administration). This is very encouraging with the only caveat that most of the medications studied in pediatric trials belong to newer classes of drugs. There remains a relative dearth of clinical trial data regarding the safety and efficacy of older, commonly used antihypertensive drugs (*e.g.*, diuretics) in children. Nonetheless pediatric providers can now rely on clinical trial data to guide many treatment decisions in children and adolescents with hypertension. FDA labeled antihypertensive drugs have all been safe, efficacious and well tolerated. No deaths and only rare serious adverse events have been reported in clinical trials, albeit most have been of shorter duration. Furthermore, these clinical trials have highlighted the differences between drug safety and efficacy in children versus adults. Many of the approved drugs have demonstrated differences in dosing when compared to adult recommendations and several drugs approved for use in adult patient populations (irbesartan, bisoprolol fumarate/HCTZ, felodipine and eplerenone) have not demonstrated efficacy in pediatric hypertension trials. These data highlight that pediatric drug dosing, safety and efficacy cannot simply be extrapolated from adult clinical trials

As the prevalence of childhood obesity and hypertension continue to rise, it is critical that providers familiarize themselves with these clinical trial data to guide appropriate treatment. Lifestyle changes should continue to form the mainstay of pediatric hypertension therapy; however the importance of medical therapy is increasingly recognized as a means to prevent end-organ damage and hope-

fully limit the long-term cardiovascular risk associated with hypertension.

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TOPIC HIGHLIGHT

WJC 6th Anniversary Special Issues (1): Hypertension

Alcohol-induced hypertension: Mechanism and prevention

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Abstract

Epidemiological, preclinical and clinical studies established the association between high alcohol consumption and hypertension. However the mechanism through which alcohol raises blood pressure remains elusive. Several possible mechanisms have been proposed such as an imbalance of the central nervous system, impairment of the baroreceptors, enhanced sympathetic activity, stimulation of the renin-angiotensin-aldosterone system, increased cortisol levels, increased vascular reactivity due to increase in intracellular calcium levels, stimulation of the endothelium to release vasoconstrictors and loss of relaxation due to inflammation and oxidative injury of the endothelium leading to inhibition of endothelium-dependent nitric oxide production. Loss of relaxation due to inflammation and oxidative injury of the endothelium by angiotensin II leading to inhibition of endothelium-dependent nitric oxide production is the major contributors of the alcohol-induced hypertension. For the prevention of alcohol-induced hypertension is to reduce the amount of alcohol intake. Physical conditioning/exercise training is one of the most important strategies to prevent/treat chronic alcohol-induced hypertension on physiological basis. The efficacious pharmacologic treatment includes the angiotensin-converting enzyme (ACE) inhibitors or angiotensin Ⅱ type 1 receptor blockers (ARBs) which have antioxidant activity and calcium channel blockers. The most effective prevention and treatment of alcohol-induced hypertension is physical exercise and the use of ACE inhibitors or ARBs in the clinic

Key words: Alcohol; Hypertension; Mechanisms; Prevention/treatment; Vascular endothelium

Core tip: This is a comprehensive review of the current mechanisms of alcohol-induced hypertension and strategies for prevention and treatment of alcohol-related hypertension. This updated review will be imperative to basic scientist in the area of cardiovascular physiology/pharmacology and clinicians in the academic, industry as well as clinics and hospitals.

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INTRODUCTION

Alcohol (ethyl alcohol or ethanol, C_2H_5OH) from fermented grain, fruit juice and honey have been used for thousands of years. Fermented beverages existed and alcoholic drinks used in early Egyptian civilization, in China around 7000 BC, in India, between 3000 and 2000 BC, in Babylon as early as 2700 BC, in Greece, and in South America[1]. In the sixteenth century, alcohol (called "spirits") was used largely for medicinal purposes[2]. At the beginning and mid of the eighteenth century, spirits was

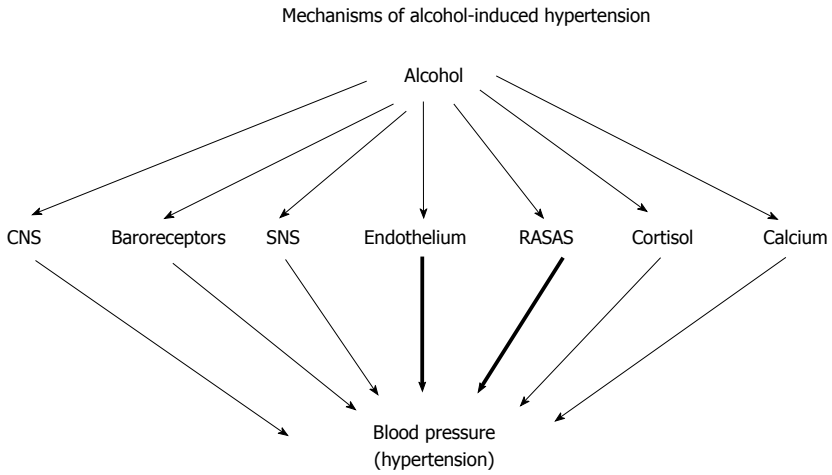

Figure 1 Mechanisms of alcohol-induced hypertension. CNS: Central nervous system; SNS: Sympathetic nervous system; RASAS: Renin-angiotensin system and aldosterone system.

used heavily in Britain. The nineteenth century brought a change in attitudes and the temperance movement began promoting the moderate use of alcohol. In 1920 the United States passed a law prohibiting the manufacture, sale, import and export of intoxicating liquors. Current research suggests that the moderate consumption of alcohol is beneficial to the cardiovascular system and lowers the blood pressure[3-5]. A preclinical study also showed a decrease in systolic blood pressure in rats fed ethanol (1.0 g/kg) for 12 wk[6]. Moderate drinking is generally considered to be: Two drinks a day for men younger than age 65, one drink a day for men age 65 and older and one drink a day for women of any age. A drink is 12 ounces (355 milliliters) of beer, 5 ounces (148 milliliters) of wine or 1.5 ounces (44 milliliters) of 80-proof distilled spirits. Low to moderate drinking has been shown to reduce the incidence of coronary heart disease[3-5] and to increase longevity. It has clearly been a major analgesic, and one widely available to people in pain[1,2,7].

Today, alcoholic beverages are consumed regularly by most of the human societies in the world. However its abuse is a major public health problem in the world. In United States alcohol abuse affects more than 20 million individuals leading to loss of 100000 lives annually[8,9]. Chronic high dose ethanol consumption most commonly causes hepatic, gastrointestinal, nervous and cardiovascular injuries leading to physiological dysfunctions[10]. A cause and effect relationship between regular alcohol consumption and blood pressure elevation (hypertension) was first suggested in 1915 by Lian *et al*[11]. Recent epidemiological and clinical studies have demonstrated that chronic ethanol consumption (more than three drinks per day, 30 g ethanol) is associated with an increased incidence of hypertension and an increased risk of cardiovascular diseases[12-17]. The magnitude of the increase in blood pressure in heavy drinkers averages about 5 to 10 mmHg, with systolic increases nearly always greater than diastolic increases[18]. Similar changes in blood pressure were also reported in preclinical studies[19-22]. In the Framingham cohort[23,24], there was an increase of 7 mmHg in mean arterial pressure when heavy alcohol users were compared with all others. In some epidemiological studies a linear dose-response relationship has been established, sometimes starting with a consumption threshold of 3 drinks per day (30 g of ethanol)[25-33]. In others, the relationship has been nonlinear, especially in women, and some authors have speculated that ingestion of smaller quantities of alcohol may reduce blood pressure[34-38]. Only a few studies have addressed the relationship between alcohol and hypertension in the elderly, and most of them have shown a strong association between hypertension prevalence and alcohol intake[39,40]. However preclinical studies have also shown a linear relationship between blood pressure and ingestion of alcohol[6]. The molecular mechanisms and possible mediators through which alcohol causes vascular injury and raises blood pressure remain elusive. This review focuses the mechanisms implicated with alcohol-induced hypertension and the strategies to control, prevent or to treat alcohol-induced elevation of blood pressure.

MECHANISMS OF ALCOHOL-INDUCED HYPERTENSION

There are several possible mechanisms through which alcohol can raise the blood pressure as shown in Figure 1.

Central nervous system in alcohol-induced hypertension

The World hypertension League speculated that the relatively greater effect alcohol on systolic blood pressure compared with diastolic blood pressure may indicate an imbalance between central nervous system factors influencing cardiac output and the peripheral vascular effects of alcohol[41,42]. There is increasing evidence that alcohol initiates central as well as peripheral reactions which in a synergistic manner have a hypertensive action. In addition, alcohol induces an increased sympathetic outflow, most probably linked to secretion of corticotropin-releasing hormone[43]. Some investigators have suggested that the association between alcohol and hypertension is related to the temporal sequence of alcohol use and blood pressure measurement[24,44]. Since many community programs require an overnight or twelve-hour fasting period, alcohol withdrawal, albeit subclinical, may be oc-

curring. Similarly, patients may abstain or diminish alcohol intake before visiting a clinic or physician. Thus, the observed elevations in blood pressure could be due to excessive central-nervous-system excitability and adrenergic discharge associated with the withdrawal period.

Baroreceptors in alcohol-induced hypertension

Alcohol diminishes the baro (presso) reflex by interacting with receptors in the brain stem, i.e. nucleus tractus solitarii and rostral ventrolateral medulla[43]. Other investigators reported that baroreceptor reflex curves, which indicate the gain in baroreceptor reflex sensitivity, were shifted up and reduced slope in ethanol fed rats when challenged with vasoconstrictors (phenylephrine and angiotensin Ⅱ) compared with controls[45]. This findings and others[42,46,47] suggest the impairment of baroreceptor control and sympathetic system. A greater decrease in heart rate in ethanol treated rats compared with control rats during β-adrenoreceptor blockade with propranolol indicates that the ethanol treated rats had an increased sympathetic activity. An increase in sympathetic activity is consistent with impairment of the baroreceptors that, when activated, inhibit the sympathetic nervous system[45,47]. However this mechanism is implicated more likely in acute alcohol-induced hypertension.

Sympathetic nervous system in alcohol-induced hypertension

Several studies reported increased sympathetic nervous system activation and discharge of sympathetic amines after alcohol consumption[43,48,49]. Alcohol may cause hypertension by affecting the autonomic nervous system[50]. However, alterations in the sympatho-adrenal function that occur during ageing may cause older people to have a different reaction to factors triggering their autonomic system than do younger individuals[51]. The increased sympathetic outflow is expected not only to induce adrenoreceptor-mediated reactions (vasoconstriction, heart rate increase) but to stimulate oxidation reactions[43]. Direct recordings of sympathetic-nerve activity suggest that short-term alcohol ingestion in humans and both short and long-term administration of ethanol in rats stimulates sympathetic-nerve discharge[47,49,50]. Moreover, in rats the alcohol-induced increases in blood pressure and sympathetic activity is centrally mediated[47]. It is possible that alcohol may stimulate adrenals to release adrenaline, resulting in increased heart rate cardiac output and systolic blood pressure[52]. Randin *et al*[53] have also reported that alcohol induces hypertension in rats by sympathetic activation that appears to be centrally mediated. This mechanism is also likely being implicated in alcohol-induced hypertension.

Renin-angiotensin-aldosterone system in alcohol-induced hypertension

The serum levels of vasoactive substances such as renin-aldosterone have been reported to be affected by alcohol ingestion *in vivo* or ethanol *in vitro*[54-56]. Antihypertensive drugs are shown to offer protection against alcohol induced responses in cultured human endothelial cells suggesting the possible involvement of renin-angiotensin system (RAS)[56]. It has been reported that a significant increase in plasma renin activity in patients consuming heavy alcohol compared to mild or moderate alcohol consumption[55,57,58]. However other reports showed no significant in plasma renin activity after alcohol consumption[48,59]. Other studies reported an expansion of the extracellular fluid after alcohol consumption which has been shown to elevate the systolic blood pressure in rats[60,61]. Chan *et al*[60] have proposed that expansion of the extracellular fluid is the result of elevated plasma vasopressin levels and plasma renin activity, indicating increased sympathetic stimulation. Recent studies have shown a significant increased in blood and aortic angiotensin II levels after alcohol ingestion in rats[62,63]. Okuno *et al*[64] have reported prolonged elevation of serum angiotensin converting enzyme (ACE) activity in alcoholics suggests that angiotensin Ⅱ levels are elevated due to activation of ACE activity. Alcohol ingestion in dogs caused sustained RAS activation with progressive increases in plasma levels of Angiotensin Ⅱ, renin activity, left ventricular ACE enzyme activity, and left ventricular myocyte Ang Ⅱ AT1 receptor expression[65]. This mechanism is more likely implicated in alcohol-induced hypertension.

Cortisol in alcohol-induced hypertension

Certain studies have implicated the role of cortisol in alcohol-induced rise in blood pressure[66-68]. Potter *et al*[66] have reported a significant rise in plasma cortisol levels following alcohol consumption and a drop in plasma cortisol levels when alcohol intake was discontinued. Increased cortisol levels in regular alcohol drinkers may be due to direct stimulation of adrenocorticotropin hormone or potentiation of corticotropin releasing hormones by arginine vasopressin[67]. The effect of blood pressure may be due to the mineralocorticoid activity of cortisol or catecholamine hypersensitivity[68]. Alcohol stimulates the secretion of corticotrophin releasing hormone in rats[69,70] leading to stimulation of cortisol secretion[71], sympathetic stimulation and hypertension in rats. However this mechanism is implicated more likely in acute alcohol-induced hypertension.

Increased intracellular calcium and vascular reactivity in alcohol-induced hypertension

Rats treated with ethanol showed constriction of blood vessels[72] due to greater shifts in the binding of the calcium ion (Ca^{2+}) in arterial and arteriolar smooth muscle cells causes increased sensitivity to endogenous vasoconstrictors. This finding is consistent with other reports showing the shifts of the extracellular Ca^{2+} to intracellular space increase the vascular sensitivity to vasoconstrictor norepinephrine[50,61]. It is proposed that alcohol increases intracellular Ca^{2+} by (1) direct upregulation of voltage-gated Ca^{2+} channels; (2) inhibition of Ca^{2+}-adenosine triphosphatase (Ca^{2+}-ATPase) that extrudes Ca^{2+} from the cells; and (3) magnesium ion (Mg^{2+}) depletion that inhibits the sodium ion (Na^{+})-potassium

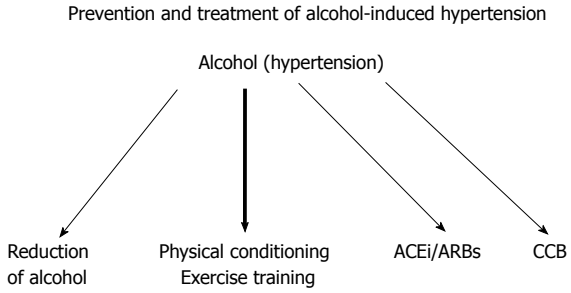

Figure 2 Prevention and treatment of alcohol-induced hypertension. ACEi/ARBs: Angiotensin converting enzyme inhibitor/Angiotensin receptor blockers; CCB: Calcium channel blockers.

ion (K^+) pump (Na^+/K^+-ATPase), causing a build up of intracellular Na^+. This reaction in turn inhibits the Na^+/Ca^{2+} exchanger, thereby increasing the intracellular calcium ion[50,61,72,73]. Chronic alcohol ingestion has been reported to induce a deficiency of blood and intracellular magnesium, which influences cellular Ca^{2+} homeostasis through attenuation of plasmalemmal ATPase activity[74]. Vasdev *et al*[75] have shown that increases in cytosolic free calcium and calcium uptake are associated with ethanol-induced hypertension in rats. Intra-arterial infusion of ethanol has been shown to reduce hand and forearm blood flow in humans[76]. This effect could be the result of a direct vasoconstriction or of a loss of endothelium dependent vasorelaxation[77]. However earlier studies in rats demonstrated no significant response of alpha-adrenergic receptor-mediated constriction of aorta after chronic ethanol ingestion in rats[45,78-80]. On the other hand, the endothelium-dependent relaxation elicited by acetylcholine was diminished in chronic alcohol-induced hypertension[77]. Our earlier study also demonstrated the role of endothelium-independent responses in the aorta of chronic alcohol treated hypertensive rats[79,80]. Inconsistencies among several reports render this mechanism of alcohol-induced hypertension less implicated.

Endothelium and oxidative stress in alcohol-induced hypertension

Imbalance of specific endogenous vasoconstrictor such as angiotensin Ⅱ, endothelin-1 and nor-epinephrine and vasodilator nitric oxide (NO) may also play an important role in alcohol-induced hypertension. Alcohol stimulates the release of endothelin 1 and 2 from vascular endothelium in a dose dependent manner[81]. Alcohol also increases the angiotensin Ⅱ levels in the blood and vessels[62,63]. Endothelin 1 and 2 as well as angiotensin Ⅱ are known to be potent vasoconstrictors of the blood vessels[63,81]. Angiotensin Ⅱ stimulates superoxide production via AT_1 receptor, by activating NADPH oxidase in the vascular wall[82,83]. Superoxide productions through NADPH oxidase activation ($p22^{phox}$ expression) has been demonstrated in rats made hypertensive with angiotensin Ⅱ infusion[84]. Chronic ethanol ingestion induces hypertension which is correlated with elevated tissue angiotensin Ⅱ levels, and activation of NADPH oxidase activity causing endothelial injury in rats[62,79,80]. It is possible that alcohol ingestion raises the blood pressure by decreasing the vasodilators such as NO in the vascular endothelium either due to inhibition of endothelial nitric oxide synthase (eNOS) or inflammatory/oxidative injury to the endothelium. Earlier studies have also shown that chronic ethanol consumption either interferes with NO production or release of NO from endothelial cells[80,85-87]. The diminished NO bioavailability may either be related to reaction with superoxide anion to form peroxynitrite radicals[88] or oxidative inactivation/uncoupling of eNOS by ethanol-induced free radicals[80,89,90]. The production of NO in the endothelium is critically dependent on the function of eNOS which is regulated by vascular endothelial growth factor[91,92]. Alcohol inhibits the enzyme that converts arginine into NO[93] as well as eNOS protein expression[80]. In the endothelium, depletion of NO production or NO reaction with superoxide anion to form toxic peroxynitrite radical which causes endothelial injury, impairment and hypertension in alcohol treated rats[20-22,62,80,94]. Recent studies have shown that chronic ethanol ingestion induces hypertension which was related to increased aortic inflammation, elevated angiotensin II levels, induction of NADPH oxidase causing endothelial injury, depletion of antioxidants, down-regulation of endothelial NO generating system and impaired vascular relaxation in rats[6,19-22,62,80]. This mechanism is most likely implicated in chronic alcohol-induced hypertension.

PREVENTION AND TREATMENT OF ALCOHOL-INDUCED HYPERTENSION

There are few strategies for the control, prevention and treatment of alcohol-induced hypertension as shown in Figure 2.

Studies have shown that a reduction in alcohol intake is effective in lowering the blood pressure both in hypertensives and normotensives and may help to prevent the development of hypertension[12,41,95,96]. Heavy drinkers who cut back to moderate drinking can lower their systolic blood pressure by 2 to 4 mm of mercury (mm Hg) and their diastolic blood pressure by 1 to 2 mmHg. Heavy drinkers who want to lower blood pressure should slowly reduce how much they drink over one to two weeks.

Another non-pharmacological prevention and treatment of alcohol-induced hypertension is physical conditioning or exercise training. There is a physiological basis for effect of physical conditioning on chronic alcohol-induced hypertension in a rat model. Exercise increases the utilization of oxygen in the body and up-regulate the antioxidant defense system in the cardiovascular system[97-100]. Exercise training also generates NO in the cardiovascular system by induction of nitric oxide synthase[19,79,90,101]. Recent studies have shown the beneficial role of physical training in the control of blood pressure in humans[97,98,102,103] and experimental animals[79,90,104,105]. Physical inactivity and overweight trigger hypertension[106,107] whereas; regular physical activity has been shown to decrease the BP and body weight[102,103]. Stud-

ies have shown that physical conditioning is beneficial in lowering the BP through suppression of weight gain in chronic ethanol treated hypertensive rats[19,79]. Physical conditioning attenuates the chronic ethanol-induced hypertension by augmenting the NO bioavailability and reducing the oxidative stress response in rats[19,79,108].

PHARMACOLOGICAL TREATMENT OF ALCOHOL-INDUCED HYPERTENSION

There are no definite clinical data available on the efficacy of specific drugs in the treatment of alcohol-induced hypertension. Randin *et al*[53] have reported that dexamethasone (2 mg per day) in human suppresses the acute alcohol-induced hypertension. It is suggested that ACE inhibitors/angiotensin Ⅱ receptor type 1 (AT_1) blockers, because of their ability to increase the cardiac output in patients with alcohol-induced cardiomyopathy will be useful in the treatment of alcohol-induced hypertension. Cheng *et al*[65] have shown that angiotensin Ⅱ type 1 receptor blockade prevents alcoholic cardiomyopathy in dogs. The calcium channel blockers, because of the probability of the involvement of calcium in the development of alcohol-induced hypertension, may also likely be the drug of choice for the treatment of alcohol-induced hypertension.

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TOPIC HIGHLIGHT

WJC 6th Anniversary Special Issues (1): Hypertension

Pediatric hypertension: An update on a burning problem

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Abstract

A large number of adults worldwide suffer from essential hypertension, and because blood pressures (BPs) tend to remain within the same percentiles throughout life, it has been postulated that hypertensive pressures can be tracked from childhood to adulthood. Thus, children with higher BPs are more likely to become hypertensive adults. These "pre-hypertensive" subjects can be identified by measuring arterial BP at a young age, and compared with age, gender and height-specific references. The majority of studies report that 1 to 5% of children and adolescents are hypertensive, defined as a BP > 95th percentile, with higher prevalence rates reported for some isolated geographic areas. However, the actual prevalence of hypertension in children and adolescents remains to be fully elucidated. In addition to these young "pre-hypertensive" subjects, there are also children and adolescents with a normal-high BP (90th-95th percentile). Early intervention may help prevent the development of essential hypertension as they age. An initial attempt should be made to lower their BP by non-pharmacologic measures, such as weight reduction, aerobic physical exercise, and lowered sodium intake. A pharmacological treatment is usually needed should these measures fail to lower BP. The majority of antihypertensive drugs are not formulated for pediatric patients, and have thus not been investigated in great detail. The purpose of this review is to provide an update concerning juvenile hypertension, and highlight recent developments in epidemiology, diagnostic methods, and relevant therapies.

Key words: Children; Hypertension; Blood pressure; Epidemiology; Diagnosis; Therapy

Core tip: It is generally presumed by cardiologists that arterial hypertension is a disease that typically develops only in adult life. However, a number of studies testify that this pathologic process can begin early in childhood, as evidenced by occasional increases in blood pressure (BP) or abnormal BP responses to physical or psychological stress. This review provides a detailed analysis concerning the epidemiology, diagnostic methods, and therapies for pediatric hypertension.

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INTRODUCTION

Although secondary arterial hypertension (HTN) was thought to be more frequent than essential arterial HTN in children, recent reports indicate that essential HTN is the most frequently manifested form of the disease during both childhood and adolescence[1]. Pediatric HTN is now commonly known worldwide to be an early risk factor for cardiovascular morbidity and mortality. The essential HTN subsequently detected in adults may have already been manifested at an early age, observed as occasional raises in blood pressure (BP) or abnormal BP response to physical or psychological stress. Similar to

other types of chronic illness, the hypertensive process likely develops several decades prior to the onset of clinical signs and symptoms[2,3]. As BP levels are typically retained throughout life, children with higher BPs are more likely to become hypertensive adults[4].

Extensive normative data on BP in children have long been available both in the United States and Europe. Pediatric BP nomograms were developed by the Task Force on BP Control in Children, commissioned by the National Heart, Lung, and Blood Institute of the National Institutes of Health, by using results obtained from 83000 children and adolescents of both genders. The percentile curves were first published in 1987 and described age-specific distributions of systolic and diastolic BPs for an age range between 5 and 17 years, with corrections for height and weight[5]. The third report from the Task Force, published in 1996, provided additional details regarding the diagnosis and treatment of HTN in infants and children[6]. In 2004, the fourth report added further information and adapted the data to growth charts previously developed from the Centers for Disease Control and Prevention[7]. In an update to the HTN guidelines published by the European Society of Cardiology in 2009, a new chapter was devoted to HTN in children, with an approach similar to the American version[8].

In accordance with the recommendations of the Task Force, BP is considered normal when the systolic and/or diastolic values are less than the 90th percentile for the child's age, sex, and height. BP is considered high for systolic and/or diastolic values > 95th percentile. For BPs between the 90th and 95th percentile, a new category (pre-HTN) has been introduced, defined as a BP ⩾ 120/80 mmHg. In cases where systolic and diastolic pressures are discrepant with respect to classification, the child's condition should be categorized using the higher value[7].

BP usually depends on the balance between cardiac output and vascular resistance. BP increases following a rise in either of these variables without a compensatory decrease from the other[9]. Factors affecting cardiac output include the following: baroreceptors, extracellular volume, effective circulating volumes of atrial natriuretic hormones, mineralocorticoids, and angiotensin, as well as contributions from the sympathetic nervous system[10]. Factors influencing vascular resistance include pressors, such as angiotensin Ⅱ, calcium (intracellular), catecholamines, vasopressin and the sympathetic nervous system, as well as depressors, such as atrial natriuretic hormones, endothelial relaxing factors, kinines, prostaglandin E_2 and prostaglandin I_2[10].

Changes in electrolyte blood concentrations (particularly changes in sodium, calcium, and potassium levels), may also affect vascular resistance. Under normal conditions, extracellular volume is maintained by the excretion of sodium in amounts equal to those ingested. Retention of sodium results in an increase in extracellular volume, and an elevation of BP. Sodium balance is restored by renal changes in both the glomerular filtration rate and the tubular reabsorption of sodium, resulting in natriuretic excretion of excess sodium. Elevated calcium concentrations can increase vascular smooth cell contractility, and stimulate the release of renin, synthesis of epinephrine, and enhance sympathetic nervous system activity and increasing BP. Reduced potassium intake can also increase BP by stimulating the production and release of renin and reducing natriuresis. The renin-angiotensin system and the hypothalamus-hypophysis-adrenal gland axis are suspected to be involved in the elevation of BP as well[11,12]. This complexity demonstrates the difficulty in identifying the mechanism that accounts for HTN, and explains why treatment is often designed to affect regulatory factors rather than the cause of the disease. For example, BP can be elevated as a result of increased sodium renal reabsorption, insulin resistance, leptin resistance, vascular resistance, and sympathetic tone caused by hyperinsulinemia.

EPIDEMIOLOGY

The prevalence of HTN in the pediatric population was examined as early as 1963[13], though the precise rates are not known. The majority of studies report rates ranging from 1% to 5%, although prevalence as high as 10% has been reported for some isolated geographic areas[14-19]. However, regression to the mean from repeated measurements included from more recent studies has placed the prevalence of HTN at less than 5%[19]. The discrepancy in reported values is likely due, in part, to the arbitrary definition of HTN and the BP measurement method[20]. The frequent use of non-specific population BP nomograms may exaggerate the prevalence of HTN in children and adolescents in some specific geographic areas. In fact, genetic and environmental differences can influence HTN incidence between regions. Although reference standards established in the US have been adopted worldwide, many local percentile curves are still being used, especially in northern Europe[14,16,20,21], and clinicians from every ethnic group or geographic area in the world should produce their own national BP nomograms relating to age, gender, and height.

A review by Chiolero *et al*[13] examined the HTN prevalence rates reported in large-scale school-based studies (> 2000 children) from all over the world published between 1980 and 2006. Most studies determined HTN from a single BP measurement, with a prevalence of isolated systolic HTN at 7.2%-19.4%. However, in the only study where three different BP measurements were used, the overall prevalence of HTN was 4.5%. While some authorities recommend only one recording, others advocate taking the average of two or three pressures, which more accurately reflects the overall BP of the individual patient[22]. According to the United States Task Force, elevated pressures must be confirmed on repeated visits before characterizing a child as having HTN, with at least three different measurements strongly recommended[7,14]. The prevalence of pediatric HTN can also be influenced by the method of measurement, as oscillometric devices vary by manufacturer and require validation and calibration, and auscultation is subject to operator-dependent

biases such as rounding errors (digit preference), expectation bias, and operator skill[23].

Recent reports on the prevalence of normal-high BP or pre-HTN (between 90th and 95th percentile) in younger individuals is concerning, as it is associated with an intermediate degree of organ damage[24]. In three different recent surveys performed in the United States, the prevalence of normal-high BP ranged from 3.4% to 31.4% in large cohorts of children and adolescents, which was largely influenced by age and weight[25-27]. In a study on high school students by McNiece *et al*[28], the prevalence of combined pre-HTN and HTN was over 30% in obese boys and 23%-30% in obese girls, depending on ethnicity. A three-year longitudinal screening of BP in Italy found that pediatric pre-HTN and HTN are equally prevalent[29,30].

DIAGNOSTIC TOOLS

Two non-invasive methods, auscultatory and oscillometric, are typically used to diagnose HTN in children and adults. When the auscultatory method is used, pediatric systolic BP is defined on the basis of the first Korotkoff sound, while the diastolic BP usually corresponds to the fifth Korotkoff sound[31]. However, a meta-analysis from the Bogalusa Heart Study indicated that the fourth Korotkoff sound is a more reliable measure of diastolic BP and a better predictor of adult HTN than the fifth[32]. Moreover, a comparison of these methods for BP measurement in the San Antonio Triethnic Children's BP Study indicated that systolic and diastolic pressure readings were 10 and 5 mmHg higher, respectively, with an oscillometric compared to an auscultatory device[33]. Thus, caution must be used when diagnosing HTN with an automated device.

The current United States Task Force recommendation for choosing an appropriate size cuff for measuring BP is a bladder width equal to 40% of the upper arm circumference (UAC). However, most physicians use the older two-thirds or three-fourths upper arm length (UAL) recommendations to choose a cuff, and significant differences have been highlighted between the methods. Specifically, systolic BP measured using the 40% UAC criterion reflects a directly measured radial arterial pressure and significantly overestimates the diastolic pressure. Using available cuffs for indirect measurements by two-thirds and three-quarters UAL criteria significantly underestimates systolic as well as diastolic BPs when compared with radial intra-arterial BP[34]. Therefore, recommendations for BP cuff selection should be reviewed. Moreover, labeling of BP cuffs for infant, pediatric, small adult, adult, and large adult patients is misleading, and such designations should be eliminated. Cuff sizes should be standardized, indicate bladder size, and be uniformly color-coded for convenience[35].

Twenty-four-hour ambulatory BP monitoring (ABPM) can more precisely characterize changes in BP during daily activities, and is superior to clinical BP monitoring in predicting cardiovascular morbidity and mortality in adults[36,37]. As children and adolescents tend to be more emotional with consequent BP raises that can indicate HTN, ABPM may differentiate those with "white coat" HTN from those with chronic HTN. As a result, ABPM is gaining acceptance as a useful modality for the evaluation of BP levels in research and clinical settings[38,39], and may help overcome some of the challenges clinicians face when attempting to categorize a young patient's BP levels[40]. ABPM is recommended for the standard assessment of pediatric patients for confirming the diagnosis of HTN (*e.g.*, exclusion of "white coat" HTN), evaluating for the presence of masked HTN, assessing BP variability, determining dipping status in patients at high risk for larger organ damage (*e.g.*, those suffering from sleep apnea), assessing the severity and persistence of HTN, and evaluating BP levels in chronic pediatric diseases associated with HTN. In addition, ABPM can be used to evaluate the effectiveness of drug therapy, monitor for drug-resistant HTN, and determine whether symptoms are a result of drug-related hypotension.

PEDIATRIC BP MONITORING

For monitoring BP in children, a suitable ABPM device should be selected, such as devices with appropriate cuff sizes that have been validated according to the standards set by the Association for Advancement in Medical Innovation or the British Heart Society. Moreover, individuals with specific training in the application and interpretation of ABPM data in pediatric patients should obtain the readings using a standard approach. Monitors should be applied to the non-dominant arm unless contraindicated (*e.g.*, the presence of an arteriovenous fistula). After application, results should be compared with resting BP measured in the clinic using the same technique as used by the ambulatory device (auscultatory or oscillometric). Calibration between methods should be considered adequate when there is agreement within 5 mmHg between the average of three clinic and three ambulatory BP measurements. Cuff placement and proper device function should be verified for values falling outside this range. Wide disagreement between resting and ambulatory device measurements of diastolic BP may occur with the use of auscultatory ABPM devices that lack pediatric settings to adjust for the larger fourth and fifth Korotkoff sound differences often seen in younger children. If this occurs, an oscillometric device may be preferred, or interpretation may be restricted only to the values for systolic BP.

Patients or their guardians should be instructed to record their antihypertensive medication intake, and activity, sleep, and wake times in a diary. As a sufficient number of valid BP recordings are needed to provide interpretable data, devices should be programmed to record BP every 20 to 30 min during waking hours and every 30 to 60 min during sleep hours. ABPM recordings should be edited for outlying values and data should be visually inspected for gross inconsistencies, such as BPs and heart rates that fall considerably outside the ranges normal for the patient's age, such as a systolic BP 60 to 220 mmHg,

diastolic BP 35 to 120 mmHg, heart rate 40 to 180 beats per minute, or pulse pressure 40 to 120 mmHg. As a general rule, the above stated limits should be programmed into the ABPM software to minimize subjective editing of ABPM data. Standard calculations should be reported (mean ambulatory systolic and diastolic BP during the 24 h, daytime, and nighttime periods). Dipping (percent day-night difference) should be determined for systolic and diastolic pressures: (mean daytime BP-mean nighttime BP)/mean daytime BP × 100. ABPM levels should be interpreted using appropriate pediatric normative data, such as gender- and height-specific data obtained from large pediatric populations using similar techniques. A diagnosis of HTN is indicated by significant abnormalities in ambulatory BP levels and loads occurring during the daytime, nighttime, or the entire 24-h period[41,42].

THERAPY

An initial attempt should be made to lower BP by means of non-pharmacologic procedures, in spite of scientific evidence underlining the limited efficacy of this type of approach. There is a strong association between BP values and body weight, and weight loss in children is correlated with lowered BPs[43-48]. Therefore, the primary objective should be to achieve and maintain a normal body weight. Regular exercise and a reduction in sedentary activities (such as watching TV or playing videogames) will result in enhanced weight loss and improved BP values[49]. In addition, the intake of sugary drinks and calorie-rich foods should be limited and fresh fruits and vegetables encouraged, to ensure a satisfying and healthy diet. The help of a dietician specializing in the treatment of children and adolescents may be particularly useful in motivating self-control[50-52].

Sodium intake should also be limited. Many studies have reported that a reduced-sodium diet decreases BP values in children by 1-3 mmHg[53-58]. A randomized trial demonstrated that adolescent BPs were significantly reduced by limiting sodium intake in early childhood[59]. Current recommendations for sodium intake are 1.2 g/d for children between the ages of 4 and 8 years, and 1.5 g/d for older children[60], which are lower than the amount of sodium present in a typical daily diet. Thus, a reduction in salt intake together with a reduced-calorie diet may enhance the effects achieved by weight loss alone[7]. Other lifestyle changes, such as improving the quality of sleep or quitting smoking, can also help to lower BP[61,62].

Pharmacologic treatment is indicated for HTN that persists despite these lifestyle changes, as well as for secondary HTN, HTN associated with organ dysfunction, and HTN in diabetic patients (type 1 and type 2), according to United States guidelines[7]. In addition, children or adolescents with dyslipidemia, although not included in the therapeutic indications, may also benefit from administration of a low-dose antihypertensive therapy[63,64]. The main therapeutic aim is to reduce BP to below the 95th percentile, or below the 90th percentile if other cardiovascular risk factors are present. The number of antihypertensive drugs specifically indicated for use in children has risen considerably in recent years, including beta-blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin-receptor blockers, calcium channel blockers, and diuretics. Trials using these drugs in children have been directed almost exclusively at assessing their efficacy in lowering BP, and show these drugs to be safe and well-tolerated with satisfactory short-term BP reduction[7]. Several classes of drugs are particularly indicated for use in hypertensive subjects with specific concomitant diseases. As an example, ACE inhibitors and angiotensin-receptor blockers are recommended for hypertensive diabetics or those with microalbuminuria, as well as in patients with chronic renal failure and proteinuria, whereas beta-blockers and calcium channel blockers are indicated for use in patients affected by migraine and headache. The lowest dose of antihypertensive drug should be used and gradually increased until the desired BP values are achieved. If peak doses are reached without any appreciable benefit, or the young patient manifests adverse effects, it may be advisable to implement a combined therapy with a second drug that enhances the efficacy of the first[7]. Particular care should be taken in monitoring children for organ dysfunction and potential adverse effects, as well as in assessing the efficacy of treatment. For example, children undergoing treatment with ACE inhibitors and/or diuretics should be carefully monitored for electrolytic balance. Combination pharmacotherapy in children has not been well studied, and is not recommended as an initial treatment. Multi-drug combinations, such as bisoprolol and hydrochlorothiazide, should only be prescribed in particularly unresponsive and severe cases[65]. A "step-down" therapy may be implemented in patients achieving satisfactory BP values over a lengthy period, such as a gradual tapering of treatment in overweight/obese children who have lost a significant amount of weight. In some cases, treatment may be withdrawn, though patients should undergo long-term follow-up to monitor for relapse[7,66].

Pharmacologic treatment is inherently more difficult in children than it is in adults. Unlike children, adults are able to learn to live with their condition, maintain treatment compliance, and are mindful of the consequences of untreated HTN[67]. The majority of drugs available for the treatment of HTN do not have pediatric formulations, and often assume a distasteful flavor when divided or pulverized, though thiazide diuretics (hydrochlorothiazide and chlorthalidone), calcium channel blockers (lercanidipine), and angiotensin receptor antagonists (candesartan) do not have any flavor and can therefore easily be administered to small children[65,68]. Drugs that are prescribed for hypertensive children should have minimal side and prolonged therapeutic effects, though slow-release formulations should be avoided as they are poorly absorbed by children and lose their prolonged effect once the tablets are split. Adverse side effects are most frequent with diuretics, followed by beta-blockers, calcium antagonists, ACE-inhibitors and fast angiotensin receptor blockers[65,68]. Moreover, similar to other classes

of compounds, antihypertensive drugs have different pharmacokinetics in children, particularly in very young children; therefore, drug dosage should be adjusted[65,68].

CONCLUSION

Cardiovascular diseases such as HTN develop slowly and their pathogenesis often begins in childhood. A routine use of specific and carefully constructed BP tables will allow pediatric clinicians and cardiologists to identify pathophysiologic conditions in their patients that may only clinically manifest after several decades. The diagnosis and treatment of pediatric high BP and HTN should therefore be considered as a preventative measure, rather than simply the tracking of an early predisposition to a fatal destiny in adulthood.

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TOPIC HIGHLIGHT

WJC 6th Anniversary Special Issues (1): Hypertension

Potential pathophysiological role for the vitamin D deficiency in essential hypertension

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Abstract

Vitamin D deficiency has been indicated as a pandemic emerging public health problem. In addition to the well-known role on calcium-phosphorus homeostasis in the bone, vitamin D-mediated processes have been recently investigated on other diseases, such as infections, cancer and cardiovascular diseases. Recently, both the discovery of paracrine actions of vitamin D (recognized as "local vitamin D system") and the link of vitamin D with renin-angiotensin-aldosterone system and the fibroblast growth factor 23/klotho pathways highlighted its active cardiovascular activity. Focusing on hypertension, this review summarizes the more recent experimental evidence involving the vitamin D system and deficiency in the cardiovascular pathophysiology. In particular, we updated the vascular synthesis/catabolism of vitamin D and its complex interactions between the various endocrine networks involved in the regulation of blood pressure in humans. On the other hand, the conflicting results emerged from the comparison between observational and interventional studies emphasize the fragmentary nature of our knowledge in the field of vitamin D and hypertension, strongly suggesting the need of further researches in this field.

Key words: Vitamin D; Hypertension; Cardiovascular disease; Renin; Angiotensin

Core tip: This review provides a comprehensive and critical analysis of the most recent studies investigating the relationship between vitamin D and essential hypertension. From the both observational and interventional studies, conflicting results have been shown. This review article provides some hypothesis to explain these discrepancies. In addition to the potential bias related to the study design, some pathophysiological explanation was suggested, especially involving the potential role of local vitamin D system as well as the fibroblast growth factor 23/klotho axis. This review aims at suggesting a careful reflection so that future studies might be designed for minimize bias and encompass the complex biology of vitamin D system.

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INTRODUCTION

Vitamin D deficiency has recently emerged as a public health problem, affecting almost 50% of the population worldwide[1]. In addition to the reduced exposition to sunlight[2], also genetic and environmental factors have been suggested as a cause of this pandemic, such as pollution, diet, sedentary life style and stress[3]. Moreover, vitamin D is no longer considered as only a pivotal mediator of calcium metabolism and skeletal health, but it also regulates several cell functions, including differentiation and metabolism. This aspect may explain the reason why hypovitaminosis D has been proved to be an independent risk factor for overall mortality in various cohort analyses[4], whereas vitamin D supplementation significantly reduced mortality[5]. Moreover, similar data were collected from different clusters of inflammatory and chronic diseases, such as infections[6], autoimmunity[7], neurodegenerative pathologies[8], as well for cancer[9]. However, a special interest was conferred to the potential relationship between vitamin D and cardiovascular (CV) disorders. Although in human cohorts low vitamin D levels were associated with impaired CV outcomes[10], a causal relationship remains unknown, and the general enthusiasm about the benefits of vitamin D supplementation have been recently replaced by words of caution.

On the other hand, novel topics that might address many question in the field of vitamin D, such as fibroblast growth factor (FGF) 23-klotho axis, non-genomic effects of vitamin D and the paracrine effects of vitamin D (also called "local vitamin D system") have been identified. In the following paragraphs, we will focus on the mechanisms triggered by vitamin D in arterial hypertension, starting from the complex interplay with the renin-angiotensin-aldosterone system (RAAS) in both basic research and clinical trials.

VITAMIN D SYSTEM AND BLOOD PRESSURE

Vitamin D

In humans, more than 80% of vitamin D requirements is produced through the ultraviolet-B (UVB)-induced conversion of 7-dehydrocholesterol to vitamin D in the skin, whereas only 10%-20% is absorbed with the diet[1]. The photosynthesis of vitamin D evolved over 750 million years ago, first in the phytoplankton and then in early plants and animals[11]. From an evolutionary stand point it is interesting to note that the first living beings synthetizing vitamin D were missing calcific skeleton. This suggests that a new recognized non-metabolic role (called "non-classical effects") of vitamin D might actually be the oldest. Regardless of the source, vitamin D requires liver hydroxylation [through 25-hydroxylase (CYP2R1 or CYP27A1)] to form 25-hydroxyvitamin D [25(OH) vitamin D or calcidiol], inactive form but used as reference for vitamin D status, because abundant, stable and easier to quantify[1]. In the kidney 25(OH) vitamin D is then hydroxylated to 1,25-dihydroxyvitamin D [1,25(OH)2 vitamin D or calcitriol] the active form of vitamin D [through 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1)]. This latter step is a pivotal effector of calcium homeostasis and thus highly controlled by the up-regulation of parathyroid hormone (PTH) and the suppression of FGF23/klotho axis[12]. Although the exact contribution of extra-renal hydroxylation in determining the circulating levels of 1,25(OH)2 vitamin D is still unknown, it has been recognized also an extra-renal activity of CYP27B1. Finally, the recent identification of a role of vitamin D binding proteins on vitamin D catabolism has further increased complexity of the system[13].

Vitamin D receptor

Vitamin D receptor (VDR) is member of nuclear hormone receptors superfamily. Following binding with 1,25(OH)2 vitamin D, VDR recruits one of the retinoid X receptors (RXR α, β or γ) forming homo- or heterodimers to promote a specific, high-affinity DNA-binding interaction. This transcriptional complex binds to repeated sequences of 6 hexamers [vitamin D response elements (VDRE)] in the promoter region of target gene[1]. VDR is believed to directly or indirectly regulate 3% to 5% of human genome and the different genomic activation of vitamin D in the different cell types involves allosteric influences, VDRE location and epigenetic modification (of both DNA and histones)[14]. In addition, VDR recognizes extra-nuclear ligands including endogenous steroids and other lipophilic compounds[15,16]. Finally, VDR may be expressed also on the cell surface membrane and within mitochondria thus might modulate non-genomic signalling pathways, such as 1,25(OH)2 vitamin D-mediated rapid-response[17]. Vitamin D are deeply involved in several patterns of CV pathophysiology, including vascular inflammation[18] and endothelial dysfunction[19] as observed in patients with chronic kidney disease (CKD)[20] and type 2 diabetes[21] as well as in asymptomatic subjects[22]. For instance, in vitro VDR activation induces nitric oxide production in endothelial cells[23] and improves the angiogenic properties of endothelial progenitor cells[24], while regulates proliferation[25], migration[26], mineralization[27] and thrombogenic protein expression[28] in vascular smooth muscle cells (VSMCs). The recent recognition of specific VDR polymorphisms and genetic susceptibility in pathophysiology of hypertension has further supported these insights[29].

Vitamin D hydroxylases

The gene encoding for CYP27B1 is widespread expressed in various tissue of endodermal, ectodermal and mesenchymal origin. Since even VDR is highly represented in tissues, an autocrine/paracrine vitamin D system has been strongly suggested. In contrast to endocrine vitamin D system, local regulation of 1,25(OH)2 vitamin D levels

is independent of PTH expression, but rather relies on environmental factors[30]. CYP27B1 expression in endothelial cell is regulated by pro-inflammatory cytokines[31], in VSMCs is under estrogenic control[32] whereas many signals regulate the expression in monocyte/macrophage, including toll-like receptor[33], interferon-γ[34], FGF23[35] and uremia[36]. Accordingly, CYP27B1$^{-/-}$ mice develop an hypertensive phenotype, also characterized by increased circulating level of renin, angiotensin (Ang) II and aldosterone, then suppressed by administration of 1,25(OH)2 vitamin D independently of serum levels of calcium or phosphorus[37].

Vitamin D and FGF23/Klotho pathways

Recently, the discovery of FGF23 has extended the complexity of the endocrine network involving the vitamin D system. As vitamin D counter-regulatory hormone, FGF23 suppresses renal synthesis of 1,25(OH)2 vitamin D by inhibiting CYP27B1 and up-regulating CYP24A1. These effects are independent of VDR but require co-factor klotho, essential for FGF23 signal transduction[38]. Overall, 1,25(OH)2 vitamin D and FGF23 are involved in a classical hormonal loop also including PTH. High levels of 1,25(OH)2 vitamin D raise the serum concentrations of both calcium and phosphate. Concomitantly, the feedback by PTH reduces only calcium levels by enhancing its urinary excretion. Increased levels of FGF suppress the expression of sodium-phosphate cotransporter NaPi-2a on renal proximal tubules, thus resulting in increased phosphaturia[39]. Therefore, phosphorus homeostasis might be maintained by 1,25(OH)2 vitamin D *via* a direct regulation on FGF23 levels.

Thus, the discovery of FGF23 might explain some paradoxical concerns on vitamin D, especially among the ambiguous results of interventional studies. A strong correlation between an increased risk of mortality and high circulating levels of both FGF23 and phosphate has been also reported[40,41], suggesting that there is a threshold in vitamin D supplementation beyond which 1,25(OH)2 vitamin D may have detrimental effects.

For instance, the age-associated suppression of Klotho expression[42] may promote a vitamin D toxicosis during therapeutic supplementation characterized by over-hyperphosphatemia and thus increased cardiovascular risk[43]. Although it is likely a failure of the normal feedback mechanism regulating vitamin D and FGF23, the molecular bases of these clinical features have not been identified yet. Furthermore, Camalier *et al*[44] recently provided evidence of both rapid and late effects induced by FGF23 on mesenchymal stromal cells, involving cell proliferation and extracellular matrix (ECM) regulation. In addition, Jimbo *et al*[45] showed that FGF23 promoted osteoblastic differentiation of aortic VSMCs from uremic rats by inducing ERK1/2 phosphorylation pathway. However, it should be noted that these features were shown only in primary rat VSMCs and other studies failed to recognize the relevance of FGF23-Klotho signalling in mouse arteries[46,47].

Ultimately, although further studies in humans are warranted, we agree with Glade M.J., who suggested that there may be an age at which vitamin D deficiency may become life-sustaining, not life-threatening[48].

PATHOPHYSIOLOGICAL PATHWAYS OF VITAMIN D IN HYPERTENSION

Although the effects of vitamin D on blood pressure have been known for several decades, some physiological aspects on the modulation of vascular cells and the vascular tone still remain to be clarified.

RAAS

RAAS plays a pivotal role in maintaining sodium and blood volume homeostasis even by modulating the renal function and blood pressure. RAAS up-regulation was shown to promote the development of hypertension and increased CV risk[49,50].

Salt- and volume-independent RAAS up-regulation (documented by an increase in renin and Ang II levels) was associated with hypertension and cardiac hypertrophy in VDR$^{-/-}$ mice[51]. Similarly, in wild-type mice, 1,25(OH)2 vitamin D inhibition (through dietary intake of strontium) increased renin expression, while 1,25(OH)2 vitamin D supplementation down-regulated RAAS in a VDR-dependent manner[51].

Also the evidence of a preserved CV function in VDR$^{-/-}$ mice undergoing RAAS inhibition (using Angiotensin converting enzyme inhibitors or Angiotensin receptor I blockers) confirmed a direct connection between RAAS and vitamin D system[52]. Interestingly, similar results were also reported in CYP27B1$^{-/-}$ mice[37]. Among the several cross-sectional and prospective studies investigating the association of vitamin D deficiency and hypertension only Forman *et al*[53] provided a mechanistic role of vitamin D system in the RAAS regulation. Lower 25(OH) vitamin D levels correlated with both higher Ang II at baseline (P = 0.03), and blunted renal plasma flow response to Ang II infusion in a cohort of 184 normotensive subjects treated with high-salt diet. These findings were confirmed in subsequent studies[54,55].

From a molecular point of view, the research group directed by Li *et al*[52] discovered a direct effect of 1,25(OH)2 vitamin D on renin gene transcription. They identified that vitamin D is capable of suppressing renin gene transcription by a cAMP response element, identified on the promoter region of *Ren-1c* gene[56]. In addition, the same authors confirmed a central role of active vitamin D by excluding the control of PTH or serum calcium levels on renin expression[57]. On the other hand, Ferder and co-workers have recently proposed a new hypothesis about the dependency instead of complementarity vitamin D system and RAAS. Overturning the classical view, the authors suggested the RAAS-induced inflammatory response as regulator of vitamin D status thus representing the "primum movens" of current vitamin D deficiency pandemic[58]. Anyway, although suggestive, this hypothesis of a reciprocal counter-regulatory

effect between vitamin D and RAAS is currently highly speculative. Research models identifying effectors shared by RAAS and vitamin D are still missing[59]. Angiotensin Ⅱ is a main mediator responsible for adverse vascular remodelling in hypertension[60]. By promoting endothelial dysfunction and vascular permeability, RAAS induces recruitment and activation of inflammatory cells within the vessel wall. This inflammatory behaviour stimulates hyperplasia and hypertrophy of VSMCs, but also their release of pro-inflammatory molecules (VCAM-1, monocyte chemoattractant protein-1, interleukin 6 and 8)[61]. Furthermore, angiotensin Ⅱ was shown to mediate the shift of VSMCs toward a fibroblast phenotype that alters the ECM composition by suppressing the activity of matrix metalloproteinases and enhancing the production of their inhibitors[62]. Among the intracellular signalling pathways involved in angiotensin Ⅱ signalling a key role is played by oxidants and their downstream signalling cascades including mitogen-activated protein kinase, protein kinase C, phospholipase A2 and the transcription factors NFκB and activator protein-1[63].

PTH

PTH is a crucial regulator of calcium and phosphate homeostasis, achieved in different ways, such as osteoclast/osteoblast activation, enhancement of intestinal and renal calcium absorption and up-regulation of CYP27B1 expression in the kidney. Although not generally accepted[64], higher PTH concentrations were associated with an increase in several CV risk factors[65], including hypertension[66-76]. Moreover, several cohorts of sporadic primitive hyperparathyroidism were found associated with arterial stiffness[77-84]. The mechanism linking PTH and blood pressure is still unclear and several pathways might be triggered. PTH up-regulates RAAS activity promoting renin release[85,86], but it also directly promotes aldosterone release from adrenal glands[87]. Also the increase of serum calcium PTH may indirectly modulate renin release[88] and aldosterone synthesis[87] in addition to activate VSMC[89]. PTH increases sympathetic activity with additional RAAS activation (increase in renin release and aldosterone secretion)[90] and vascular contractility[90]. Finally, a cellular interaction through the PTH/PTH-related protein receptor expressed on endothelial cells[91], VSMC[92] and inflammatory cells[93] may directly affect the vascular function.

CLINICAL STUDIES

The association between vitamin D levels and blood pressure was previously reported, observing higher blood pressure trends in the winter months and location further from the equator[94]. Many clinical studies have subsequently provided consistent results but this topic is still widely debated, especially after the results observed in the interventional clinical trials.

Cross-sectional studies

A large number of cross-sectional studies investigated the relationship between vitamin D deficiency and blood pressure, as well as the prevalence of hypertension. Table 1 summarizes the studies having 25(OH) vitamin D as reference for the vitamin D status[1].

The most relevant results were acquired from the national health and nutrition examination survey (NHANES), widely representative of non-hospitalized United States civilian population. First Martins *et al*[95] showed an increased prevalence of hypertension associated with low serum 25(OH) vitamin D levels in 15088 subjects from this cohort. In addition, the very large sample size of this cohort allowed to recognize the inverse relationship between 25(OH) vitamin D and raised blood pressure also in several subgroups (such as African Americans and older people[96,97], children/adolescents[101,112], Hispanic people[113], in addition to observed an increased prevalence of pre-hypertension in 25(OH) vitamin D deficient subjects[121]). Other cross-sectional cohort studies with large sample size supporting these findings were the German National Health Interview and Examination Survey (4030 subjects)[98], the 1958 British birth cohort (6810 subjects)[100], and the Tromsø Study (4125 subjects)[104] as well as the cohorts collected from Israel people (34874 subjects)[108] and Copenhagen population[123]. Other smaller cohorts supporting these insights were collected in Europe[73,103,109,111,122,126], North America[110,118,120,124], Oceania[102] and Asia[72,105,115]. Despite the large numbers of subjects and their worldwide distribution, a clear relationship between vitamin D and blood pressure has not yet been established so far. In fact, among the studies listed in Table 1, seven did not confirm this association[64,67,70,72,73,119,123]. These conflicting results are in accordance with some unanswered questions in the field of vitamin D biology. In fact, despite the standardization of the season of subject recruitment, the latitudes, where studies were carried out, determine a confounding effect related to the pivotal role of sunlight exposure and consequent vitamin D synthesis within the skin[2]. Another potential bias is that differences in serum 25(OH) vitamin D levels might depend on the age. Elderly subjects have a reduced skin synthesis and intestinal absorption of vitamin D in addition to spend less time outdoors, limiting sunlight exposure[127]. Regardless of the latitude and season, only few studies have estimated sun exposure and dietary intake (as well as a possible supplementation) of vitamin D, especially in the elderly population. Moreover, racial differences should be recognized, since the black population correlated with a higher incidence of vitamin D deficiency (and also hypertension), because of their high skin content of melanin[128]. In this regard, it should be emphasized that most of the negative studies were made up from Caucasian[67,73,123] Hispanic[119] and Chinese[64,72] cohorts. Finally, there is still much debate about which cut-off value defines 25(OH) vitamin D deficiency. However, among the results reported in Table 1 most of the studies showed the first quartile or proposed a cut-off closed to 30 nmol/L. In addition, for higher mean 25(OH) vitamin D levels, blood pressure poorly correlated with vitamin D but rather with PTH

Table 1 Cross-sectional studies evaluating vitamin D blood pressure

Ref.	Year	Study design (sample size)	Country (ethnicity) Age	Correlation (lower reference range of 25(OH) vitamin D)	Findings
Snijder *et al*[67]	2007	Cross-sectional from the LASA (1205 subjects more than 65 yr old)	Netherlands (caucasian) men and women ≥ 65 yr	No (I quartile: < 25 nmol/L)	25(OH) vitamin D was not associated with systolic or diastolic BP or prevalence of hypertension. Instead, PTH correlated with both BP and hypertension incidence
Martins *et al*[95]	2007	Cross-sectional from the 1988-1994 NHANES (15088 subjects)	United States (Caucasian and African Americans and other) men and women age stratified	Yes (I quartile: < 52.5 nmol/L)	Adjusted inter-quartile analysis showed an increased prevalence of hypertension in in the lower quartile of 25(OH) vitamin D (OR = 1.30, 95%CI; 1.13-1.49; $P < 0.05$)
Scragg *et al*[96]	2007	Cross-sectional from the 1988-1994 NHANES (12644 subjects not treated with anti-hypertensive drugs)	United States (Caucasian and African Americans and other) men and women age stratified	Yes (I quintile: < 40 nmol/L)	Adjusted inter-quintile analysis of 25(OH) vitamin D showed significant inverse correlation with both systolic ($P < 0.01$) and diastolic ($P < 0.05$) BP. This association was stronger in more than 50 years old and black people
Judd *et al*[97]	2008	Cross-sectional from the 1988-1992 NHANES (7699 non-hypertensive subjects)	United States (White and black people) men and women age stratified	Yes (Vitamin D deficiency defined as < 50 nmol/L)	Lower 25(OH) vitamin D concentrations were associated with a higher blood pressure category in white people ($P < 0.01$) but after adjustment for age the association was no longer significant
Hintzpeter *et al*[98]	2008	Cross-sectional from GNHIES (4030 adults)	Germany (Caucasian) men and women 18-79 yr	Yes (Vitamin D deficiency defined as < 12 nmol/L[99])	According to 25(OH) vitamin D levels, in multivariate analysis there was a relationship between 25(OH) vitamin D and hypertension bot in men (OR = 0.97, 95%CI: 0.94-0.99; $P < 0.05$) and in women (OR 0.96, 95%CI: 0.93-0.99; $P < 0.05$)
Hypponen *et al*[100]	2008	Cross-sectional from 1958 British birth cohort (6810 subjects)	United Kingdom (Caucasian) men and women 45-47 yr	Yes (I tertile: < 45 nmol/L)	The lower 25(OH) vitamin D tertile was associated with hypertension (OR 0.72, 95%CI: 0.61-0.86; $P < 0.01$)
Reis *et al*[101]	2009	Cross-sectional from the 2001-2004 NHANES (3577 non-pregnant adolescents without diagnosed diabetes)	United States (Caucasian and African Americans and other) male and female adolescent 12-19 yr	Yes (I quartile: < 37.5 nmol/L)	25(OH) vitamin D was inversely associated with systolic BP ($P < 0.05$) also in the adjusted odds ratio for the interquartile comparison (OR = 2.36, 95%CI: 1.33-4.19; $P < 0.05$)
Pasco *et al*[102]	2009	Cross-sectional (861 subjects)	Australia (Caucasian) women: 20-92 yr	Yes (I tertile 25(OH)D: < 30 nmol/L)	In this cohort there was a significant inter-tertile difference in mean BP ($P < 0.001$) as well as in anti-hypertensive medication use ($P < 0.01$)
Almirall *et al*[103]	2010	Cross-sectional (237 subjects more than 64 years old)	Spain (Caucasian) men and women 64-93 yr	Yes (cut-off for vitamin D deficiency: < 62.5 nmol/L)	A significant negative association was observed between serum 25(OH) vitamin D levels and both systolic ($P < 0.05$) and diastolic BP ($P < 0.05$) also in multivariate analysis
Jorde *et al*[104]	2010	Cross-sectional from the Tromsø Study (4125 subjects not treated with anti-hypertensive drugs)	Norway (Caucasian) Men and women age stratified	Yes (I quartile: < 41.4 nmol/L)	At adjusted inter-quartile analysis serum 25(OH) vitamin D was inversely correlated with systolic BP ($P < 0.01$)
Kim *et al*[105]	2010	Cross-sectional (1330 subjects)	South Korea (Asian)	Yes (I quintile: < 29.7 nmol/L)	At adjusted inter-quintile analysis, both systolic and diastolic BP decreased linearly with increasing of 25(OH) vitamin D (quintile 1-5; P for trend < 0.01). Moreover, inter-quintile comparison of BP had OR of 0.42 (95%CI: 0.24-0.73; $P < 0.05$)
Zhao *et al*[106]	2010	Cross-sectional from the 2003-2006 NHANES (5414 subjects not assuming anti-hypertensive drugs)	Men and women < 40 yr United States (Hispanic, Caucasian and African Americans) men and women ≥ 20 yr	Yes (I quintile: < 37.5 nmol/L	Across 25(OH) vitamin D quintiles systolic and diastolic BP decreased linearly and inversely (P < 0.01). Moreover, the prevalence ratio for hypertension was lower in the highest quintile (OR = 0.82, 95%CI: 0.73-0.91; $P < 0.05$)
Fraser *et al*[107]	2010	Cross-sectional from the 2001-2006 NHANES (3958 subjects)	United States (Caucasian and African Americans and other) men and women ≥ 20 yr	Yes (linear correlation)	25(OH) vitamin D has an inverse linear correlation with systolic blood pressure in various adjusted models ($P < 0.05$)

Steinvil *et al*[108]	2011	Cross-sectional case-control study (34874 subjects of which 8387 hypertensive)	Israel men and women 38-72 yr	Yes (vitamin D deficiency defined as < 37.5 nmol/L)	The age-adjusted OR for hypertension among normal and deficient serum 25(OH) vitamin D was 1.19 (95%CI: 1.09-1.31; $P < 0.01$) in women, whereas in men there was not statistical difference
Burgaz *et al*[109]	2011	Cross-sectional from the ULSAM (833 adult men)	Sweden (Caucasian) Men 71 yr	Yes (vitamin D deficiency defined as < 37.5 nmol/L)	Adjusted logistic regression confirmed the association between 25(OH) vitamin D concentration < 37.5 nmol/L and hypertension (OR = 3.3, 95%CI: 1.0-11.0; $P < 0.05$)
Bhandari *et al*[110]	2011	Cross-sectional (2722 subjects of which 1415 hypertensive)	United States (Caucasian and African Americans and other) men and women mean age 58.5 yr	Yes (I quartile: < 37.5 nmol/L)	The prevalence rate of hypertension was inversely correlated with serum 25(OH) vitamin D. Inter-quartile comparison showed an adjusted OR of 2.70 (95%CI: 1.41-5.19; $P < 0.05$)
Pacifico *et al*[111]	2011	Cross-sectional case-control study (452 children and adolescent of which 304 over-weight/ obese and 148 normal weight)	Italy (Caucasian) Male and female children	Yes (I tertile of 1,25(OH)2 vitamin D: < 42.5 nmol/L)	1,25(OH)2 vitamin D was inversely correlated with systolic BP both in the whole population ($P < 0.01$) and over-weight ($P < 0.01$) population as well as in control group ($P < 0.01$). Regardless of model for adjusted analysis, the OR for hypertension among tertile categories had a P value < 0.05.
Williams *et al*[112]	2011	Cross-sectional from 2003-2006 NHANES (5617 adolescent)	United States (Caucasian and African Americans and other) male and female children 12-19 yr	Yes (linear correlation)	In this cohort, 25(OH) vitamin D showed a linear inverse association with systolic BP in multivariate analysis ($P < 0.01$).
Forrest *et al*[113]	2011	Cross-sectional from 2005-2006 NHANES (4495 adults subjects of which 1482 hypertensive)	United States (Caucasian and African Americans and other) Men and women age stratified	Yes (vitamin D deficiency defined < 50 nmol/L[114])	Vitamin D deficiency independently correlated with prevalence of hypertension ($P < 0.01$).
He *et al*[70]	2011	Cross-sectional from 2003-2006 NHANES (7561 of which 1849 treated with anti-hypertensive drugs)	United States (Caucasian and African Americans and other) Men and women age stratified	No (I quintile: < 33 nmol/L)	25(OH) vitamin D was inversely associated with systolic BP. However, 25(OH) vitamin D lost its statistical significance in a multivariate analysis including PTH. Instead, PTH maintained a strong correlation with BP in multivariate analysis regardless of covariates.
Dorjgochoo *et al*[115]	2012	Cross-sectional study from two, population-based, prospective cohort studies (1460 subjects of which 547 hypertensive)	China (Asian) men and women 40-74 yr	Yes (lowers range defined by I quintile 23.5 nmol/L and cut-offs of 37.5 nmol/L[116] and 27.5 nmol/L[117])	Among men cohort, BP was inversely and significantly correlated with 25(OH) vitamin D ($P < 0.05$). Moreover, prevalence of hypertension was inversely associated with non-deficient status of vitamin D (adjusted OR = 0.29, 95%CI: 0.10-0.82; $P < 0.05$)
Sakamoto *et al*[118]	2013	Cross-sectional from the AHS-2 (568 subjects)	United States (equally matched Caucasian and African Americans) men and women 30-95 yr	Yes (vitamin D deficiency defined as < 50 nmol/L)	Regardless of adjusted analysis models, Caucasian people showed a linear inverse correlation between 25(OH) vitamin D and BP ($P < 0.05$). Also the comparison between vitamin D deficient and non-deficient showed statistical difference ($P < 0.05$).
Li *et al*[64]	2012	Cross-sectional (1420 subjects of which 487 hypertensive)	China (Asian) Men and women ≥ 65 yr	No (I quartile: < 42 nmol/L)	Serum 25(OH) vitamin D levels were not associated with risk of hypertension in single and multiple regression models. Similarly, PTH is not independently associated with BP or risk of hypertension
Caro *et al*[119]	2012	Cross-sectional (219 subjects of which 115 hypertensive)	Puerto Rico (Hispanic) Men and women 21-50 yr	No (cut-off used to define non optimal: 75 nmol/L)	Vitamin D status was not found to be associated with BP
Chan *et al*[72]	2012	Cross-sectional (939 men aged 65 yr and older)	China (Asian) men ≥ 65 yr (age strafied)	No (I quartile: < 63 nmol/L)	Vitamin D status was not found to be associated with BP. Instead, PTH was directly and independently associated with BP also in multivariate analysis.
Parikh *et al*[120]	2012	Cross-sectional (701 adolescents)	United States (Caucasian and African Americans) Male and female 14-18 yr	Yes (I tertile: < 54.8 nmol/L)	Serum 25(OH) vitamin D has a linear inverse correlation with both systolic ($P < 0.05$) and diastolic ($P < 0.01$) BP. However, in the adjusted analysis only the relationship with systolic BP remained significant.
Sabanayagam *et al*[121]	2012	Cross-sectional from NHANES III (9215 subjects of which 3712 with pre-hypertension)	United States (Caucasian and African Americans and other) men and women age stratified	Yes (I quartile: < 44.25 nmol/L)	In this cohort the systolic BP are inversely correlated with the vitamin D status ($P < 0.05$) and lower values of 25(OH) vitamin D were associated with increase prevalence of pre-hypertension (adjusted OR = 1.48, 95%CI: 1.16-1.90; P value for trend < 0.01).

van Ballegooijen *et al*[122]	2012	Cross-sectional from the Hoorn study (256 subjects)	The Netherlands (Caucasian) men and women 50-75 yr	Yes (I quartile: < 60.8 nmol/L)	In this cohort there was an inverse correlation between 25(OH) vitamin D and both systolic and diastolic BP (*P* value for trend < 0.01 for both)
Skaaby *et al*[123]	2012	Cross-sectional 4330 subjects)	Denmark (Caucasian) men and women 30-60 yr	No (I quartile: < 33 nmol/L)	Mean 25(OH) vitamin D levels did not differed between hypertensive and normotensive subjects. There was not increased prevalence of hypertension in vitamin D deficient subjects
Kruger *et al*[124]	2013	Cross-sectional form the PURE study (291 African women)	All over the world countries (African) women > 47 yr	Yes (vitamin D deficiency defined as < 30 nmol/L[125])	Both systolic and diastolic BP correlated linearly and inversely with serum 25(OH) vitamin D level ($P < 0.05$ for both). However, only systolic BP maintain statistical significance in multivariate analysis ($P < 0.05$).
Mateus-Hamdan *et al*[73]	2013	Cross-sectional (284 geriatric patients of which 106 hypertensive)	France men and women mean age 85 ± 6 yr	No (linear correlation)	Means PTH but not 25(OH) vitamin D levels are significant different in hypertensive compared to normotensive patients.
Ke *et al*[126]	2013	Cross-sectional from the ATBC (2271 subjects of which 1430 hypertensive)	Finland (Caucasian) men and women 50-69 yr	Yes (I quartile: < 25 nmol/L)	Serum 25(OH) vitamin D level has a significant and inverse association with systolic BP ($P < 0.05$), also if stratified in groups. Moreover, the lower group was associated with increased prevalence of hypertension in multivariate analysis (*P* value for trend < 0.05).

LASA: Longitudinal aging study amsterdam; 25(OH)D: Cholecalciferol; BP: Blood pressure; PTH: Parathyroid hormone; NHANES Ⅲ: Third United States national health and nutrition examination survey; OR: Odds ratio; GNHIES: German national health Interview and examination survey; ULSAM: Uppsala Longitudinal study of adult men; AHS-2: Adventist health study-2; PURE study: Prospective urban and rural epidemiology study; ATBC: Alpha-tocopherol and beta-carotene study.

levels[67,70].

Longitudinal studies

Few studies have investigated the incidence of hypertension in vitamin D-deficient subjects. In addition, no study among them had this aim as a primary outcome, suggesting some potential limitation in the statistical power estimation. In addition, the majority of the cohorts investigated was limited to the Caucasian race and female gender, further limiting the generalizability of the results. However, we believe that the main limitation is represented by the lack of prospective risk evaluation in the elderly. In fact, even if the follow-up is extended over 65 years, this overlap does not recognize the critical alterations in vitamin D metabolism during aging. Taking those important limitations into the account, Table 2 summarizes the most important longitudinal observational studies, starting from the results of health professionals' follow-up study (HPFS) and the nurse health study (NHS)2.

Forman *et al*[129] firstly reported an increased risk of incident hypertension in 1811 subjects selected from these two matched cohorts at 4-year follow-up (pooled RR = 3.18; 95%CI: 1.39-7.29, $P < 0.05$). In addition, the investigators extended this risk prediction, as a surrogate, to the overall study population including 38388 man from HPFS (adjusted RR = 2.31; 95%CI: 2.03-2.63, $P < 0.05$) and 77531 women from the NHS 2 (adjusted RR = 1.57; 95%CI: 1.44-1.72, $P < 0.05$). Afterwards, the same authors also designed a prospective nested case-control study including 1484 normotensive women from the NHS 2 that confirmed the previous results (interquartile OR = 1.66; 95%CI: 1.11-2.48, *P* value for trend = 0.01)[132]. Also the Intermountain Heart Collaborative Study Group provided similar results prospectively analyzing a large electronic medical database of a general healthcare population. In addition to recognize a wide prevalence of vitamin D deficiency, very low levels of 25(OH) vitamin D were directly associated with an increased risk of developing CV disease, including hypertension (HR = 1.62; 95%CI: 1.48-2.02, $P < 0.01$)[133]. Significant association between vitamin D deficiency and incidence of hypertension was also observed in a smaller subgroup analysis from both woman cohort of Michigan Bone Health and Metabolism Study (OR = 3.0; 95%CI: 1.01-8.7, $P < 0.05$)[134] and for male population of Physicians' Health Study (HR = 0.69; 95%CI: 0.50-0.96, $P < 0.05$)[136]. On the other hand, other large sample size studies such as subgroup analyses from Ely study[131], Tromsø study (burdened with a 40% dropout rate)[104], Women's Health Initiative[135] and Alpha-Tocopherol and Beta-Carotene study cohort[126], as well as cohort of general Copenhagen population[123] did not confirm any association between vitamin D levels and incidence of hypertension.

Randomized clinical trials

Table 3 summarizes randomized interventional clinical trials investigating the link between vitamin D and blood pressure.

Although most of the studies reported a significant serum 25(OH) vitamin D increase after supplementation, they are impeded by several limitations, mostly related to study design issues. The first one consists in the limited number of trials investigating blood pressure as a primary outcome. In addition, only few studies focused on vitamin D-deficient cohorts, more suitable for investigating the effectiveness of a supplementation with vitamin D. In this regard, a subgroup analysis of vitamin D-deficient

Table 2 Longitudinal studies addressing the association between vitamin D and blood pressure

Ref.	Year	Study design and follow-up (sample size)	Country (ethnicity) Age	Correlation (lower reference range of 25(OH) vitamin D)	Findings
Forman *et al*[129]	2007	Prospective observational nested case-control study from HPFS and NHS-2 4 yr (1811 subjects)	United States (Caucasian) men 47-82 yr women 43-68 yr	Yes (vitamin D deficiency defined as < 37.5 nmol/L[130])	Multivariate RR of incident hypertension among vitamin D deficient subject was 3.18 (95%CI: 1.39-7.29; $P < 0.05$)
Forouhi *et al*[131]	2008	Prospective observational from the Ely study 10 yr (534 subject)	United Kingdom (Caucasian) men and women 40-69 yr	No (vitamin D deficiency defined as < 25 nmol/L)	There were not significant changes in BP during the follow-up
Forman *et al*[132]	2008	Prospective observational nested case-control study from the NHS 2 7 yr (1484 normotensive women)	United States (Caucasian) women: 32-52 yr	Yes (Ⅰ quartile: < 21 nmol/L)	Median 25(OH) vitamin D were lower in women developing hypertension (P < 0.01). Moreover, interquartile analysis showed significant and inverse correlation between 25(OH) vitamin D and hypertension (OR = 1.66, 95%CI: 1.11-2.48; *P* value for trend < 0.05)
Jorde *et al*[104]	2010	Prospective observational from the Tromsø Study 14 yr (4125 subjects not treated with anti-hypertensive drugs)	Norway (Caucasian) men and women 25-84 yr	No (Ⅰ quartile: < 41.4 nmol/L)	At adjusted analysis, 25(OH) vitamin D did not predict future hypertension or increase in BP: Moreover there was not any association between change in serum 25(OH) vitamin D and BP
Anderson *et al*[133]	2010	Prospective observational average 1.3 yr (maximum 9.1 yr) (41497 subjects)	United States men and women 34-76 yr	Yes (vitamin D deficiency defined as < 37.5 nmol/L)	Lower 25(OH) vitamin D levels were associated with higher incidence of hypertension (HR = 1.62, 95%CI: 1.48-2.02; $P < 0.01$)
Griffin *et al*[134]	2011	Prospective observational from MBHMS 14 yr (559 women)	United States (Caucasian) women 24-44 yr	Yes (vitamin D deficiency defined as < 80 nmol/L)	25(OH) vitamin D insufficiency has an increased risk of systolic hypertension at multivariate analysis (OR = 3.0, 95%CI: 1.01-8.7; $P < 0.05$)
Margolis *et al*[135]	2012	Prospective observational from the WHI 7 yr (4863 post-menopausal women)	United States (Caucasian, African, Hispanic, Asian and others) women 50-79 yr	No (Ⅰ quartile: < 34.4 nmol/L)	There was not significant linear or nonlinear trend in the risk of incident hypertension
Wang *et al*[136]	2012	Prospective observational form PHS 15.3 yr (1211 normotensive men)	United States men 40-84 yr	Yes (Ⅰ quartile: < 39.9 nmol/L)	There was significant difference only between Ⅰ and Ⅲ quartile (HR = 0.69, 95%CI: 0.50-0.96; $P < 0.05$)
Skaaby *et al*[123]	2012	Prospective observational 5 yr (4330 subjects)	Denmark (Caucasian) men and women 30-61 yr	No (Ⅰ quartile: < 33 nmol/L)	Multivariate logistic regression analyses did not show any association between 25(OH) vitamin D incidence rate of hypertension.
Ke *et al*[126]	2013	Prospective observational from the ATBC 4 yr (2271 subjects of which 1430 hypertensive)	Finland (Caucasian) men and women 50-69 yr	No (Ⅰ quartile: < 25 nmol/L)	25(OH) vitamin D did not predict future hypertension.

HPFS: Health professionals' follow-up study; NHS 2: Nurse heatlh study 2; 25(OH)D: Cholecalciferol; RR: Relative risk; BP: Blood pressure; OR: Odds ratio; HR: Hazard ratio; MBHMS: Michigan bone health and metabolism study; WHI: Women's health initiative; PHS: Physicians' health study; ATBC: Alpha-tocopherol and beta-carotene study cohort.

subjects, from a sample of 112 Danish hypertensive patients randomized to high-dose 25(OH) vitamin D supplementation (75 μg/d) versus placebo, showed a significant decrease of 24-h systolic and diastolic blood pressure values ($P < 0.05$)[155]. These findings confirmed previous results from other small sample size cohorts of vitamin D-deficient patients[141,142,150]. For this reason, the recently results by Forman *et al*[157] from the largest published cohort of hypertensive patients ($n = 283$) randomized to vitamin D supplementation versus placebo appear of particular interest. The oral administration of 25(OH) vitamin D (25 to 100 μg/d) significantly decreased the blood pressure levels. Unfortunately, these studies present additional limitations, such as taking into account the different approaches used for vitamin D supplementation. Although sunlight exposition might be the more physiological way, the ultraviolet (UV)-B rays-induced skin synthesis of vitamin D is hard to quantify and thus poorly investigated[140,151]. Oral supplementation has been preferred because easier to manage (despite some variability in intestinal absorption may exist) if provided through diet regimen[147], nutritional supplements[146] or direct vitamin D administration (daily intake[137-139,141,143,144,152-157] or loading dose[142,148-150,158]). Finally, it should be reported

Table 3 Randomized clinical trial investigating the protective effect of vitamin D supplementation on blood pressure

Ref.	Year	Study design	Country (ethnicity) Age	Intervention	Findings
Lint *et al*[137]	1988	(sample size) Prospective randomized double-blind placebo-controlled trial (65 men with glucose intolerance of which 26 hypertensive)	Sweden (Caucasian) 61-65 yr	(follow-up) α-calcidol 0.75 μg (12 wk)	In hypertensive patients supplementation has addictive effect to concomitant antihypertensive therapy in reducing BP ($P < 0.01$). In the whole population there was only non-significant trend in BP lowering
Pan *et al*[138]	1993	Prospective randomized double-blind 2 × 2 interventional trial (58 institutionalized elderly persons)	Taiwan (Asian) not provided	calcium 800 mg/d or 1,25(OH)2 vitamin D 5 μg/d or calcium 800 mg/d + 1,25(OH)2 vitamin D 5 μg/d, or placebo (11 wk)	Any type of supplementation failed to reduce BP
Scragg *et al*[139]	1995	Prospective randomized double-blind placebo-controlled trial (189 elderly subjects)	United Kingdom (not provided) 63-76 yr	25(OH) vitamin D 2.5 μg/d or placebo (5 wk)	Although treatment was effective in increasing serum 1,25(OH)2 vitamin D ($P < 0.01$) and decreasing PTH ($P < 0.01$), there was not difference in BP change
Krause *et al*[140]	1998	Prospective randomized double-blind controlled trial (18 patients with untreated mild essential hypertension)	Germany (Caucasian) 26-66 yr	Full body UVB or UVA thrice weekly (6 wk)	In accordance with a 162% rise in plasmatic 25(OH) vitamin D ($P < 0.01$) and 15% fall in serum PTH ($P < 0.01$), the UVB group showed also a reduction in 24-h ambulatory systolic and diastolic BP ($P < 0.01$)
Pfeifer *et al*[141]	2001	Prospective randomized double-blind controlled trial (148 elderly subject with 25(OH)D < 50 nmol/L)	Germany (Caucasian) 70-86 yr	Calcium 600 mg × 2/d or calcium 600 mg + 25(OH) vitamin D 10 μg twice daily (8 wk)	In accordance with a 72% rise in plasmatic 25(OH) vitamin D ($P < 0.01$) and 17% fall in serum PTH ($P < 0.05$), combined supplementation significantly reduced systolic BP ($P < 0.05$)
Sudgen *et al*[142]	2008	Prospective randomized double-blind placebo-controlled trial (34 elderly type 2 diabetic patients with 25(OH)D < 50 nmol/L)	United Kingdom (not provided)	Loading dose ergocalciferol 2500 μg or placebo (8 wk)	Supplementation significantly rise plasmatic 25(OH) vitamin D ($P < 0.01$) and reduced systolic BP, whereas there was only a trend in diastolic BP decrease
Alborzi *et al*[143]	2008	Prospective randomized double-blind placebo-controlled trial (24 elderly type 2 diabetic patients with 25(OH)D < 50 nmol/L)	mean 64 years United States (Caucasian and African Americans) 56-80 yr	Paricalcitol 1 or 2 μg/d or placebo (4 wk)	Any dose of paricalcitol failed to reduce BP
Margolis *et al*[144]	2008	Prospective randomized double-blind controlled trial (36282 n post-menopausal women from WHI study)	United States (Caucasian, Asian, Hispanic, African American) 50-79 yr	Calcium 500 mg × 2/d or calcium 500 mg + 25(OH) vitamin D 5 μg twice daily (7 yr)	There was no significant difference in over time change of BP in the whole population. In addition, supplementation failed to reduce the risk of developing hypertension in non-hypertensive patients at baseline
Nagpal *et al*[145]	2008	Prospective randomized double-blind placebo-controlled trial (71 older overweight men)	India (Indian population) 36-54 yr	25(OH) vitamin D 3000 μg every 2 wk for 3 times or placebo (7 wk)	Supplementation failed to reduce BP
Daly *et al*[146]	2009	Prospective randomized double-blind controlled trial (124 community-dwelling men)	Australia (Caucasian) 55-69 yr	Milk fortified with calcium (500 mg) and 25(OH) vitamin D (10 μg) twice a day or standard milk (2 yr)	Supplementation failed to reduce BP
Hilpert *et al*[147]	2009	Prospective randomized double-blind controlled trial (23 hypertensive adults)	United States (not provided)	Dairy-rich, high fruits and vegetables diet or a high fruits and vegetables diet or an average Western diet (5 wk)	High fruits and vegetables diet dairy-rich or not significantly reduced BP ($P < 0.05$). Moreover, in dairy-rich, high fruits and vegetables diet there was a greater lowering of intracellular calcium ($P < 0.01$), strongly associated with fall in diastolic BP ($P < 0.05$)
Witham *et al*[148]	2010	Prospective randomized double-blind placebo-controlled trial (56 patients with history of stroke and baseline 25(OH)D < 75 nmol/L)	United Kingdom (not provided) 53-79 yr	Loading dose ergocalciferol 2500 μg or placebo (8 and 16 wk)	Supplementation significantly increased serum 25(OH) vitamin D to both controls ($P < 0.01$). However, treatment failed to reduced BP

Witham *et al*[149]	2010	Prospective randomized double-blind placebo-controlled trial (61 patients with type 2 diabetes and baseline 25(OH)D < 100 nmol/L)	United Kingdom (not provided) 55-76 yr	Loading dose ergocalciferol 2500 μg or 5000 μg or placebo (8 and 16 wk)	Supplementation significantly increased serum 25(OH) vitamin D to both controls (P < 0.01 for both). However, supplementation failed to reduced BP
Judd *et al*[150]	2010	Prospective randomized double-blind controlled trial (9 patients with baseline 25(OH)D within 25 and 75 nmol/L in addition to systolic BP between 130 and 150 mmHg)	United States (African American) mean 45 yr	loading dose ergocalciferol 2500 μg or placebo weekly for 3 wk or 25 (OH) vitamin D 0.5 μg twice a day for 1 wk (3 wk)	Only supplementation with 25(OH) vitamin D decrease by 9% mean systolic BP (P < 0.01) in accordance with rise of serum 25(OH) vitamin D (P < 0.05)
Scragg *et al*[151]	2011	Prospective randomized double-blind controlled trial (119 patients with baseline 25(OH)D < 50 nmol)	New Zealand (Pacific islander, Caucasian and Maori) 23-87 yr	24 whole body exposures of either UVB or ultraviolet A (6 and 12 wk)	In the UVB arm there was a significant increase in serum 25 (OH) vitamin D after both 6 and 12 wk (P < 0.01 for both). However, treatment failed to reduced BP
Salehpour *et al*[152]	2012	Prospective randomized double-blind placebo-controlled trial (77 pre-menopausal overweight and obese women)	Iran (Arabian) 30-46 yr	25 (OH) vitamin D 25 μg daily or placebo (12 wk)	Supplementation significantly rise plasmatic 25 (OH) vitamin D (P < 0.01) and fall PTH (P < 0.01). Moreover, although treatment improved lipid profile, there was no effect on BP
Gepner *et al*[153]	2012	Prospective randomized double-blind placebo-controlled trial (110 post-menopausal women with baseline 25(OH)D within 10 and 60 nmol/L)	United States (not provided) 60-67 yr	25 (OH) vitamin D 62.5 μg daily or placebo (16 wk)	Supplementation, although significantly raised serum 25(OH) vitamin D (P < 0.01), failed in improving BP control assessed by changes in FMD, PWV and Aix
Wood *et al*[154]	2012	Prospective randomized double-blind placebo-controlled trial (305 healthy post-menopausal women)	United Kingdom (not provided) 48-72 yr	25 (OH) vitamin D 10 μg or 25 μg/d or placebo (1 yr)	Supplementation failed in improving CV risk profile, including BP control
Larsen *et al*[155]	2012	Prospective randomized double-blind placebo-controlled trial (112 hypertensive patients)	Denmark (Caucasian) 48-72 yr	25 (OH) vitamin D 75 μg/d or placebo (20 wk)	Supplementation significantly rise plasmatic 25 (OH) vitamin D (P < 0.01) and fall PTH (P < 0.01) but failed in improving BP control. However, in a post-hoc subgroup analysis of patient with 25 (OH) vitamin D deficiency at baseline supplementation significantly decrease 24-h systolic and diastolic BP (P < 0.05)
Zhu *et al*[156]	2013	Prospective randomized double-blind placebo-controlled trial (43 healthy subjects)	China (Asian) 20-22 yr	Calcium 600 mg + 25 (OH) vitamin D 3.12 μg daily or placebo, in addition to 500 kcal/d of caloric deficit (7 yr)	Except a reduction in visceral fat mass, supplementation failed in improving CV risk profile, including BP control
Forman *et al*[157]	2013	Prospective randomized double-blind placebo-controlled trial (283 healthy black subjects)	United States (African American) mean 51 yr	25 (OH) vitamin D 25 μg or 50 or 100 μg/d or placebo (12 and 24 wk)	Supplementation significantly decrease BP consistent with increasing dose (P < 0.05). Moreover, there was linear correlation between systolic BP decrease and rise of serum 25 (OH) vitamin D (P < 0.05)
Witham *et al*[158]	2013	Prospective randomized double-blind placebo-controlled trial (159 with isolate systolic hypertension)	United States (not provided) mean 77 yr	Loading dose 25 (OH) vitamin D 2500 μg or placebo (12, 24 and 36 wk)	Supplementation significantly rise plasmatic 25 (OH) vitamin D (P < 0.01) but failed in improving BP control. Moreover, treatment failed to achieve secondary outcomes including 24-h blood pressure, arterial stiffness and endothelial function

α-calcidol: Synthetic analog of 1,25(OH)2D; BP: Blood pressure; 1,25(OH)2D: Calcitriol; UVB: 94.5% UVA and 3.5% UVB; UVA: 99.5% UVA and 0.05% UVB; 25(OH)D: Cholecalciferol; PTH: Parathyroid hormone; WHI: Women's Health Initiative Calcium/vitamin D trial; HyD: 25(OH)D metabolite with hydrophilic properties and much shorter half-life; FMD: Brachial artery flow-mediated vasodilation; PWV: Carotid-femoral pulse wave velocity; Aix: Aortic augmentation index; CV: Cardiovascular.

the failure of Women's Health Initiative study to prove changes in blood pressure in a very large sample size of post-menopausal women (n = 36282) randomized to receive calcium versus calcium plus 25(OH)D over 7-year follow-up[144].

Meta-analyses of clinical studies

Five meta-analyses were recently performed to quantify the prospective associations of vitamin D status with the risk of hypertension. Pittas *et al*[159] included the results of four observational longitudinal cohorts with 32181 subjects with a follow-up of 7 to 10 years. The pooled analysis showed an increased risk of developing hypertension in vitamin D-deficient subjects (RR = 1.76; 95%CI: 1.27-2.44, P < 0.05). Conversely, another meta-analysis of ten randomized clinical trials failed to prove the effectiveness of vitamin D supplementation in promoting blood pressure decrease[159]. Therefore, this mismatch between

observational studies and randomized interventional clinical trials is retrieved in other meta-analyses The lack of relationship in interventional studies was reported by Witham *et al*[160] and Wu *et al*[161], while pooled analysis of observational studies showed a strong association between vitamin D status and blood pressure[162]. In particular, the meta-analysis of observational longitudinal studies by Kunutsor *et al*[163] recently reported that subjects in the higher tertiles of vitamin D levels have a 30% lower risk of developing hypertension as compared to those in the bottom tertiles (pooled RR = 0.70; 95%CI: 0.58-0.86, $P < 0.05$).

OPEN ISSUES AND PERSPECTIVES

Many questions recently emerged from efficacy and safety in interventional trials using vitamin D supplementation. In experimental mouse models, excessive intake of vitamin D induces vascular and soft-tissue calcifications. Thus, in human beings, caution has to be used on the pro-calcifying effects of exogenous vitamin D. In addition to derangement in calcium homeostasis, it should take into account the detrimental effects of vitamin D-induced phosphate overload involving also FGF23/klotho axis. On the other hand, the definition of the optimal vitamin D status from a CV point of view remains matter of debate and general consensus is still missing. "Bone health-driven" recommendations agree to define insufficient a 25(OH) vitamin D levels < 20 ng/mL, suggesting a target of 30 ng/mL. Similarly, reports from large cohorts (such as NHANES[164] and The Framingham offspring study[165]) showed a linear inverse association with CV outcome for 25(OH) vitamin D levels up to 30ng/mL. Considering hypertension, the results from the Vitamin D and Omega-3 Hypertension Trial (VITAL Hypertension) that is still enrolling patients[166] might clarify this point. Finally, the "local vitamin D system" is emerging as a pivotal topic that might explain the conflicting results between observational and interventional trials[167].

CONCLUSION

Neither the European society of Cardiology nor American Heart Association have published CV-focused algorithms regarding vitamin D deficiency and this is because the first results from randomized clinical trials have provided more questions than answers. Certainly, several factors involved in vitamin D biology are under-recognized or hard to assess, including physical activity, sunlight exposure, health status or dietary habits. Moreover, several confounding factors have not been considered in several studies, such as comorbidities, concomitant medications or differences in gender, age and race. In addition, also vitamin D compounds proposed were highly variable, ranging from native (cholecalciferol or ergocalciferol) or synthetic (α-calcidol) inactive vitamin D to active vitamin D (calcitriol) up to selective VDR activators (paricalcitol). However, it is likely that other unidentified factors are also involved in vitamin D biology, such as the possible relationship with other endocrine networks, emphasizing the need of pre-clinical studies.

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TOPIC HIGHLIGHT

WJC 6th Anniversary Special Issues (1): Hypertension

Device-guided breathing exercises for the treatment of hypertension: An overview

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Abstract

The American Heart Association considers device-guided breathing as a reasonable treatment modality in their statement on non-pharmacological options for lowering blood pressure. This review discusses all randomized controlled trials that have investigated the effects of device-guided breathing on blood pressure in patients with hypertension. Thirteen studies were included in this review. In total, 627 patients were included, of which 365 patients were allocated to device-guided breathing. Only 6 studies used acceptable control groups: listening to music, meditative relaxation exercises, or a sham-device. Two sponsored trials showed beneficial effects of device-guided breathing, both used listening to music as a control group. The remaining 4 studies, which had no employees of the manufacturer listed as co-author, observed no beneficial effects on blood pressure. There is only 1 study that used a sham device as a control group. All other studies were to some extend methodologically flawed. Based on the studies with an acceptable methodological quality, there is no clear evidence supporting a short-term beneficial effect on blood pressure by using device-guided breathing.

Key words: Hypertension; Device-guided breathing; Review

Core tip: This review discusses all randomized controlled trials that have investigated the effects of device-guided breathing on blood pressure. There were 6 studies with an acceptable control group. Two (manufacturer sponsored) trials showed beneficial effects of device-guided breathing, both used listening to music as a control group. The remaining 4 studies observed no beneficial effects. We conclude that there is no sufficient evidence for recommending device-guided breathing in the treatment of hypertension.

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INTRODUCTION

Treatment of hypertension includes both pharmacological and non-pharmacological interventions. Accepted non-pharmacological interventions are sodium restriction, losing weight, increasing physical activity, smoking cessation and optimizing alcohol consumption[1-3]. In a scientific statement from the American Heart Associa-

tion (AHA) regarding non-pharmacological options for lowering blood pressure, device-guided slow breathing is described as a reasonable treatment modality to reduce blood pressure (Class Ⅱ A, Level of *Evidence B*)[4]. Device-guided slow breathing aims at lowering the respiratory frequency into a so-called "therapeutic breathing zone" (less than 10 breaths per minute) through biofeedback by using an electronic device. Exercises are regarded as successful if the total exercise time is at least 45 min per week, preferably 15 min daily[4]. Sympathetic overactivity is hypothesized as an important contributing factor in the development of hypertension[5-7]. Efforts aimed at reducing this autonomic imbalance may indeed be an effective therapy for hypertension. Slow and regular breathing, guided by musical tones, will lead to a reduction of sympathetic activity and also to an increase in heart rate variability[5]. The baroreceptors measure blood pressure in the carotid arteries and the aorta, and an increase in pressure leads to parasympathetic activation and vice versa (negative feedback mechanism). As an increase in heart rate variability will lead to an increased baroreflex sensitivity[5], device-guided breathing may lead to lower blood pressure values.

The conclusions of the writing group of the AHA statement were based on a meta-analysis[8] and several other studies[9-19]. After the publication of the guideline, two additional studies have been published[20,21]. The overall effect estimate in the meta-analysis showed a small beneficial blood pressure lowering effect [a reduction of 3.7 mmHg in systolic blood pressure (SBP)], but the authors of the meta-analysis stated that the results of the overall effect estimates should be interpreted with caution because of methodological flaws in most studies. Beneficial effects were not observed after excluding studies with high risk of bias or studies that were sponsored by or involved the manufacturer of the device[8]. A previous editorial already emphasized that an independent double-blind study with a proper control group, preferably a sham device, would be necessary to answer the question whether device-guided breathing has any effect on blood pressure[22]. Recently, an investigator-initiated double-blind and sham-controlled trial was performed[20]. This review discusses all randomized controlled trials (RCTs) that have investigated the effects of device-guided breathing on blood pressure in patients with hypertension.

PREVIOUS STUDIES

Thirteen studies, of which the study and patient characteristics are presented in Table 1, were included in this review. In total, 627 patients were included, of which 365 patients were allocated to device-guided breathing. Except for 1 study in which a bi-level positive pressure device (BiPAP®) was used[19], all other studies used the Resperate® device. The Resperate® device uses a form of biofeedback with "breathe in" and "breathe out" instructions according to the listeners breathing rate to guide the respiration into a lower frequency by prolonging expiration. The BiPAP® device was used for the treatment of patients with obstructive sleep apnea and it was also capable of guiding patients' respiratory rate to less than 10 breaths per minute. Three studies had no control group[11,12,19], 4 studies compared the intervention to usual care or frequent blood pressure measurements[13,14,17,21], 4 studies compared the intervention to listening to music[9,10,15,16], 1 study compared the intervention to meditative relaxation exercises[18], and 1 study used a sham-device in the control group[20]. Except for 3 studies[15,16,20], all other studies were sponsored by or involved the manufacturer of the Resperate® and BiPAP® devices. According to the meta-analysis by Mahtani *et al*[8], the Anderson paper was also not sponsored by the manufacturer[18]. However, the acknowledgements section of this manuscript states that Drs. B. Gavish, an employee of the company that manufactures the Resperate® device, had reviewed the paper.

EFFECTS OF DEVICE-GUIDED BREATHING

Table 1 presents an overview of the effects of device-guided breathing on blood pressure. Only 4 studies reported between-group-differences including the 95% confidence intervals[9,15,16,20]. Significant decreases in blood pressure were observed in all 3 studies without a control group[11,12,19]. A significant between-group-difference was observed in 2 out of 4 studies that compared device-guided breathing to daily blood pressure measurements[13], and usual care[17]. Studies comparing device-guided breathing to usual care cannot differentiate the 3 possible mechanisms through which the Resperate® could have a blood pressure lowering effect: (1) effects of guided slowing of breathing itself; (2) listening to music; and (3) sitting still. Conclusions regarding the isolated effect of device-guided breathing are only valid when a study has an appropriate control group to disentangle these 3 effects. Therefore, this review will further focus on the 6 studies that used acceptable control groups: listening to music, meditative relaxation exercises and a sham-device[9,10,15,16,18,20]. Two sponsored trials showed beneficial effects of device-guided breathing, both used listening to music as a control group[9,10]. In the study by Schein *et al*[9] device-guided breathing was not effective in lowering SBP compared to the control group. This study pre-defined a 5 mmHg reduction in diastolic blood pressure (DBP) as clinically relevant. The difference in DBP change between both groups was 4.4 mmHg in favour of the intervention group (P = 0.008). Although a second study failed to predefine a clinically relevant difference, it showed a significant decrease in office SBP compared to a Walkman group (between-group-difference 4.6 mm Hg, P = 0.001)[10]. The remaining 4 studies, which had no employees of the manufacturer listed as co-author, observed no beneficial effects on blood pressure[15,16,18,20]. Only the study by Landman *et al*[20] described the presence of 2 negative side-effects, but this was insufficient to conclude

Table 1 Study and patients characteristics

Ref.	Study group		Period (wk)	Study arm		Endpoint	Results (mean)		
	Disease, therapy, patients	Number (I/C)		Intervention	Control		Intervention (mmHg)	Control (mmHg)	Difference intervention *vs* control (mmHg)
Schein *et al*[9], 2001; Israel	HT, medication, BP ⩾ 140/90, 25-75 yr	32/33	8	Resperate® 10 min/d	Walkman 10 min/d	SBP DBP	156.6 > 141.4 96.7 > 86.7[a]	154.7 > 143.4 93.4 > 87.8	-2.9 (-2.8-10.6) -4.4[a] (1.1-7.6)
Grossman *et al*[10], 2001; Israel	HT, medication, BP ⩾ 140/90, 25-75 yr	18/15	8	Resperate® 10 min/d	Walkman 10 min/d	Clinic, SBP DBP Home, SBP DBP	160 > 152.5 95 > 91 157 > 152.0 94 > 91.3	155 > 152.1 94 > 92.5 151 > 149.8 90 > 90.9	-4.6[a] -2.5 -3.8 -3.6[a]
Rosenthal *et al*[11], 2001; Israel	HT, medication, BP 130/85-180/110, 25-75 yr	13/-	8	Resperate® 15 min/d	-	24 h, SBP DBP	137.1 > 129.9[a] 82.5 > 80.2	- -	- -
Viskoper *et al*[12], 2003; Israel	HT, medication, SBP 140-160 or DBP 90-100, 40-80 yr	17/-	8	Resperate® 15 min/d	-	Home, SBP DBP Clinic, SBP DBP	156.4 > 150.0[a] 88.5 > 85.9[a] 155.4 > 142.5[a] 88.9 > 82.0[a]	- - - -	- - - -
Meles *et al*[13], 2004; Italy	HT, 40-75 yr + 1) not treated, SBP 140-159 or DBP 90-99; OR = 2) medication and BP > 140/90	48/31	8	Resperate® 15 min/d	BP 1/d	Home, SBP DBP Clinic, SBP DBP	137 > 131.6[a] 83 > 79.8[a] 141.4 > 135.9 88.1 > 84.5[a]	126 > 124.1 79 > 78.0 133.2 > 133.0 85.9 > 86.8	-3.5[a] -2.2[a] -5.3 -4.5[a]
Elliot *et al*[14], 2004; United States	HT, medication, SBP 140-179, DBP < 110, 40-75 yr	89/60	8	Resperate® 15 min/d	BP 3/d	Clinic, SBP DBP Home, SBP DBP	150.3 > 139.7 84.7 > 81.5 145.8 > 145.3 85.9 > 85.3	149.8 > 140.6 86.8 > 83.6 141.3 > 141.9 83.7 > 83.5	-1.4 0.0 -1.1 -0.4
Logtenberg *et al*[15], 2007; The Netherlands	T2DM, HT, medication, SBP 140-160, > 18 yr	15/15	8	Resperate® 15 min/d	Discman 15 min/d	Clinic, SBP DBP Home, SBP DBP	153.5 > 146.0 83.0 > 82.0 - -	150.4 > 138.2 87.0 > 81.5 - -	4.7 (-11.7-2.3) 4.6 (-10.4-2.3) 1.0 (-7.8-5.8) 1.3 (-5.8-3.2)
Altena *et al*[16], 2008; The Netherlands	HT, medication SBP 140-160, > 18 yr	15/15	8	Resperate® 15 min/d	Discman 15 min/d	Clinic, SBP DBP Home, SBP DBP	-9.8 -4.6 -2.5 -4.9	-5.6 -2,0 -2.9 -3.4	-4.2 (-12.4-3.9) -2.6 (-8.4-3.3) 0.5 (-3.7-4.8) -1.8 (-8.4-4.8)
Schein *et al*[17], 2009; Israel	T2DM, HT, medication, SBP >130	33/33	8	Resperate® 15 min/d	Usual care	Clinic, SBP DBP	150 > 140 81 > 77	147 > 149 81 > 80	-12[a] -3
Anderson *et al*[18], 2010; United States	Stage 1 HT or pre-hypertension, no medication, no CVD or T2DM.	20/20	4	Resperate® 15 min/d	Meditative exercise 15 min/d	Clinic, SBP DBP 24h, SBP DBP	141.8 > ? 88.2 > ? 138.2 > 137.7 84.6 > 83.8	140.1 > ? 85.2 > ? 137.3 > 137.8 80.4 > 81.8	- - -1 -2.2
Bertisch *et al*[19], 2011; United States	HT and OSA, medication or untreated, BP 120/80-160/100, 20-75 yr	25/-	8	BiPAP® 15 min/d	-	Clinic, SBP DBP	140 > 130.4[a] 82.7 > 80.2	- -	- -
Landman *et al*[20], 2013; The Netherlands	T2DM, HT, medication, SBP 140-160, ⩾ 18 yr	24/24	8	Resperate® 15 min/d	Sham-Device 15 min/d	Clinic, SBP DBP Home, SBP DBP	151.6 > 145.6 82.1 > 76.2 ? ?	151.2 > 142.8 80.7 > 77.0 ? ?	2.4 (-6.5-11.2) -2.3 (-6.7-2.2) -3.0 (-13.2-7.2) 0.1 (-6.9-7.1)
Howorka *et al*[21], 2013; Austria	T2DM, HT, medication, BP < target value, 18-78 yr	16/16	8	Resperate® 12 min/d	Usual care	24h, SBP DBP Daytime SBP DBP	126.1 > 123.2[a] ? 129.3 > 127.1 ?	? ? ? ?	? (P = 0.18) ? (P = 0.43)

[a]P < 0.05 *vs* control. I: Intervention; C: Control; HT: Hypertension; SBP: (Systolic) blood pressure; DBP: Diastolic blood pressure; T2DM: Type 2 diabetes mellitus; CVD: Cardiovascular disease; OSA: Obstructive sleep apnea.

that there was a causal relationship with device-guided breathing.

METHODOLOGICAL QUALITY

In order to compare the studies, we assessed the methodological quality using the criteria as described by van Tulder *et al*[23] (Table 2). The quality of the study by Anderson et al. was low; they used an open randomisation procedure without any further explanation regarding this procedure and blinding[18]. After carefully evaluating the studies by Schein *et al*[9] and Grossman *et al*[10] several methodological questions remained unanswered. It was stated in the Schein *et al*[9] study that the study had a double-blind study design[9]. Randomisation was performed by a third party and a special technician delivered and

Table 2 Randomized controlled trials with an active control group: methodological quality

Criteria	Schein[9]	Grossman[10]	Logtenberg[15]	Altena[16]	Anderson[18]	Landman[20]
Randomization adequate	+/-	+	+	+	-	+
Treatment allocation concealed	+	?	+	+	-	+
Groups similar at baseline	+	+	+	+	+	+
Patient blinded	+/-	+/-	+	+	-	+
Care provider blinded	+/-	+/-	-	-	-	+
Outcome assessor blinded	-	?	-	-	-	+
Co-interventions avoided	+	+/-	?	+	?	+
Compliance acceptable	+	?	+	+	+	+
Withdrawal/drop-out rate acceptable	+	+	+	+	+	+
Timing of outcome assessment similar	+	+	+	+	+	+
Intention to treat analyses	+/-	+	+	+	-	+

explained the device and study procedures. Although the doctor was not aware of the group assignment, patients had weekly follow-up meetings including blood pressure measurements by that same person. Patients were requested not to talk about the specific device with their doctor or to other persons who may be participating in the study. As the patients saw their doctor very regularly it is not unlikely that the doctor became aware of group assignment. Therefore, from a methodological point of view, the authors could have opted for another person performing the outcome measurements. An alternative method would have been to check the success of the blinding procedure. The authors did not explain their rationale behind this randomisation procedure. Furthermore, there were several primary endpoints instead of 1 primary endpoint and 2 secondary endpoints. Also, 5% of all blood pressure data were excluded in an unconventional and post-hoc defined 'end of treatment period' analysis.

Grossman *et al*[10] did not describe whether treatment allocation was concealed and who performed the outcome measurements. Also, data on compliance and whether the blinding procedure was a success, were not provided. Two patients in the control group started lifestyle modification programmes, but analyses without these patients did not change the results.

The Logtenberg, Altena *et al*[16] and Landman *et al*[20] studies have one important limitation in common: the width of the 95%CI of the change of office-measured SBP between groups[15,16,20]. These studies were powered to detect an absolute reduction of 10 mmHg in SBP. In all these studies the limits of the confidence intervals exceeded the boundary of 10 mmHg. The 95%CI in the Logtenberg *et al*[15] and Landman *et al*[20] studies ranged from -2.3 mmHg to 11.7 mmHg, and -6.5 mmHg to 11.2 mmHg, respectively, with a direction in favour of the control group[15,20]. This means that clinically relevant disadvantageous effects of device-guided breathing could not be ruled out. For the Altena *et al*[16] study, the confidence interval ranged from -12.4 mmHg to 3.9 mmHg with a direction in favour of the intervention group[16]. Logtenberg *et al*[15] did not provide data on avoiding co-interventions, whereas Altena *et al*[16] reported that 1 patient in the control group had a change in antihypertensive therapy (per-protocol analyses showing the same results). HbA1c level was higher in the intervention group of the Landman *et al*[20] study, but additional analyses in which adjustments for age, gender, body mass index and HbA1c were done did not relevantly change the results[20]. The adjusted differences in SBP and DBP were 1.1 mmHg (95%CI: -7.6-9.8, in favour of the control group) and 3.5 mmHg (95%CI: -0.4-7.4, in favour of the intervention group), respectively. Finally, the Logtenberg *et al*[15] and Altena *et al*[16] studies had a single-blind design.

Sample size calculations were described in 4 studies[9,15,16,20], and lacking in the Anderson *et al*[18] and Grossman *et al*[10]studies[10,18]. Although Grossman *et al*[10] mentioned that the group size was large enough, they didn't provide a calculation[10]. The Logtenberg study based the calculation on mean SBP and standard deviation (SD) in their clinic[15]. Altena *et al*[16] used the mean blood pressure and SD that were observed in the Logtenberg *et al*[15] study. The most conservative and optimal calculation was performed in the Landman study, as they based their sample size on the highest SD of the change in SBP in the Logtenberg *et al*[15] (SD 9.4 mmHg) and Altena *et al*[16] (SD 10.9 mm Hg) studies[20]. Comparable to their data analysis, Schein *et al*[9] used an unconventional method for the estimation of their sample size. The standardised detectable difference was based on a previous study[24] while they could have used the change in blood pressure and its SD.

DISCUSSION

Out of the 13 RCTs published, there were only a few studies with an acceptable methodological quality. All studies had a short follow-up period. In order to exert effects on cardiovascular morbidity by using device-guided breathing, the device has to be used for many months and preferably years. None of the studies investigated whether the device could be used for prolonged periods. There is 1 meta-analysis, without any involvement of the manufacturer, that showed a small beneficial effect on blood pressure with unclear clinical relevancy of using device guided breathing[8]. As was discussed by the authors of this meta-analysis, the overall effect estimate could have been biased due to inclusion of inadequately controlled trials and sponsored studies. In studies with

an acceptable methodological quality, no beneficial effects were seen. Sensitivity analysis showed that studies, performed without involvement of the manufacturer, showed no beneficial effects of device-guided breathing[8]. Since the meta-analysis was published, 1 additional study has been completed. This study, which had a successful double-blinding procedure and a sham control group, showed no beneficial effects and even possible adverse events[20]. Unfortunately, the writing group of the AHA guideline on non-pharmacological hypertension treatment finished writing the guideline before publication of this latest trial. As this latest study has the highest level of evidence, the writing group from the AHA was asked to reconsider their recommendation from Class Ⅱ A, Level of Evidence B into class Ⅲ, Level of Evidence B (evidence that treatment is not effective)[25]. The committee responded that they didn't believe that the recommendation should be changed[26]. Despite the fact that the latest study showed possible adverse events, the writing group focussed on a small positive general effect estimate from the meta-analysis by Mahtani *et al*[8] and a meta-analysis that was performed by themselves[4]. This positive recommendation by the guideline committee does not seem to be in line with the evaluation of the authors of the Mahtani *et al*[8] study who criticized the methodological quality of most studies and the sponsor involvement in the discussion section of that paper[8]. Since 1 member, who was involved in evaluating the topic of device-guided breathing for the AHA guideline, previously received funding from the manufacturer of the Resperate® device, the response of the AHA guideline committee is of potential concern[4]. We agree with Mahtani *et al*[8] that there is a real possibility that bias was introduced in the overall effect estimate from combining not adequately controlled studies and by including studies with a high level of sponsor involvement.

CONCLUSION

We conclude that, based on studies with acceptable methodological quality, there is no evidence for a short-term beneficial effect on blood pressure by using device-guided breathing. A meta-analysis of individual patient data combining studies with adequate control groups should be performed in the near future. Since there are no trials, not even uncontrolled, with sufficient follow-up on the feasibility and safety of using the device for many months or years, this device cannot safely be advised for treating hypertension in daily practice.

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TOPIC HIGHLIGHT

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Abstract

The influences of life habits on the cardiovascular system may have important implications for public health, as cardiovascular diseases are among the leading causes of shorter life expectancy worldwide. A link between excessive ethyl alcohol (ethanol) consumption and arterial hypertension was first suggested early last century. Since then, this proposition has received considerable attention. Support for the concept of ethanol as a cause of hypertension derives from several epidemiologic studies demonstrating that in the general population, increased blood pressure is significantly correlated with ethanol consumption. Although the link between ethanol consumption and hypertension is well established, the mechanism through which ethanol increases blood pressure remains elusive. Possible mechanisms underlying ethanol-induced hypertension were proposed based on clinical and experimental observations. These mechanisms include an increase in sympathetic nervous system activity, stimulation of the renin-angiotensin-aldosterone system, an increase of intracellular Ca^{2+} in vascular smooth muscle, increased oxidative stress and endothelial dysfunction. The present report reviews the relationship between ethanol intake and hypertension and highlights some mechanisms underlying this response. These issues are of interest for the public health, as ethanol consumption contributes to blood pressure elevation in the population.

Key words: Ethanol; Hypertension; Calcium; Nitric oxide; Oxidative stress

Core tip: After a century of study, the relationship between chronic ethanol consumption and hypertension is well established. This review provides a description of the main studies that showed a relationship between chronic ethanol consumption and hypertension in humans. We also discuss studies using animal models of ethanol-induced hypertension, describing the main mechanisms by which ethanol consumption leads to hypertension.

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INTRODUCTION

Hypertension is a major independent risk factor for cardiovascular disease. In ethanol-consuming populations, the amount of ethanol consumed has a significant impact on blood pressure values, the prevalence of hypertension, and cardiovascular and all-cause mortality. The observa-

Table 1 List of the main epidemiological studies describing the relationship between ethanol consumption and hypertension

Ref.	Yr	Study	Subjects	Age (yr)
Lian[1]	1915		150	42-43
Clark *et al*[2]	1967	Los angeles heart	865	21[1]
Gyntelberg *et al*[3]	1974	Copenhagen	5249	40-59
Klatsky *et al*[4]	1977	Kaiser-Permanente Ⅰ	83947	15-79
Dyer *et al*[5]	1977	Chicago W. Electric	1899	40-55
Arkwright *et al*[6]	1982	Perth	491	20-45
Milon *et al*[7]	1982	Lyon	1134	20-59
Klatsky *et al*[10]	1986	Kaiser-Permanente Ⅱ	66510	-

[1]Mean age.

tion that the excessive consumption of ethyl alcohol (ethanol) is associated with high blood pressure is nearing its centennial mark[1]. In the last century, numerous epidemiologic studies have found an association between ethanol consumption and arterial hypertension[2-6]. It is estimated that 5% to 24% of hypertension cases are associated with ethanol consumption[7,8]. However, although the link between ethanol consumption and arterial hypertension is well established, the mechanism through which ethanol increases blood pressure remains elusive. The effects of ethanol on the cardiovascular system are complex, and attempts to evaluate the possible mechanisms underlying ethanol-induced hypertension in humans are hindered by several limitations. These difficulties include differences in the duration of ethanol use, the timing and frequency of blood pressure measurements, variability in the type and frequency of ethanol intake, age, gender, ethnicity, salt use, body mass index and comorbid conditions.

Animal models of alcoholism may be relevant to understanding the mechanisms by which ethanol consumption increases blood pressure. Data support the involvement of increased sympathetic activity, stimulation of the renin-angiotensin-aldosterone system, increased intracellular Ca^{2+} in smooth muscle with a subsequent increase in vascular reactivity, oxidative stress and endothelial dysfunction. In this review, we will discuss the relationship between ethanol intake and hypertension and some of the possible mechanisms underlying this response. For the present review, a MEDLINE-based search was conducted using the following keywords: "alcohol", "alcoholism", "ethanol", "blood pressure", "hypertension", "nitric oxide", "oxidative stress", "calcium", "endothelial dysfunction" and "vascular reactivity". Articles were further limited to those published in English (except the classic article published in French by Camille Lian) and containing abstracts. Reasons for the exclusion of articles include unclear ethanol dose or ingestion period. Information analysis started with the title, followed by the abstract and, finally, the complete report.

ETHANOL CONSUMPTION AND HYPERTENSION IN HUMANS (TABLE 1)

In 1915, the French army physician Camille Lian studied approximately 150 French career soldiers (42 and 43 years old), relating their drinking to high blood pressure. The results of this study showed a clear threshold relationship of heavy drinking to hypertension, which was defined as 150/100 mmHg, and very heavy drinking increased the risk further. The moderate drinkers consumed 2 L of wine per day, the heavy drinkers consumed more than 2 L per day, and the very heavy drinkers consumed 3 or more liters per day. This was the first report on this relationship, but the result was ignored for approximately 50 years. In the 1960s and 1970s, findings among smaller patient populations corroborated the initial results described by Lian[2,3].

In this review, for the purpose of standardization, the levels of ethanol consumption in humans have been expressed as the number of standard drinks per day (1 standard drink is defined here as the equivalent of 14 g of ethanol). A landmark observational study published in 1977, the Kaiser-Permanente Multiphasic Health Examination Data, reported differences in systolic blood pressure as high as 11 mmHg in individuals consuming 6 or more drinks per day compared with non-drinkers[4]. This study was based on self-administered questionnaires from more than 80000 men and women and showed that a threshold of 3 or more drinks per day was a risk factor for hypertension across races and in both sexes. Moreover, the study found a relationship between the amount of ethanol consumed and blood pressure. This observation was corroborated by other studies. For example, among Danish men aged 40-59 years, the differences in blood pressure between those consuming 6 or more drinks per day and those consuming fewer drinks per day were 8 mmHg (systolic) and 4.5 mmHg (diastolic)[3]. Systolic pressure increased progressively with increasing ethanol consumption among 491 Caucasian males aged 20-45 years. Importantly, the effect of ethanol on systolic blood pressure was independent of the effects of age, obesity, cigarette smoking and physical activity[9].

The second Kaiser-Permanente study reconfirmed the relationship of higher blood pressure to ethanol use[10]. Data from approximately 80000 persons, collected in the United States from 1978 to 1981, revealed a direct positive relationship between the regular consumption of alcoholic beverages and higher blood pressure, independent of potential confounding factors, including age, body weight and smoking status. One important finding of this study was that at 1 to 2 drinks per day, there was a slight but significant increase in blood pressure, indicating that the threshold was lower than that reported in the first Kaiser-Permanente study. The change in the threshold values between the two studies was the result of the division of lighter drinkers into several categories in the second study. As observed previously in the first Kaiser-Permanente study, systolic and diastolic blood pressures substantially increased at 3 to 5 and 6 or more drinks per day.

In his review of studies examining the prevalence of hypertension in ethanol consumption groups, MacMahon (1987) analyzed 29 cross-sectional studies and 6 prospec-

tive studies conducted in populations from a variety of geographic regions, including North America, Australia, Japan, Europe and New Zealand. Most of these studies reported a significant positive association between hypertension and ethanol consumption[11]. The association was shown to be independent of confounders such as age, body mass index, smoking status and exercise. In general, the studies highlighted that the increase in systolic pressure was greater than that in diastolic pressure and that there was a trend toward a greater effect of ethanol on blood pressure in older men compared with younger men. Finally, the studies showed that at 3 to 4 drinks per day, the prevalence of hypertension was approximately 50% greater than that in non-drinkers, and at 6 to 7 drinks per day, the prevalence was 100% greater.

The exact threshold for the effect of ethanol on blood pressure is not clear. In fact, the threshold question is controversial, as epidemiologic studies could not resolve the question of a possible threshold for the apparent risk of hypertension. While several studies have suggested little or no effect of up to 1 or 2 drinks per day on blood pressure[2-4,12], others have shown a progressive linear association[6,7,13]. The first Kaiser-Permanente study described a threshold relationship at 3 to 5 drinks a day for men, with a substantial increase in systolic blood pressure at 6 drinks a day[4]. However, the threshold was found to be at a much lower drinking level than that described in the first Kaiser-Permanente study. Significantly higher systolic pressures were found in Caucasian males who consumed 2 or fewer drinks a day[9]. The second Kaiser-Permanente study described that at 1-2 drinks per day, there was a slight but significant increase in blood pressure[4]. A slight increase in blood pressure was found in men reporting as few as 1 to 2 drinks per day in that survey.

The contribution of ethanol consumption to the prevalence of hypertension is dependent upon the population studied and varies widely in different populations. In developed countries such as the United States and England, it has been estimated that as much as 30% of hypertension may be attributed to ethanol consumption[14]. Other studies suggested this proportion to be smaller. The Australian Risk Factor Prevalence Study[15] estimated that 7% of the prevalence of hypertension could be attributed to ethanol consumption, while the first Kaiser Permanente Study estimated a proportion of 5%[4]. In these two studies, it was estimated that a maximum of 11% of hypertension in men could be attributed to the consumption of ethanol. A French epidemiological study estimated that 24% of the prevalence of hypertension in French men could be attributed to ethanol consumption[7]. Similar results were found in a cross-sectional study in Sidney, where it was estimated that 24% of hypertension may be attributed to ethanol consumption[16].

The estimate is somewhat lower in women and higher in men[4,10]. In the Risk Factor Prevalence Study[15], ethanol consumption accounted for no more than 1% of hypertension in women. The reasons for the gender difference in the proportion of hypertension prevalence associated with ethanol consumption are not fully understood, but they are most likely attributed to the less consumption of ethanol by women than men[11].

The mechanism(s) by which ethanol consumption leads to elevations in blood pressure is uncertain. A small number of studies in humans have attempted to address this question. The role of catecholamines in mediating the effects of ethanol on blood pressure has been investigated in humans. In this regard, increases in plasma adrenaline[17] and noradrenaline[18] were described in humans after ethanol ingestion, and it was suggested that activation of the adrenergic system may be responsible for the increased blood pressure. On the other hand, Potter *et al*[19] did not observe changes in catecholamines levels after ethanol consumption. Moreover, these authors reported that plasma renin and cortisol levels were not affected by the consumption of ethanol[19]. Arkwright *et al*[9] observed that, although blood pressure was higher among ethanol drinkers, there were no changes in plasma adrenaline, noradrenaline, cortisol and renin in these subjects. Conversely, Ibsen *et al*[20] showed increased plasma renin levels among heavy ethanol drinkers. Potter *et al*[19] found that plasma cortisol, but not plasma rennin, increased after ethanol consumption. The reason for the inconsistencies among these results is uncertain, and further studies on the mechanisms underlying the pressor effects of ethanol in humans would be of value. The results of these studies raise a number of possibilities concerning the involvement of humoral mechanisms in the pressor effects of ethanol. However, the available data in humans are not sufficient to allow substantive conclusions. In light of the need for careful investigation of the mechanisms underlying the effects of ethanol on blood pressure, experimental models were created and are used for this purpose.

ANIMAL MODELS OF ETHANOL-INDUCED HYPERTENSION

Most experimental studies corroborate the findings of the epidemiological studies described above, confirming that ethanol consumption is associated with increased blood pressure levels and an increased prevalence of hypertension. Chan and Sutter[21] found that treatment of male Wistar rats for 12 wk with a solution of ethanol (20% *v*/*v*) resulted in mild hypertension. An increase of approximately 25% in mean arterial blood pressure (from 98 to 122 mmHg) was described later by these authors using the same experimental model[22]. Similarly, Abdel-Rahman *et al*[23] observed an increase in systolic blood pressure after 12 wk of ethanol feeding (20% *v*/*v*) in Wistar and Sprague-Dawley rats. Blood pressure was significantly higher at week 6 in Sprague-Dawley ethanol-fed rats (from 106 to 147 mmHg) and at week 8 in Wistar ethanol-fed rats (from 117 to 149 mmHg). The authors also found that ethanol-fed rats had a higher sympathetic activity, as beta-blockade with propranolol decreased heart rate to

a greater degree in ethanol-fed rats than it did in control rats[23]. Strickland and Wooles[24] showed that the systolic and diastolic pressures of ethanol-fed (ethanol 20% *v/v*) Sprague-Dawley rats became significantly greater at 4 wk and continued to increase throughout the remainder the study. The systolic blood pressure of ethanol-fed rats was increased by 6.6 mmHg at 4 wk and by 33.8 mmHg at 22 wk compared with the controls. The difference in diastolic blood pressure between the control and ethanol-fed rats was 5.8 mmHg at 4 wk, and this difference increased to 47 mmHg by 22 wk of ethanol feeding[24]. Vasdev *et al*[25-27] described an increase in systolic blood pressure in male Wistar rats after 1 wk of treatment with ethanol. The rats were given 5% ethanol in their drinking water for 7 wk, and the systolic blood pressure in the ethanol-treated rats was found to be significantly higher than that in the controls after 1 wk or longer[25-27]. Interestingly, the discontinuation of ethanol treatment for 7 wk did not reverse the hypertension or the adverse renal vascular changes in ethanol-induced hypertensive rats[25].

In the study of Utkan *et al*[28], systolic blood pressure was recorded weekly using the tail-cuff method in Wistar rats treated with ethanol (7.2% *v/v*) for 4 wk. There was a mild but significant elevation of systolic blood pressure in the ethanol-fed rats by week 1 compared to baseline measurements, and this difference remained higher at later times. This study showed that the hypertensive state associated with ethanol intake can be observed in the early stages of ethanol consumption. A possible explanation for such a finding could be the higher blood ethanol levels found in this study (293.6 ± 5.2 mg/dL)[28]. Brown *et al*[29] showed that ethanol-consuming Sprague-Dawley rats exhibited elevated systolic blood pressures compared with the control group (151.6 ± 0.6 *vs* 132.9 ± 2.7 mmHg). In this study, the blood ethanol levels averaged 63.8 ± 2.5 mg/dL.

In a previous study, we compared the effects of ethanol intake (20% *v/v*) for 2, 6 and 10 wk on arterial blood pressure in conscious Wistar rats[30]. The baseline systolic, diastolic and mean arterial pressure values of ethanol-treated rats were increased (approximately 20%) after the 3 different periods of treatment. Because blood pressure was already elevated in the 2-wk-treated rats, our results supported the notion that the hypertensive state associated with ethanol intake can occur in the early stages of ethanol consumption. This finding contrasted those of previous studies, which have reported that blood pressure elevation occurred late during chronic ethanol treatment[23,24,28]. Blood ethanol content is a potential explanation for the disparity among reports.

Using this same model of ethanol feeding, we investigated the effects of ethanol treatment for 2 and 6 wk on both blood pressure and vessel reactivity. Mild hypertension was observed in chronically ethanol-treated rats, which was due to increases in both systolic and diastolic pressures. Chronic ethanol consumption in rats increased the contractile response of the aorta and mesenteric arterial bed[31-33]. In addition to its hypertensive effect, ethanol consumption can also modulate the response to vasoactive agents *in vivo*. Data from our group showed that chronic ethanol consumption increased blood pressure as well as the pressor response induced by phenylephrine and endothelin-1[30,34].

The studies using animal models established a positive correlation between the duration of ethanol consumption and the increase in blood pressure, showing that the period of exposure to ethanol is an important factor in the development of hypertension[23,24]. Additionally, there is evidence that blood ethanol concentration contributes to the increase in blood pressure in animal models of alcoholism, where higher blood ethanol concentrations may account for the earlier development of hypertension. Previously, we showed that increased blood pressure, concomitant with ethanol feeding, was observed in 2-wk ethanol-treated animals, in which the blood ethanol content was 1.67 ± 0.21 mg/mL[30]. Abdel-Rahman *et al*[23] reported a blood ethanol concentration of 0.53 ± 0.04 mg/mL in 12-wk-treated rats. Additionally, Abdel-Rahman *et al*[23] (1985), who did not detect blood pressure changes after ethanol treatment, reported a blood ethanol concentration of 0.34 ± 0.04 mg/mL in rats treated with ethanol for 30 d[35].

Several mechanisms have been postulated for the hypertensive response to chronic ethanol consumption. Evidence suggests the existence of a myogenic mechanism(s) that involves alterations in the contractile/relaxant properties of vascular smooth muscle. In fact, the majority of studies describing the effects of ethanol on arterial blood pressure also evaluated the effects of ethanol on vascular responsiveness[24,28,29,31-33].

MECHANISMS UNDERLYING ETHANOL-INDUCED HYPERTENSION (TABLE 2)

Myogenic mechanism

Much of the research investigating the chronic effects of ethanol on the cardiovascular system has addressed vascular responsiveness to vasoconstrictor agents. In this regard, enhanced vascular reactivity to vasoconstrictor agents or impairment of vascular relaxation is described to contribute to the cardiovascular complications associated with chronic ethanol consumption. The initial studies in this field showed enhanced vascular reactivity to α_1-adrenoceptor agonists in different arteries from ethanol-fed rats. Pinardi *et al*[36] found that chronic ethanol consumption significantly enhanced the contractile response induced by phenylephrine of endothelium-intact aortic rings. Noradrenaline-induced contraction of the superior mesenteric artery was shown to be greater in rings from ethanol-treated rats[37]. Likewise, there was an ethanol-associated increase in the maximal contractile response to phenylephrine, a selective α_1-adrenoceptor agonist, in endothelium-denuded aortic rings[38]. Later, Ladipo *et al*[39] demonstrated that chronic ethanol consumption increased the sensitivity of rat aortic rings to noradrenaline. At this point, although it was well estab-

Table 2 Summary of the main mechanisms underlying ethanol-induced hypertension

Ref.	Mechanism
[17,18]	Increase in sympathetic nervous system activity
[20]	Stimulation of the renin-angiotensin-aldosterone system
	Myogenic mechanism:
[31,32,36-42]	Enhanced vascular reactivity to vasoconstrictor agents
[33,41,44-46]	Impairment of the vascular relaxation
	Oxidative stress:
[70-77]	Increase in reactive oxygen species generation
[81,82,85-87]	Reduction of antioxidant systems
[28,44,52,95-102]	Decrease of nitric oxide bioavailability and endothelial dysfunction

lished that chronic ethanol consumption enhanced α_1-induced contraction, the mechanisms underlying this response were poorly understood. Moreover, the experiments designed to study the vascular effects of chronic ethanol consumption on α_1-induced contraction used only one period of treatment[21,28,29]. Based on these observations, we proposed a study to investigate the time-course of changes in vascular reactivity to phenylephrine in aortas from chronically ethanol-treated rats as well as to evaluate in detail the mechanisms underlying the effects of long-term ethanol consumption on α_1-induced contraction. Chronic ethanol consumption produced an increased responsiveness to phenylephrine in aortas, although there was no relationship between the period of treatment (2, 6 and 10 wk) and the magnitude of the enhancement of α_1-induced contraction[40]. Importantly, the increased responsiveness to phenylephrine was also observed after endothelial denudation, further suggesting that the increased sensitivity to α_1-adrenergic agonists was not dependent on the presence of the endothelium. The enhanced vascular response to phenylephrine observed in the aorta of ethanol-treated rats was maintained by two mechanisms: an increased release of thromboxane A2, a vascular smooth muscle-derived vasoconstrictor prostanoid, and an increased extracellular Ca^{2+} influx. One interesting finding of this study was that the increased response to phenylephrine was not the result of a nonspecific increase in rat aorta reactivity induced by chronic ethanol intake, as the contractile responses to endothelin-1 or KCl were not affected by the ethanol treatment. In fact, while studying the effect of ethanol consumption on the reactivity of rat carotids to endothelin-1, we found an increase in endothelin-1-induced contraction in this artery with no change in the contraction induced by phenylephrine[41,42]. The hyperactivity to endothelin-1 in the rat carotid was not different among the three periods of treatment (2, 6 and 10 wk) used in our study. The potentiation of endothelin-1-induced contraction in the rat carotid was caused by reduced expression of pro-relaxation endothelial endothelin receptor type B (ET_B) receptors.

Most of the experiments designed to study the relationship between alterations in vascular functionality and increases in blood pressure induced by ethanol consumption used conduit vessels, such as the aorta. However, while the aorta does not offer substantial resistance to blood flow, the contribution made by vessels of smaller diameter to peripheral vascular resistance is much greater. In rats, the mesenteric circulation receives approximately one-fifth of the cardiac output[43], and thus, regulation of this bed provides a significant contribution to the regulation of systemic blood pressure. To further analyze this aspect, we evaluated whether alterations in the reactivity of the mesenteric arterial bed could account for the hypertensive state associated with ethanol consumption[31]. Chronic ethanol consumption produced an endothelium-dependent increased responsiveness to phenylephrine in a perfused mesenteric arterial bed isolated from rats treated with ethanol for 6 wk but not from rats treated for 2 wk. However, increased blood pressure was observed in ethanol-treated animals after 2 wk, whereas altered responsiveness to phenylephrine was only observed in rats treated for 6 wk. These observations supported the notion that the altered responsiveness of resistance arteries was not the cause, but rather the consequence, of the increased blood pressure associated with ethanol intake[31,32]. The increased vascular response to phenylephrine observed in the mesenteric arterial bed was maintained by two mechanisms: an increased release of endothelial-derived vasoconstrictor prostanoids and a reduced modulatory action of endothelial nitric oxide (NO); the latter is likely associated with a reduced expression of the enzyme eNOS (endothelial NO synthase)[32].

Impairment of vascular relaxation may also contribute to the cardiovascular complications associated with chronic ethanol consumption. Long-term ethanol consumption significantly reduced acetylcholine-induced relaxation in the aortic rings from rats treated with ethanol for 12 wk[44] and 8 wk[45]. In the rat carotid, the relaxation induced by IRL1620, a selective endothelin ET_B receptor agonist, was reduced after treatment with ethanol; this effect was mediated by a mechanism involving the down-regulation of endothelial ET_B receptors[41]. More recently, we found that chronic ethanol consumption reduced the endothelium-dependent relaxation induced by the peptide adrenomedullin in the rat aorta[46].

In resistance arteries, Hatton *et al*[37] showed an increased response of mesenteric arteries to noradrenaline in rats treated with ethanol for 18 wk. The finding that the relaxation induced by acetylcholine, but not by sodium nitroprusside, was reduced in the mesenteric arterial bed from ethanol-treated rats indicated that chronic ethanol consumption decreased the action of NO or its endothelial cell receptor-stimulated production/release[32]. Similarly, ethanol consumption was also found to reduce the endothelium-dependent relaxation induced by adrenomedullin in the rat mesenteric arterial bed[33]. The vascular relaxation induced by adrenomedullin in the rat mesenteric arterial bed is endothelium-dependent and involves the activation of the NO-cyclic guanosine monophosphate pathway[47]. In our study, no differences in adrenomedullin-induced relaxation were detected in control and ethanol-exposed tissues after incubation with

L-nitro-arginine methyl ester, a NOS inhibitor, suggesting that the reduced adrenomedullin responsiveness of the mesenteric arterial bed from ethanol-treated rats was due to an impaired modulation of adrenomedullin-induced relaxation by NO[33].

The vascular endothelium and vascular smooth muscle cells are important targets for the effects of ethanol consumption. These effects are complex, and the identification of biochemical/molecular mechanisms that could explain such effects is warranted. A number of mechanisms have been postulated to explain the pathogenesis of high-dose ethanol toxicity in the vasculature. These mechanisms include an increase in intracellular Ca^{2+} levels with a subsequent increase in vascular reactivity, oxidative stress and a reduction in NO bioavailability. These processes will be discussed in the following sections.

Alterations in Ca^{2+} levels

One of the mechanisms by which chronic ethanol consumption leads to alterations in vascular responsiveness is by increasing the intracellular Ca^{2+} levels in vascular smooth muscle cells. Ca^{2+} is a cation of critical importance for many cellular control mechanisms, including muscle contraction. During excitation, the intracellular Ca^{2+} concentration increase by either (1) Ca^{2+} entry through the plasma membrane through voltage- or ligand-gated ion channels, or (2) release from intracellular stores (sarcoplasmic reticulum or mitochondria).

Some studies have provided evidence that ethanol consumption increases the intracellular Ca^{2+} concentration. This response may result from a direct effect of ethanol on plasma membrane permeability, Na^+ transport and Na^+-Ca^{2+} exchange, and/or impaired Ca^{2+} transport due to a secondary abnormality, such as Mg^{2+} depletion, which is described in alcoholics[48]. Increased Ca^{2+} influx results in increased vascular contractility and reactivity, and those responses increase vascular tone and peripheral vascular resistance, thereby elevating blood pressure[49]. Tirapelli *et al*[40] described an increased phenylephrine-induced contractility of arteries from ethanol-treated rats. SQ29548, a potent and selective thromboxane A2 receptor antagonist, reduced the maximal $CaCl_2$ response of aortic rings from ethanol-treated rats, suggesting that the enhanced response to extracellular Ca^{2+} was modulated by PGH_2/TXA_2. Based on these results, it was concluded that prostanoids mediate the enhanced reactivity to phenylephrine by mechanisms that alter the mobilization of or sensitivity to extracellular Ca^{2+}[40].

The effect of chronic ethanol administration on blood pressure and its relation to Ca^{2+} were also investigated by Hsieh *et al*[50] in 7-wk-old Wistar rats that had received 15% ethanol in their drinking water. The blood pressure in ethanol-treated rats was significantly higher than in the controls. The extracellular fluid volume was increased in ethanol-treated rats, and the blood pressure significantly correlated with increases in the intracellular Ca^{2+} concentration. These results suggest that increased intracellular Ca^{2+} and augmented body fluid volume contributed to the development of ethanol-induced hypertension. It was also suggested that these responses were partly mediated by Mg^{2+} depletion and suppressed Na^+ pump activity[50]. In fact, these factors appear to be all-important in the etiology of hypertension[51].

In 2008, Tirapelli *et al*[52] reported an increased responsiveness to KCl of arteries from female rats chronically treated with ethanol. Because KCl-induced contraction depends almost exclusively on Ca^{2+} influx through the activation of voltage-sensitive channels[53], it was suggested that ethanol consumption increases the Ca^{2+} influx through these channels. Vasdev *et al*[54] observed that ethanol consumption (10% ethanol in drinking water-6 wk) increased systolic blood pressure and that this response was associated with an increased Ca^{2+} uptake by aortas from ethanol-treated animals. These findings suggested that increases in cytosolic free Ca^{2+} and in Ca^{2+} uptake in the vasculature are associated with ethanol-induced hypertension. Two years later, these authors reported that verapamil, a Ca^{2+} channel blocker, reversed the increase in systolic blood pressure and aortic Ca^{2+} uptake induced by chronic ethanol consumption. In addition to the effects observed previously, the authors observed smooth muscle cell hyperplasia in small arteries and in renal arterioles from ethanol-treated rats[25].

In a clinical study, it was demonstrated that both systolic and diastolic blood pressures were significantly higher in individuals drinking 275 g ethanol per week[55]. In these subjects, increased plasma Ca^{2+} levels were correlated with increased diastolic blood pressure. An increment in diastolic pressure of 6.9 mmHg correlated with increments of 0.1 mmol/L in plasma Ca^{2+} concentration. Those findings suggested that regular ethanol consumption predisposes to hypertension by facilitating Ca^{2+} accumulation in cells involved in blood pressure regulation[55]. Taken together, the above-mentioned studies suggest a role for Ca^{2+} in ethanol-induced hypertension. In this scenario, ethanol consumption would alter Ca^{2+} influx/permeability in the vasculature with a consequent increase in vascular contractility and peripheral resistance, which in turn would be responsible for the increase in blood pressure associated with ethanol consumption.

Oxidative stress

Reactive oxygen species (ROS) are reactive chemical entities produced as intermediates in reduction-oxidation (redox) reactions. Perturbations of the balance between ROS production and scavenging by antioxidant systems result in oxidative stress and presumably in pathophysiologic changes. Oxidative stress is a common mediator of pathogenicity in cardiovascular diseases, such as hypertension[56,57]. ROS have an important pathophysiological role in inflammation (by influencing platelet aggregation and migration of monocytes), hypertrophy, proliferation, fibrosis, angiogenesis, processes that are involved in cardiovascular remodeling and endothelial dysfunction[58-61].

The role of ROS in the pathophysiology of hypertension is well established[62-64]. The causal relationship between ethanol, ROS and hypertension most likely occurs at the vascular level, where ethanol promotes oxidative

stress, endothelial dysfunction, vascular inflammation, increased vascular reactivity and structural remodeling. Together, these responses lead to increased peripheral resistance and therefore to increased blood pressure[65, 66]. It is known that ROS modulate specific cellular pathways (redox signaling), leading to changes in gene transcription and in functional oxidative modifications of cellular proteins that cause cellular dysfunction[56,67,68]. Thus, oxidative stress not only causes direct and irreversible oxidative damage to macromolecules, but it also affects redox-dependent signaling in the vasculature[69]. ROS generation by ethanol is important to its pathophysiology in the cardiovascular system, as ethanol is extensively metabolized into acetaldehyde in the liver, mainly by the enzyme alcohol dehydrogenase[70]. Acetaldehyde, in turn, is oxidized to acetate by acetaldehyde dehydrogenase, which results in the generation of ROS and decreased NO levels[71].

In addition to the ROS generated during ethanol metabolism, some studies have shown the involvement and contribution of the nicotinamide adenine dinucleotide phosphate [NAD(P)H] oxidases to dysfunctions promoted by chronic ethanol consumption in several tissues[72-76]. Increased vascular oxidative stress induced by ethanol consumption is related to the activation of the enzyme NAD(P)H oxidase, and this mechanism is involved in the increased blood pressure caused by chronic ethanol consumption. NAD(P)H oxidase is the main source of ROS in endothelial and smooth muscle vascular cells[65], and it is considered a key factor in the vascular dysfunctions induced by ethanol. Husain *et al*[77] demonstrated that chronic ethanol consumption leads to an increased NAD(P)H oxidase activity and ROS generation that leads to membrane lipid peroxidation. The authors also observed increased phenylephrine-induced contraction and reduced acetylcholine-induced relaxation in aortas from ethanol-treated rats[77]. These data suggest that the initial step in the cardiovascular dysfunction associated with chronic ethanol consumption involves the formation of ROS, and this process can be mediated by the enzyme NAD(P)H oxidase. Moreover, this enzyme has been implicated in the activation of xanthine oxidase and the uncoupling of eNOS, which leads to ROS overproduction[78].

The antioxidant enzymes are the first line of defense against ROS-induced oxidative tissue injury. In vascular tissue, the enzymatic antioxidant system mainly consists of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), thioredoxins and peroxiredoxins. The non-enzymatic antioxidants include ascorbate, tocopherol, glutathione, bilirubin and uric acid[79,80]. The antioxidant mechanisms antagonizing the consequences of chronic ethanol consumption have particularities related mainly to the type of tissue studied, the duration of treatment and the concentration of ethanol used. Das and Vasudevan[81] showed that ethanol consumption increased SOD activity and decreased CAT activity in a time- and dose-dependent manner[81]. Husain *et al*[82] demonstrated increased SOD activity in the liver of rats treated with ethanol[82]. It is known that SOD activity is modulated by increased ROS generation and by lipid peroxidation[83,84]. In rats, chronic ethanol treatment led to increased CAT activity and impaired the maintenance of the glutathione redox cycle in renal tissue, with an increase in GPx activity and a decrease in GSH (reduced glutathione) levels[84].

In clinical studies, increased plasma activity of SOD and GPx was observed in subjects who regularly consume ethanol[85,86]. Husain *et al*[87] demonstrated that chronic ethanol consumption by rats significantly depressed both cytosolic CuZn-SOD and mitochondrial Mn-SOD activities in the plasma, indicating an inability of the cells to scavenge superoxide anion. Moreover, plasma CAT and GPx activities were also significantly decreased in ethanol-treated rats. The inhibition of these enzymes may increase superoxide anion availability, which can react with NO to form peroxynitrite[87].

The role of oxidative stress in ethanol-induced hypertension is complex and may involve increases in ROS generation or reductions in antioxidant systems. The increase in oxidative stress promoted by ethanol is associated with endothelial dysfunction, vascular inflammation and increased vascular reactivity. These processes may contribute directly or indirectly to increased peripheral resistance and therefore to increased blood pressure.

NO bioavailability

In 1980, Furchgott *et al*[88], in classic study, discovered that endothelial cells produce an endothelium-derived relaxing factor (EDRF) in response to stimulation by acetylcholine. In 1987, Palmer *et al*[89] and Ignarro *et al*[90] identified EDRF as NO, a free radical that diffuses to underlying smooth muscle to induce vasodilatation[89,90]. These findings marked the beginning of a major worldwide expansion of research into the role of NO in vascular physiology and pathophysiology.

The endothelium plays a pivotal role as a sensor, transducer, and integrator of signaling processes regulating vascular homeostasis, and it is known that vascular diseases, including hypertension, are characterized by impaired endothelium-derived NO bioactivity. The effect of ethanol on the function of the endothelial is complex[91]. Appreciating the importance of NO in the maintenance of vascular tone, some studies have examined the mechanisms underlying the impairment of NO-mediated vasodilatation by chronic ethanol consumption[92]. In theory, such a decrease in NO bioactivity could result from reduced NO production or from the inactivation of NO[93]. NO is produced by NOS (nitric oxide synthase) *via* one of three isoforms: the neuronal NOS (nNOS/NOS1), inducible NOS (iNOS/NOS2), and the endothelial NOS (eNOS/NOS3)[94]. Ethanol exerts different effects on these isoforms in a variety of cells and tissues. Tirapelli *et al*[52] demonstrated that chronic ethanol consumption reduced the vascular expression of eNOS in female rats. Conversely, iNOS expression in arteries from ethanol-treated rats was significantly increased compared with control tissues. This response could be the result of a compensatory mechanism, where increased iNOS expression could induce a substantial and sustained release of NO

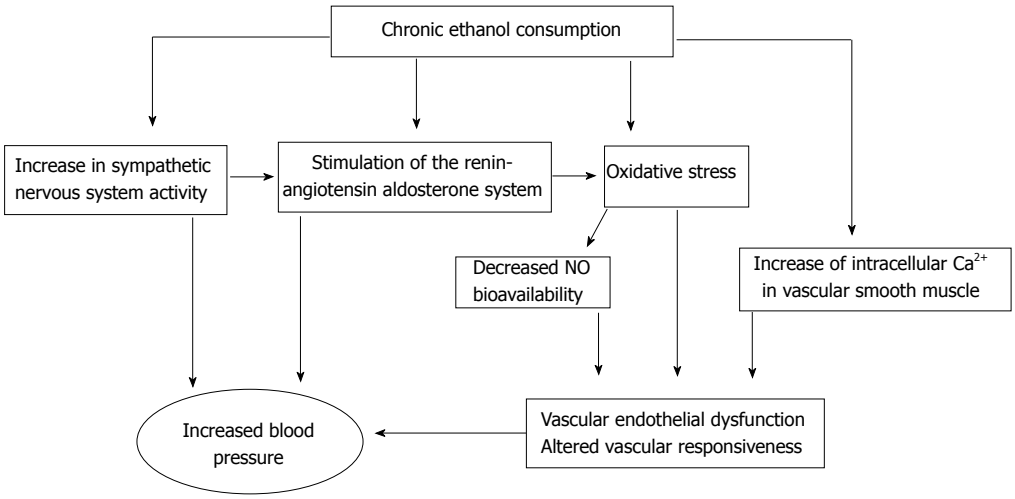

Figure 1 Summary of the basic pathophysiological mechanisms underlying ethanol-induced hypertension.

to compensate for the reduction of eNOS expression[52]. In the rat liver, ethanol decreased eNOS expression and activity[95]. Krecsmarik *et al*[96] demonstrated that chronic ethanol consumption induced an increase in iNOS activity and a decrease in nNOS expression in the rat gastrointestinal tract[96]. Moreover, chronic ethanol treatment reduced the eNOS-dependent relaxation of cerebral arterioles in rats[97].

The effect of ethanol on endothelial NO bioavailability appears to be related to the dose of ethanol. In this sense, it was shown that low concentrations of ethanol induced an increased release of endothelial NO due to the activation and expression of NOS[98,99]. Utkan *et al*[28] described that chronic ethanol consumption potentiates endothelium-dependent relaxation in aortic rings, most likely through interference with the synthesis and/or release of NO or adaptive alterations in muscarinic receptors on the endothelial cells[28].

While low concentrations of ethanol are described to increase endothelial NO production, the chronic consumption of high doses of ethanol impairs endothelial function in association with reduced NO bioavailability. Husain *et al*[44,100] described down-regulation of the NO-generating system, leading to impaired vasorelaxation and hypertension. Male Fisher rats orally administered 20% ethanol (4 g/kg - 12 wk) showed increased systolic and diastolic blood pressures and impaired vascular relaxation compared with controls. The expression of eNOS in the thoracic aorta isolated from ethanol-fed rats was down-regulated, leading to a depletion of aortic NO. This process may alter resistance vessel architecture, reducing its dilatory capacity[44,100]. In 2004, Kuhlmann *et al*[101] reported that high concentrations of ethanol decreased NO synthesis in and proliferation of endothelial cells from human umbilical veins.

The concentration of plasma asymmetric dimethylarginine (ADMA) in alcoholics is higher than in non-alcoholic subjects[102]. ADMA is an endogenous inhibitor of NO production, which is generated from the methylation of arginine residues by arginine methyltransferases and subsequent proteolysis. In this sense, increased ADMA levels could also contribute to the reduced bioavailability of NO in alcoholics.

NO, which is constantly formed, readily reacts with reactive molecules, such as superoxide anion[103,104]. Most of the cytotoxicity attributed to NO is due to peroxynitrite, which is produced from the reaction between NO and superoxide anion[105]. This loss of NO that occurs in the reaction with superoxide anion deprives vascular smooth muscle cells of NO. Ethanol reduces the bioavailability of NO through both the inhibition of eNOS and through the formation of peroxynitrite, which can lead to cellular damage[106].

CONCLUSION

The link between hypertension and chronic ethanol consumption is well established, and the mechanism by which ethanol increases blood pressure is complex. There appears to be more evidence implicating the sympathetic nervous system, the renin-angiotensin-aldosterone system, increased intracellular Ca^{2+} in vascular smooth muscle, oxidative stress, decreased NO bioavailability and endothelial dysfunction than there is evidence for the other mechanisms suggested, but this issue remains an open one. After a century of study, it is established that chronic ethanol consumption leads to hypertension and that this process is a multi-mediated event involving the aforementioned mechanisms (Figure 1). Thus, it is of great importance to invest in implementing strategies that help to prevent alcoholism, thus reducing the risk of ethanol-associated cardiovascular diseases.

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TOPIC HIGHLIGHT

WJC 6th Anniversary Special Issues (1): Hypertension

Asserted and neglected issues linking evidence-based and Chinese medicines for cardiac rehabilitation

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Abstract

High blood pressure is among the most prevalent chronic disease in adults that impacts on the quality of life of patients, which are often subjected to physical rehabilitation. Chinese medicine intervention in patients with hypertension presents promising albeit inconclusive results, mostly due to methodological issues. This paper discusses asserted and neglected issues linking evidence-based and Chinese medicines as related to systemic arterial hypertension, as well as their impact on the physical rehabilitation of those patients. On the one hand, natural history of hypertension, pulse palpation, and herbal therapy are among the asserted issues because of the scientific evidence collected about them, either in favor or against its integration to the current medical practice. On the other hand, anatomical variations of vessels and comparative physiology are among the most commonly neglected issues because previous researches on integrative medicine ignored the possible effects of these issues as related to the study's outcome. The asserted issues highlighted in this paper stimulate the increasing use of Chinese medicine for health care and the continuity of research on integrative medicine in the cardiovascular field for rehabilitation. The neglected issues poses additional challenges that must not be overlooked in future research on this topic so that the integration of both traditional and current knowledge may be of benefit to the population with cardiovascular disease.

Key words: Cardiovascular disease; Hypertension; Chinese medicine; Rehabilitation; Integrative medicine

Core tip: Integrative medicine might provide better clinical results than evidence-based or Chinese medicines isolated for patients undergoing cardiac rehabilitation. The asserted issues highlighted in this paper (natural history of hypertension, pulse palpation, and herbal therapy) stimulate the increasing use of Chinese medicine for health care and the continuity of research on integrative medicine in the cardiovascular field. Conversely, some neglected issues (anatomical variations of vessels and comparative physiology) poses challenges that must not be overlooked in future research on this topic so that the integration of both traditional and current knowledge may be of benefit to the population with cardiovascular diseases.

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INTRODUCTION

High blood pressure is a major public health problem

worldwide. Hypertension is among the most prevalent chronic, non-contagious disease in adults[1], despite the trend to decrease its prevalence in some countries[2]. The natural history of this disease still needs elucidation: although most of its modifiable and non-modifiable risk factors are well known, the etiology of primary systemic arterial hypertension (SAH) remains uncertain[3]. The long-term impact of hypertension on health is nevertheless evident. Small, middle and large-sized arteries are the earliest body structures affected by time-sustained levels of high blood pressure[4]. Such arterial remodeling process contributes to the pathophysiology of this condition in target-organs others than the arteries such as the skeletal muscle[5], heart, kidneys, brain, and eyes[6]. Without early and proper intervention, organic functions start to deteriorate such that they are detectable by either laboratorial or imaging exams as a complement to the clinical examination of signs and symptoms[3]. On a timely fashion, functional capacity may be compromised at the systemic level[7] with possible impacts on the quality of life of these patients[8], which often are subjected to physiotherapy and cardiac rehabilitation.

Chinese medicine comprises a phenomenological, philosophic, and systematic traditional health care system developed through almost five millennia[9]. Because Chinese medicine was rooted in a sociocultural environment that differed from the European medicine at its early beginning, it is reasonable to expect differences on both medical practices and respective evolution of medical theories. Nevertheless, recent randomized clinical trials, systematic reviews, and meta-analyses on the efficacy of Chinese medicine interventions in patients with SAH were conducted[10] with promising albeit inconclusive results. In general, those studies help answering questions raised from the clinical point-of-view, such as "Is Chinese medicine intervention effective for reducing or controlling blood pressure levels". Investigating this point-of-view leaves opened the traditional point-of-view, which raised questions such as "Are there actual subtypes of hypertension as related to Chinese medicine" or "Is the theory of pattern differentiation for diagnosis relevant for guidance on therapeutic intervention".

In other words, one may argue what are the scientific evidences for the statements found in the Chinese medicine literature, specially the most antique ones. On the one hand, diving into the traditional Chinese medical literature one can find a number of traditional assertions calling for scientific evidence, if any. On the other hand, researchers often assume that some of these traditional factors may not have a detectable effect on their study' s outcome. As it was argued that integrative medicine might provide better clinical results than either one isolated[11], a comprehensive overview of the asserted and neglected issues between evidence-based and Chinese medicines is necessary for both clinicians and researchers. Therefore, this paper discusses the asserted and neglected issues linking evidence-based and Chinese medicines as related to SAH, as well as their possible impact on the physical rehabilitation of those patients.

ASSERTED ISSUES

In this section, the natural history of SAH, pulse palpation, and herbal therapy are discussed. These topics are considered as asserted issues because of the scientific evidence collected either in favor or against their integration into the current medical practice. However, they should not be considered as final positions because there are lacunas that still need to be addressed in future studies. Table 1 presents summary information about the studies cited in this section.

Natural history of SAH

The epidemiological concept of natural history of diseases also applies to Chinese medicine, with proper correspondence due to their inherent conceptual differences. The Chinese medicine counterpart of an ongoing morbid process is called *zheng* or pattern. It is worth noticing that a pattern encompasses other information than just signs and symptoms in the Western sense: behavior, emotional states, self-awareness of social status, and physical constitution are among other manifestations considered for diagnosis or "pattern differentiation"[9]. Regardless of these differences, Chinese medicine theory presents basic elements of the natural history of diseases such as the existence of protection and risk factors for patterns, a clinical horizon for the onset of manifestations, and health outcomes such as cure, permanent or temporarily disability, and death.

As a matter of fact, there is evidence supporting that most clinical manifestations observed in patients with SAH and that are used for pattern differentiation are actually associated with target-organs damage (TOD). For instance, the clinical manifestations of cerebrovascular disease are strongly associated (Pearson correlation coefficient = 0.718, $P < 0.001$) to those of "Obstruction of phlegm and dampness of Heart/Liver/Gallbladder"[12]. Moreover, long-term SAH can lead to myocardial ischemia, conduction defects, arrhythmias, and ventricular hypertrophy[13]. The brain is another target-organ usually damage by the SAH; cognitive disturbances in the elderly are, at least in part, hypertension-related[14-16]. High risk of stroke, cognitive decline, and dementia are also associated to SAH[17-19]. Some mild retinal changes are largely nonspecific except in young patients, hemorrhages, exudates and papilledema, are only present in severe hypertension and are associated with increased cardiovascular risk[3]. All the above-cited TOD eventually manifests signs and symptoms, which should be early detected in the natural history of SAH. Therefore, it is possible to assert that there is a relationship between Chinese medicine patterns and the clinical presentation of SAH-including its related comorbidities.

Most importantly, it is also possible to infer that patients with SAH are candidates for cardiac rehabilitation, even from the traditional Chinese medicine point-of-view. Recent systematic reviews found that Chinese medicine mind-body exercises such as *qigong*[20] and *taijiquan*[21] can be of benefit for patients undergoing antihypertensive

Table 1 Summary description of studies on the asserted issues linking evidence-based and Chinese medicines

Ref.	Study characteristics	Main results	Main limitation
Natural history of patterns			
Luiz *et al*[12]	Cross-sectional observational design Forty-three patients with hypertension grades Ⅰ, Ⅱ and Ⅲ	Patterns were strongly or moderately associated with target-organ damage Manifestations were at most weakly associated with hemodynamic variables	Target-organ damages were not confirmed by laboratory or imagery methods Patients were under antihypertensive drug therapy
Chan *et al*[20]	Systematic review (8 studies) Seven randomized controlled trials and one non-randomized controlled clinical trial	Qigong improved physical symptoms in patients with coronary artery disease Qigong improved functional capacity of cardiac patients Qigong reduced blood pressure levels No adverse effects reported	Overall poor quality of most studies included in the review Study heterogeneity
Yeh *et al*[21]	Systematic review (26 studies) Nine randomized controlled trials, thirteen non-randomized controlled trials, and four observational studies	*Taijiquan* reduced blood pressure levels No adverse effects reported	Overall poor quality of most Chinese studies included in the review Study heterogeneity
Pulse palpation			
Luiz *et al*[12]	Cross-sectional observational design Forty-three patients with hypertension grades Ⅰ, Ⅱ and Ⅲ	Frequency analysis of clinical manifestations and pulse images of patterns Most frequent pulse image: wiry pulse (52% of the cases)	Patients were under antihypertensive drug therapy
Ferreira *et al*[25]	Cross-sectional observational study Twenty-nine healthy subjects and twenty-three patients with hypertension grades Ⅰ, Ⅱ or Ⅲ	Higher pulse wave velocity and lower arterial compliance of the brachial-radial artery segment in hypertension	Arterial tonometry was subjected to transducer set-up and manual positioning Patients were under antihypertensive drug therapy
Ferreira *et al*[26]	Cross-sectional observational study Sixty-three healthy subjects and fifty-two patients with hypertension grades Ⅰ, Ⅱ or Ⅲ	Lower arterial compliance of the brachial-radial artery segment in hypertension Hypertrophic remodeling of medium-sized arteries in hypertension	Arterial tonometry was subjected to transducer set-up and manual positioning Patients were under antihypertensive drug therapy
Ferreira *et al*[27]	Cross-sectional observational study Sixty-three healthy subjects and fifty-two patients with hypertension grades Ⅰ, Ⅱ or Ⅲ	Impaired flow-mediated vasodilation in hypertension	Arterial tonometry was subjected to transducer set-up and manual positioning Patients were under antihypertensive drug therapy
Ferreira *et al*[28]	Cross-sectional observational study Sixty-three healthy subjects and fifty-two patients with hypertension grades Ⅰ, Ⅱ or Ⅲ	Increased peripheral vascular resistance immediately after ischemic occlusion Slower response to flow-mediated vasodilation	Arterial tonometry was subjected to transducer set-up and manual positioning Patients were under antihypertensive drug therapy
Lu[29]	Cross-sectional observational study Fifty-nine patients with hypertension grades Ⅰ, Ⅱ or Ⅲ	Higher amplitudes for harmonics #0 (heart), #1 (liver), #3 (spleen), #4 (lung), and #6 (gallbladder) in hypertension	Poor description of the studied sample Patients were under antihypertensive drug therapy
Ferreira[30]	Computational simulation study Model of the radial artery during "simultaneous pressing"	Lack of correspondence between pressure pulse spectral harmonics and Chinese medicine theory of pulse palpation	No experimental data from patients with hypertension
Herbal therapy			
Xiong *et al*[32]	Narrative review	Herbal therapy may potentially reduce blood pressure variability, inhibit sympathetic activity, prevent target-organ damage, and improve insulin resistance	Potentially biased (selection and report bias) Some results outcome from animal studies not yet tested in humans

treatment. The benefits of *qigong* practice may include the alleviation of physical symptoms related to cardiovascular disease (CVD) (*e.g.*, 63% of the group presented relieving of coronary artery disease symptoms) and the control of blood pressure (*e.g.*, 88% of the group presented lower blood pressure levels) after 1-year practice, and the increase in functional capacity (*e.g.*, 13.7% higher six-minute walk distance after a 16-wk *qigong* training program)[20]. Likewise, the benefits of *taijiquan* practice may include a reduction in systolic and diastolic blood pressures (3-32 mmHg and 2-18 mmHg, respectively)[21]. However, it is not clear whether the effects on blood pressure are due to the traditional aspects of Chinese medicine practice or to the increased physical activity itself, or both. Nevertheless, further research is necessary to determine whether Chinese medicine therapy indicated from pattern differentiation is of benefit to patients with SAH, either at secondary or tertiary level of prevention.

Pulse palpation

Clinical examination in Chinese medicine is not different from that practiced in evidence-based medicine: inspec-

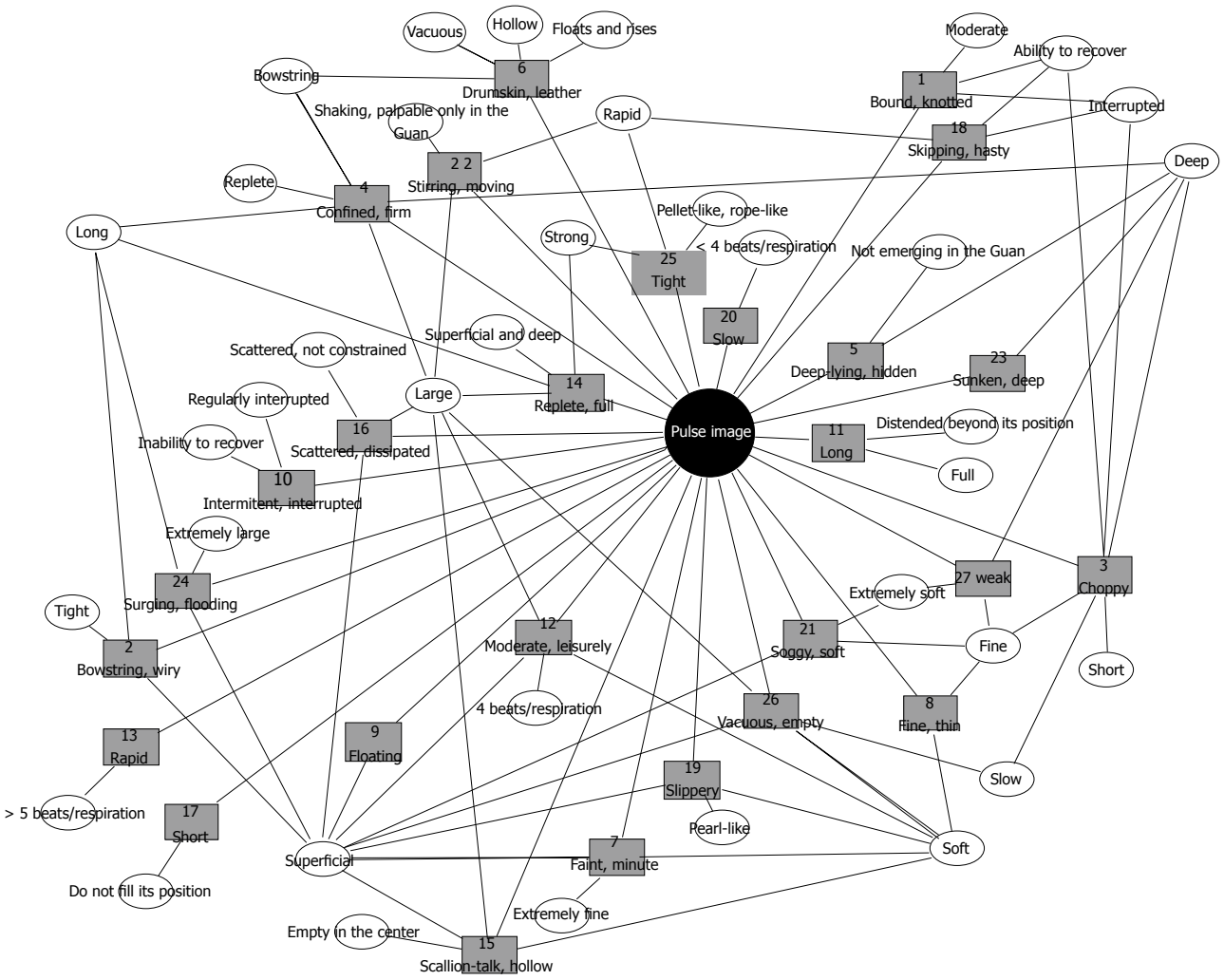

Figure 1 Pulse image network. The classic pathologic 27 pulse images (greyish, rectangular nodes) described by common attributes (whitish, ellipsoid nodes) derived from categories (frequency, rhythm, wideness, depth, and qualities). Notice that there are pulse images described by exclusive attributes, while other pulse images are described by shared attributes.

tion, auscultation and olfaction, inquiry and palpation. The most striking difference is that even today Chinese medicine health providers do not make use of any complementary exam or equipment (*e.g.*, arterial tonometry, imaging or laboratorial data), thus relying exclusively on the subjective assessment of the five senses for confirmation or exclusion of possible patterns. Among these examinations, pulse palpation is probably the most famous and intriguing one, since antiquity until present days[22].

Fundamental attributes of the arterial pulse such as frequency, rhythm, wideness, and depth are shared between Chinese and evidence-based medical practices. Descriptions of abnormal pulses as palpated at either the radial or carotid artery are established for clinical diagnosis of patients with cardiovascular diseases (CDV)[23]. Chinese medicine practitioners also make use of subjective attributes to describe their feeling of the pulse – the so-called *pulse image*[22]. Figure 1 exhibits the network of all 27 pathological pulse images from descriptions arranged by attribute[22] as generated by Cytoscape 3.0.0[24]. It can be observed that there are pulse images described by exclusive attributes (*e.g.*, "rapid" or "short" pulse), while other pulse images are described by shared attributes (*e.g.*, "weak" or "fine" pulse). In particular, the "deep", "fast", "slippery", "strong", "thin" and "wiry" pulse images are frequently observed in patters related to SAH (*e.g.*, wiry pulse = 52%, thin pulse = 25.6%, deep pulse = 7%)[12]. Therefore, it is possible to assert that there is a relationship between the abnormal pulses and pulse images, although no evidence on this specific relationship in patients with SAH have been presented so far using quantitative pulse wave analysis.

In the last decades, pulse wave analysis using radial artery tonometry along with mathematical simulation and modeling has been used for the noninvasive assessment of both anatomic and functional status of arteries[23]. For instance, previous studies showed that patients with SAH may present increased pulse wave velocity and decreased radial artery compliance[25], medium-sized arteries hypertrophic remodeling[26], and impaired flow-mediated vasodilation characterized by smaller and slower radial artery vasodilation[27,28]. These adaptive characteristics

Table 2 Summary description of studies on the neglected issues linking evidence-based and Chinese medicines

Ref.	Study characteristics	Main results	Main limitation
Anatomical variations of vessels			
Chen *et al*[43]	Cross-sectional observational study One hundred healthy subjects, forty-six with pancreatitis, forty-two with duodenal bulb ulcer, twenty-two with appendicitis, and third-eight with acute appendicitis	Accuracy of 82% for classification of normal or abnormal pulses using an auto-regressive model for analysis of wrist pulse signals (blood flow signal) and a support vector machine	Ultrasound-based blood flow measurements was subjected to manual positioning and operator experience Only one position was investigated (above the styloid process) Pattern differentiation was performed (in either group) and the results were not related to Chinese medicine theory
Huang *et al*[44]	Cross-sectional observational study Thirty normal subjects and thirty patients with palpitation	Higher spectral harmonic energy ratio in patients	Only 10 s were evaluated at each position Palpitation was only characterized by the evidence-based medicine and no correspondence to patterns was established Pattern differentiation was performed in either group and the results were not related to Chinese medicine theory Lack of relationship between spectral harmonic energy ratio and Chinese medicine theory for pulse palpation
Hu *et al*[45]	Cross-sectional observational study Six normal subjects (all male)	No significant difference was observed on pulse waveform parameters obtained with single or array sensors Significant differences were observed among depths	Only one position was investigated (above the styloid process) Pattern differentiation was performed in either group and the results were not related to Chinese medicine theory

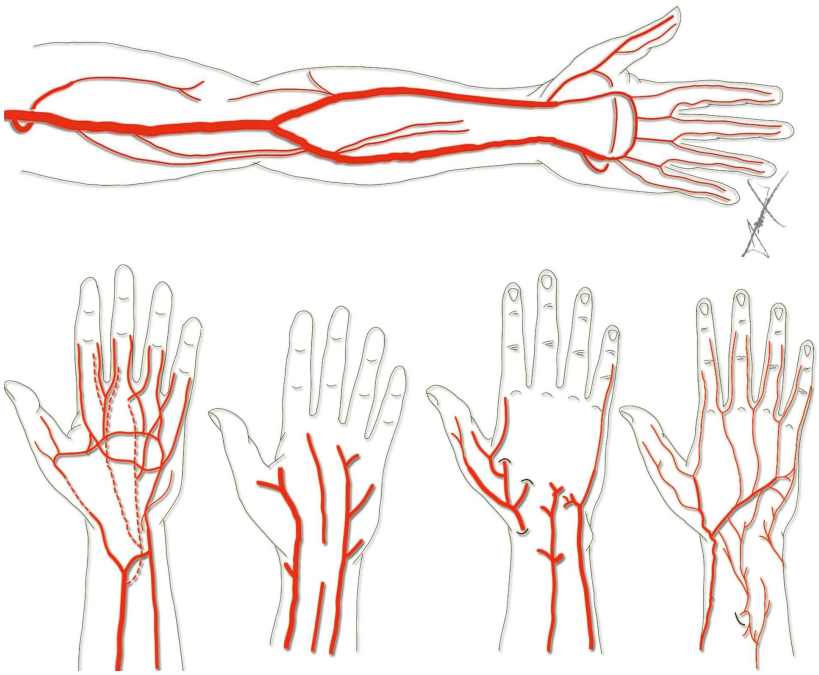

Figure 2 Anatomical drawings on variations of the course of the radial artery. Top: Most frequent arterial pattern of the radial artery. Bottom: Examples of anatomical variations of the radial artery at the wrist.

may strongly impact on the perception of the pulse as palpated at the radial artery and are reflected in the pulse waveform signal as collected using arterial tonometry. For instance, a study showed that some spectral harmonics of the pressure pulse waveform (C0, C1, C3, C4 and C6) are higher in patients with SAH as compared to health controls[29]. However, a more recent study[30] failed to found a relationship between the traditional method of 'simultaneous pressing' for wrist pulse palpation and the spectral harmonics assigned to the respective internal organs.

Herbal therapy

In the context of therapeutics for SAH, it was recently proposed to merge the ancient knowledge with the current one, yielding "the earlier the better for treating who and what are not yet ill"[31]. This proposal also reflects the epidemiologic interpretation of traditional Chinese medicine while it is in agreement with the natural history of patterns related to SAH.

The use of herbs, minerals, and animal parts to compose medicinal formulas is acknowledged as the oldest therapeutic method in Chinese medicine. Considerable advances were recently achieved in the field of antihypertensive drugs, with several drug classes available for optimization of blood pressure control[3]. However, limited efficacy for reducing blood pressure levels and side ef-

fects are among the factors that lead researchers to study other therapeutic resources, including natural compounds used in traditional medicine recipes. A large number of information about cardioprotective food is currently available and the United States Food and Drugs Administration approved and recommended some of them, even though studies are not definitive about them.

More specifically related to Chinese medicine, a recent systematic review summarized evidences in favor of Chinese herbal therapy for patients with patterns related to SAH[32]. There are formulas that have been used widely in clinical practice for treatment of hypertension such as the *Banxia Baishu Tianma Tang* (Decoction of Pinellia ternate, Atractylodes and Gastrodia elata), *Da Chaihu Tang* (Major Bupleurum Decoction), *Liu Wei Dihuang Wan* (Pill of Rehmannia), and *Banxia Baishu Tianma Tang* (Decoction of Pinellia ternate, Atractylodes macrocephala, and Gastrodia elata). The general effects observed in previous studies include the reduction of blood pressure variability, inhibition of the activity of sympathetic nerve, blocking of the renin-angiotensin system, improvement of endothelial function and insulin resistance, and prevention of TOD[32]. Altogether, it is possible to assert that ancient Chinese medicine practitioners were aware of the potential benefits of herbs on the cardiovascular system. Despite these whole-body effects, there are still some challenges for a large-scale usage of herbal therapy for Chinese medicine patters related to SAH including the quality control of compounds, interaction among formula's compounds, and dose-response effects.

NEGLECTED ISSUES

In this section, the anatomical variations of vessels and comparative physiology are discussed. These issues are considered neglected because previous researches on integrative medicine ignored these aspects as related to the studies' main outcomes. Thus, these issues must be considered in future studies as factors for analysis and not as issues that could be assumed negligible. Table 2 presents summary information about the studies cited in this section.

Anatomical variation of vessels

The radial artery is classically described at the wrist as passing deep to the tendons of the anatomical snuffbox (Figure 2, top). However, variations in the arterial pattern-*i.e.*, number and/or course of the arteries-of the upper limb have been observed frequently either in routine dissections or in clinical practice[33] and are of both clinical and surgical significances[34-39]. Variations in the origin and proximal course of this artery are the most common anomalies found in the forearm (Figure 2). For instance, a study with 150 routine dissections of the brachio-antebrachial arterial axis from adults cadavers and 10 from full-term fetuses found that 7 cases showed high origin of the radial artery, and were divided into 2 groups where one had the presence of a median artery (3 cases) and the other had the absence of the artery (4 cases)[40]. Moreover, radial artery tortuosity, hypoplasia, and stenosis were observed in patients undergoing transradial coronary intervention[41].

Chinese medicine literature states that the wrist pulse is generally felt above the styloid process of the radius and nearby proximal-distal regions in the arterial course, and that it is possible not to feel the pulse at these locations; in this case, one can feel the pulse at the external aspect of the wrist-and most importantly, it is not a sign of disease[42]. Thus, ancient Chinese medicine scholars were aware of the existence of anatomical variations of arteries and on the distinction between pulse images resulting from normal variations and morbid patterns.

Studies have been focusing on the modernization of Chinese medicine by incorporating devices (*i.e.*, pressure sensors) and automated methods (*i.e.*, software tools) to acquire pressure data from the radial artery[43-45]. However, it is intriguing that in spite of the above-cited traditional and current knowledges, none of these studies considered the anatomical variation as a confounding factor for either qualitative or quantitative pulse image analysis. Patients with hypertension are at an increased risk of presenting radial artery tortuosity[46]. Because the geometrical characteristics of the radial artery determine the transmission of the pressure pulse waveform along the vessel[26], it is expected that patients with SAH present pulse image characteristics due to arterial tortuosity, vascular remodeling, or both. Therefore, the anatomical variation of the radial artery cannot be neglected in future studies on pulse image analysis since it may help explain the qualitative or quantitative observed pulse image.

Comparative physiology

Recognized as the Father of western Medicine, Hippocrates (460-375 BC) and Huangdi (2695-2589 BC), reference inside the oldest known treatise of medicine in existence (the *Huangdi Neijing*) had in common in their discussions the use of acupuncture for treatment of various diseases, including coronary artery disease[47]. Hippocrates advocated the theory of four humors-earth, air, fire and water-when trying to explain the pathogenesis of a disease, analogous to the five-phase theory of Huangdi–wood, fire, earth, metal and water. This example of comparative reasoning can be extended to all major fields of medical knowledge in Chinese and evidence-based medicines: anatomy, physiology, semiology, pathophysiology, and therapy. It is acknowledged that there are important conceptual differences between these medical practices as related to the body structures[48], but strong similarities are empirically present at the functional level. As related to the circulatory system, Chinese medical theory also recognize its role on several functions such as the whole-body integration for distribution of substances, regulation of body temperature, and the relationship between circulation and life support[48].

Researchers are investigating Chinese medicine searching for anatomical and/or physiological explana-

tions for the phenomena related to the safety-efficacy of interventions in the patients with SAH and other CVD[10]. However, it is apparent that no comparative analysis have been systematically performed between Chinese and evidence-based medical theories. More specifically, it is not a matter of translation of terms from Chinese to English, but to properly transpose the interpretation of Chinese medicine knowledge to its counterpart in evidence-based medicine. For instance, such comparative reasoning may help explain: (1) the strong association observed between descriptions of TOD and patterns in patients with SAH; and (2) the similarities and dissimilarities between abnormal pulses, quantitative pulse waveform analysis, and qualitative pulse images. Therefore, it is recommended to not neglect the study of a comparative physiology between these two medical practices since it may improve our understanding on the natural history of SAH and the potential benefits of an integrated approach to patients undergoing cardiac rehabilitation programs.

DISCUSSION

Complementary and alternative medicine (CAM) are increasingly available and used for health care. A study[49] that analyzed data on CAM use among patients with CVD found that 36% of patients with CVD had used CAM in the previous 12 mo and 10% respondents used CAM specifically for their cardiovascular conditions–among which 5% for hypertension, 2% for coronary disease, and 3% for vascular insufficiency. The same study showed that cardiac patients use mind-body therapies including deep-breathing exercises, group support, hypnosis, meditation, relaxation, *taijiquan*, *yoga*, and *shiatsu*, among others[49]. Acupuncture, herbal Chinese medicine, moxibustion, cupping, Chinese massage, *qigong* and *taijiquan*, and dietary therapy[50], when associated to antihypertensive medication significantly reduced systolic blood pressure (-8 mmHg) and diastolic blood pressure (-4 mmHg) with no heterogeneity detected, although given the poor methodological quality and small sample sizes of most acupuncture trials, the notion that acupuncture may lower high blood pressure remains inconclusive[51].

In summary, the asserted issues highlighted in this paper stimulate the increasing use of Chinese medicine for health care and the continuity of research on integrative medicine in the cardiovascular field. Conversely, the neglected issues poses additional challenges that must not be overlooked in future research on this topic so that the integration of both traditional and current knowledge may be of benefit to the population with CVD.

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REVIEW

Alterations in cell adhesion proteins and cardiomyopathy

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Abstract

Cell adhesive junction is specialized intercellular structure composed of cell adhesion proteins. They are essential to connect adjacent heart muscle cell and make heart contraction effectively and properly. Clinical and genetic studies have revealed close relationship between cell adhesive proteins and the occurrence of various cardiomyopathies. Here we will review recent development on the disease phenotype, potential cellular and molecular mechanism related to cell adhesion molecules, with particular disease pathogenesis learned from genetic manipulated murine models.

Key words: Cardiomyopathy; Adherens junction; Desmosome; Intercalated disc; Arrhythmia

Core tip: Cell adhesive junction is a specialized intercellular structure in the heart, and essential to maintain heart contractile function. Alterations in adhesive proteins have been found to lead to various forms of cardiomyopathy. However, the molecular and cellular mechanisms underlying heart muscle dysfunction caused by those cell adhesive molecules have not been completely understood. This review provides most recent development on cellular composition of the cell adhesion proteins and their related gene mutations, disease phenotypes, potential mechanisms involved in cardiomyopathies.

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INTRODUCTION

The walls of the heart are primarily composed of elongated cardiac muscle cells, which are branched and connected each other. The site where one cardiomyocyte joins with another is called intercalated disc (ID), a specialized intercellular junctional structure found only in cardiac tissue. These structures are highly specialized and enable coordinated function of the heart mechanically to allow heart to beat[1]. Original description of the ID identifies three structures, adherens junctions, desmosomes, and gap junctions. Recognition of the area composita and the determination of interactions between intercellular adhesion molecules, gap junctions, and ion channels suggest that the ID functions as a single unit where macromolecular complexes interact to maintain synchrony of the heart (Figure 1)[2]. Alterations in adhesive proteins located at ID region have been found to lead to various forms of cardiomyopathy, often accompanied with life threaten arrhythmia and heart failure.

In this review, we will discuss the composition of adhesive junctional complexes, recent discovery on the relation of cell adhesion gene mutations and disease phenotypes and possible molecular mechanisms underlying cardiomyopathy.

CHARACTERIZATION OF CARDIOMYOPATHY AND RELATED GENETIC MUTATIONS

According to new proposed classification in 2008, cardio-

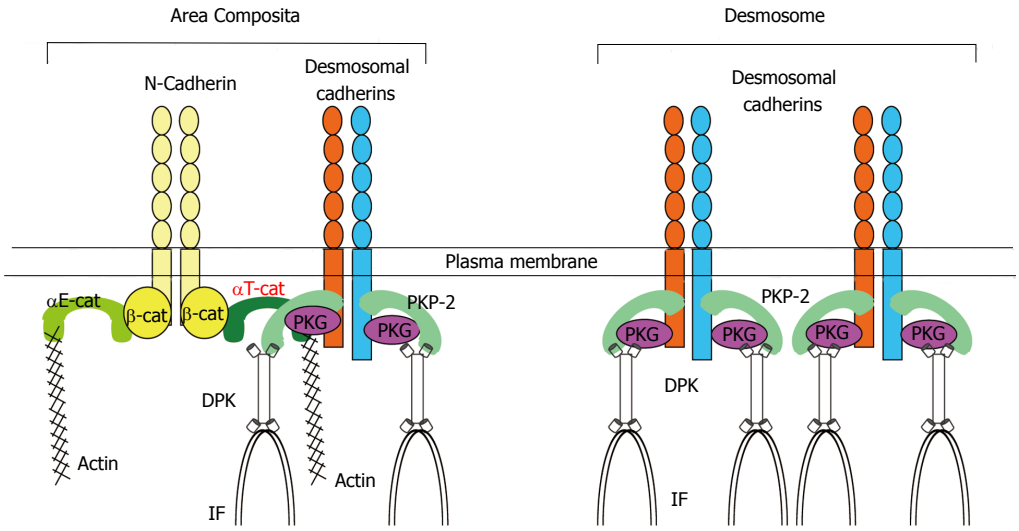

Figure 1 Components of area composita in the heart. Area composita is a mixed-type junctional structure composed of both desmosomal and adherens junctional proteins. Both αE-catenin and αT-catenin are present in the area composita at the cardiac intercalated disc. However, only αT-catenin was shown to interact directly with desmosomal protein PKP2. PKP2: Plakophilin-2; DPK: Desmoplakin; IF: Intermediate filaments.

myopathy defines as a myocardial disorder in which the heart muscle is structural and functionally abnormal[3]. Cardiomyopathies are grouped into specific morphological and functional phenotypes, including dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), arrhythmogenic cardiomyopathy (AC), and restricted cardiomyopathy (RCM). Each phenotype is sub-classified into familial and non-familial forms[3]. The causes of the cardiomyopathy are diverse, including genetic and spontaneous mutations of muscle proteins, hypertension, ischemia, and inflammation. Affected individuals may have a relative benign course, or develop progressive heart failure and experience sudden death, due to abnormal electrical rhythm and mechanical contractility caused by damaged heart muscle. Cardiomyopathy is most commonly diagnosed through *in vivo* imaging with either echocardiography or cardiac magnetic resonance image (MRI), which provide functional information to complement the structural changes from whole organ level.

DCM refers to enlargement of the heart, often affecting all four chambers. The prevalence of DCM is not completely known. At least 25% of patients in Western populations have evidence for familial disease with predominantly autosomal dominant inheritance. These mutations include genes encoding cytoskeletal, sarcomeric protein, Z-band, nuclear membrane and ID proteins[4].

In contrast, HCM is characterized by increased left ventricular wall thickness, often targeting the septum that separates left ventricle from right ventricle. The prevalence of HCM is approximately 1:500 of general populations[3,5]. Familial HCM are often caused by mutations in genes encoding cardiac sarcomeres, and often associated with congenital syndromes, inherited metabolic disorders, and neuromuscular diseases.

RCM is the most elusive, in part because the heart may appear morphological close to normal with minor increased wall thickness or modestly decreased left ventricle ejection fraction. RCM is the least common type of cardiomyopathy and the exact prevalence of RCM is unknown. Familial RCM often occurs in autosomal dominant inheritance caused by mutations in the troponin I gene or intermediate filament desmin[3].

AC also known as arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited arrhythmogenic disorder with estimated prevalence of 1 in 5000, and a frequent cause of sudden arrhythmic death in young[6]. AC is defined histologically by the presence of progressive replacement of right ventricular myocardium with adipose and fibrous tissue often confined to "a triangle of dysplasia" comprising the right ventricular inflow, outflow, and apex. These pathologic abnormalities result in functional and morphological abnormalities not only in right ventricle but also in left ventricle or both, and can be present on the absence of clinically detectable structure changes. 50% of patients carry gene mutations encoding the desmosomal complexes of the ID. Majority of cases are caused by autosomal dominantly inherited mutations although autosomal recessive forms of AC are recognized[2,3].

In practice, there is extensive overlap between these four cardiomyopathy phenotypes; for example, HCM, or AC may progress into a dilated ventricle with systolic dysfunction.

CELL ADHESION JUNCTION STRUCTURE AND COMPOSITION

Cardiac ID contains two adhesive junctions, adherens junction and desmosome, which couples cardiac muscle cells *via* actin cytoskeleton and intermediate system, respectively[1]. The classic cadherin N-cadherin is single transmembrane protein responsible for Ca^{2+}-dependent homophilic cell-cell adhesion. The cadherin adhesive ac-

tivity is regulated by a group of proteins that bind its cytopslasmic domain, called catenins. β-catenin or γ-catenin (plakoglobin) directly binds to C-terminal region of cadherin, whereas α-catenins link cadherin/catenin complex to actin cytoskeleton[7]. It has been shown that N-cadherin-mediated adhesion is essential for embryonic heart morphogenesis and development[8,9].

Plakoglobin (PG) is the only ID component found in both adhesive junctions, and also functions as a signaling protein to modulate the Wnt/β-catenin signaling pathway. PG and its homologous protein β-catenin owe 88% amino acid identity and share common protein partners[10]. The majority of PG and β-catenin is engaged at adherens junctions and/or desmosomes. Redistribution from junction to cytosol can markedly alter their signaling activities.

There are three α-catenin subtypes in mammals: αE-catenin, αN-catenin and αT-catenin[11]. αE-catenin is ubiquitously expressed and it is essential for early embryonic development[12]. αN-catenin expression is restricted to neural tissue[13]. αT-catenin is a recently identified novel member of the α-catenin family with restricted expression in testis, cardiac muscle and neurons[14,15]. Both αT-catenin and αE-catenin are expressed in the heart and localize to the ID. αT-catenin and αE-catenin contain vinculin homology domains, and share 57% overall amino acid identity[14,16]. Besides structural role in the AJ junction, α-catenins also play an important role in cell signaling. For example, αE-catenin has been implicated in sensing cell density in epidermis and restricting basal cell proliferation in neural progenitor cells[17,18]. Loss of αE-catenin triggers severe epidermal hyperproliferation and tumors in mice[17]. A role for α-catenins in regulating proliferation in the heart is currently under investigation.

Recently, a novel, exclusive type of hybrid adhering junction is identified in the mammalian heart referred to as area composita (Figure 1)[16,19]. Immunoelectron microscopy showed that the desmosomal proteins, such as desmoplakin (DSP) are not only restricted to the classic desmosomal junctions but also detected in large adherens-like junctional structures[19,20]. Typical components of the classic adherens junction, including N-cadherin, β-catenin was shown to co-localize with desmosomal proteins in the majority of the area composita[19,21]. Interestingly, the area composita is not found in lower vertebrates[22], suggesting its role in supporting the increased mechanical load on the mammalian heart by anchoring both actin and intermediate filaments over an extended junctional area of the ID. More recently, yeast two-hybrid and co-immunoprecipitation showed that αT-catenin interacts directly with desmosomal plakophilin-2 (PKP2) at area composita[16]. However, αE-catenin lacks plakophilin-binding domain, and the interaction of αE-catenin with PKP2 is not observed in the heart[16].

Recent studies have identified a novel ID protein, Xin. Xin is a muscle specific protein (mXin) associated with adherens junction through its interaction with β-catenin and actin cytoskeleton[23]. The human homolog of mXinα, Cmyα1, maps to chromosomal region 3p21, a region linked to familial DCM. However, mXinα associated mutations in human have not been identified.

Desmosome consists of desomosomal cadherins, armadillo family protein plakoglobin and plakophilin, and the plakin protein DSP[7]. Desmosomal cadherins are transmembrane proteins and form Ca^{2+}-dependent heterotypic cell-cell adhesive interactions. In the heart, only desmoglein 2 (DSG2) and desmocollin 2 (DSC2) are expressed. Both plakoglobin and PKP2 bind cytoplasmic DSG2 and DSC2, and regulate cadherin adhesive activity and implicate in signaling. DSP links membrane desmosomal cadherins to cytosplasmic intermediate filaments[7].

Gap junctions are intercellular channel that allow ions to travel from cell to cell and electrically couple myocytes. Six connexin molecules interact with one another to form a channel. Compared to noncardiac tissue, the ID contains extremely large connexin 43-containing gap junction plaques in the heart, reflecting its important function in electrically coupling of cardiomyocytes[24,25].

ALTERATIONS IN CELL ADHESIVE PROTEINS AND RELATED HUMAN CARDIOMYOPATHY

Role of adherens junction-associated proteins in human cardiomyopathy and heart failure

Studies performed in humans have demonstrated that alterations and/or mutations in the ID components are associated with a spectrum of human cardiomyopathy (Table 1). Cardiac myofibril disarray is a common feature of HCM. Studies on cases with HCM reveal the ID frequently irregular or redistributed from perpendicular to parallel of myofibril[26], with presence of decreased immunoactive signal of N-cadherin. Degenerating cardiomyocytes occasionally can be seen in HCM heart forming vacuole-like structures accompanied with strong positive staining for N-cadherin. Examination of 62 end-stage explants hearts with previous existing cardiomyopathies shows general reduction of cadherin/catenin components, often accompanied with tight junction protein claudin-5 or gap junction connexin 43 reduction[27]. A genetic screen on the entire coding region of N-cadherin gene from 96 Japanese healthy individuals identified eight sequence polymorphisms. Three of the five single-nucleotide polymorphism has an amino acid substitution, including Ala826Thr substitution in exon 15 which is located in N-cadherin binding domain of Shc[28]. Shc is an adaptor protein and has been shown to participate in signaling pathway that control cell growth. Although germline mutation in gene encoding N-cadherin has not been detected in the familial HCM patients[28], these data indicate ID components may play a role in the pathogenesis of human cardiomyopathy.

Characterization of the cell adhesion protein expression in myocardial infarct rupture patients demonstrates a significantly reduced expression of αE-catenin in both total tissue level and in the ID of infarct rupture area[29]. In contrast, other junctional components are not sig-

Table 1 Adhesive proteins-associated cardiomyopathies in human and murine models

Adhesive junctional component (Gene)	Cardiac phenotype			
	Human cardiomyopathy	Ref.	Mouse model of cardiomyopathy	Ref.
N-cadherin (CDH2)	DCM, HCM, heart failure	[26-28]	GOF: DCM, cardiac calcification	[42,58]
			LOF: DCM, ventricular arrhythmia, sudden cardiac death	[43,44]
			HET: Normal cytoarchitecture, induced arrhythmia	[25]
β-catenin (CTNNB1)	HCM, heart failure	[27,61]	GOF: DCM, premature death	[53]
			LOF: Normal cardiac function, blunt response to induced hypertrophy	[50,51]
			HET: Normal cardiac structure, reduced response to hypertrophy	[52]
Plakoglobin (JUP)	AC, Naxos disease	[30,31]	GOF (wild-type): adipocyte accumulation, inflammation	[46]
		[27,32,34,35]	GOF (Naxos mutation): adipocyte accumulation, inflammation, cardiac dysfunction, apoptosis	[47,48]
			LOF (perinatal): early onset of cardiomyopathy, severe ventricular arrhythmia	[45]
			LOF (adult): dilated cardiomyopathy, apoptosis, inflammation, fibrosis	[62]
			HET: Aged animals with right ventricular dilation, arrhythmia	[49]
αT-catenin (CTNNA3)	DCM, AC	[9,36]	LOF: DCM, arrhythmia, area composita defects	[56]
αE-catenin (CTNNA1)	Post-MI ventricular rupture Heart failure	[29]	LOF: Progressive DCM, RV dilation, MI-induced ventricular rupture	[54]
			HET: MI-induced ventricular rupture	[29]
mXinα (mXinα, Cmya1)	None	None	LOF: HCM, arrhythmia, ID defects	[23]
Desmoglein2 (DSG2)	AC	[63]	LOF: Dying cardiomyocytes with calcification, complete dissociation of intercalated discs, fibrotic replacement	[64]
			GOF (N266S): Sudden death, ventricular arrhythmias, cardiac dysfunction, biventricular dilatation, aneurysms	[59]
			GOF (N271S): Intercellular space widening, fibrosis, increased arrhythmia, lower sodium channel current density	[65]
Desmocollin2 (DSC2)	AC	[66-68]	None	
Plakophilin2 (PKP2)	AC	[63,69]	HET (haploinsufficiency): Impaired ventricular conduction, sodium channel dysfunction	[60]
Desmoplakin (DSP)	AC, Carvajal syndrome, heart failure	[38,41,56]	LOF: Impairs cardiac morphogenesis and leads to high embryonic lethality	[46,47,57]
			GOF (R283H): Apoptosis, fibrosis, lipid accumulation, ventricular enlargement and cardiac dysfunction	[58]
			HET: Excess adipocytes, fibrosis, increased apoptosis, cardiac dysfunction, and ventricular arrhythmias	[57]

DCM: Dilated cardiomyopathy; HCM: Hypertrophic cardiomyopathy; AC: Arrhythmogenic cardiomyopathy; LOF: Loss-of-function; GOF: Gain-of-function; HET: Heterozygous; ID: Intercalated disc.

nificantly changed in the injured area. This is consistent with the observation that αE-catenin heterozygous mice exhibit ventricular rupture post myocardial infarction[29]. These results suggest that patients with an intrinsic defect in their cell adhesion complex may predispose myocardial rupture after experiencing ischemic stress.

Plakoglobin (PG) encoded by the *JUP* gene is the first component of the desmosome to be implicated in the pathogenesis of AC[30]. Studies of individuals from the Greek island of Naxos identified an autosomal recessive form of AC with palmoplantar keratoderma and woolly hair referred to as Naxos disease. Gene sequencing revealed a homozygous 2-bp deletion (2157-2158delGT) in the *JUP* gene in affected individuals[31]. A study of a German family reported the first dominantly inherited *JUP* gene mutation (S39_K40insS) to cause AC without cutaneous abnormalities[32]. Both mutant forms of PG failed to localize properly at the ID, and the junctional components DSP and Cx43 were significantly reduced at the ID in these patients. Ultrastructural investigation showed ID remodeling with mislocalization and a decreased number of desmosomes[6,32]. Importantly, a reduced immunoreactive PG signal at the ID is a consistent feature in patients with dominant mutations in a variety of desmosomal genes, making PG a potential diagnostic tool for AC in affected individuals[33]. Recently, additional mutations in the *JUP* gene have been identified, including homozygous Q539X, S24X and missense 468G > A mutations. These young patients showed skin but not heart abnormalities although further examination will be required to rule out no cardiac phenotype[34, 35].

The human αT-catenin gene, CTNNA3, has been mapped to chromosome 10q21, a region linked to autosomal dominant familial DCM[9]. Although genetic screening has not detected any DCM-linked CTNNA3 mutations to date, CTNNA3 is considered a candidate gene and may be the potential cause of this disease[9]. Utilizing denaturing high-performance liquid chromatography and direct sequencing, for the first time two gene mutations in αT-catenin have been identified from 76 AC patients who did not carry any mutations in the desmosomal genes commonly mutated in AC[36]. Mutation c.281T > A (p.V94D) is located in N-teminal β-catenin binding domains of αT-catenin. Over expression p.V94D mutant in heart muscle cells shows diminished interaction of αT-catenin and β-catenin, whereas mutation c.2293_2295delTTG (p.del765L) at C-terminal of αT-catenin results in deletion of leucine in position 765 of

αT-catenin protein. The p.del765L mutant protein shows a much stronger dimerization potential and forms aggresomes in a nonmuscle cell line. Whether the area composita assembly and function is perturbed in CTNNA3 mutant AC patient heart remains unclear. Nevertheless, this is the first report on the involvement of area composita gene in AC and may suggest the pathogenesis of this disease extend beyond desmosomes. Clinically, the affected individuals showed severe right ventricle dilation, intramural and epicardial fibrosis in left ventricle, reduced right ventricular ejection fraction, and sustained ventricular tachycardia with left bundle-branch block[36]. Since the frequency of CTNNA3 mutations in AC patients is not rare, systematic screening for this gene should be considered to improve the clinical management of AC families.

Role of desmosome-associated proteins and human arrhythmogenic cardiomyoapthy

To date, human genetics studies have identified 12 independent loci and 8 disease genes for AC[2]. Five of 8 causative genes encode major components of the cardiac desmosomes, namely plakoglobin (JUP), DSP, PKP2, DSG2, and desmocollin-2 (DSC2). Up to 50% of AC probands harbor a mutation in 1 of these genes[2,37]. Mutations in desmosomal genes with recessive and dominant patterns of inheritance are associated with cutaneous disease, cardiac disease, or both. Mutations in desmosomal cadherins DSG2 and DSC2 account for 10% of cases of AC[38,39]. The phenotype includes characteristic histological and clinical feature of AC, with prominent left ventricular involvement in many cases. Heterozygous PKP2 mutations account for the highest proportion of cases, and the reported prevalence is about 43% among US studies[38, 40]. DSP is the first gene to be implicated in autosomal dominant AC mutations. In 2002, a missense mutation (S299R) in DSP was identified in an Italian AC family. The patients show classic AC phenotype with arrhythmia of right ventricular origin with instances of ventricular fibrillation and sudden cardiac death[41]. Interestingly, recent genetic analysis has identified AC patients with mutations in more than one desmosomal gene supporting a multigenic etiology to this disease (Table 1).

ALTERATIONS IN CELL ADHESION PROTEINS IN GENETICALLY MANIPULATED ANIMAL MODELS OF CARDIOMYOPATHY

Despite human genetics studies have been successful in identifying disease-causing genes, multiple interacting factors, including genetic background; various environmental stimuli (hemodynamic stress, inflammation, and metabolism) can influence the ultimate clinical outcome and diagnosis. In past decades, genetically engineered mouse models have been widely used and provided invaluable resources for understanding pathogenesis of cardiomyopathy (Table 1).

Role of adherens junction-associated proteins in animal models of cardiomyopathy

N-cadherin is the only classical cadherin expressed in the myocardium, and plays a key role in maintaining cardiac structure integrity. Ectopic expression of epithelial cadherin (E-cadherin) in the myocardium causes early onset of HCM, cardiac calcification, and increased mortality. Overexpression of N-cadherin in adult mouse heart leads to dilation of the left ventricle, redistribution of β-catenin, Cx43 and upregulation of pathological marker atrial natriuretic factor[42]. Induced deletion of N-cadherin specifically in the adult mouse heart (N-cad CKO) results in disassembly of the ID structure, dilation of ventricular and atrial chambers, reduced wall thickness, and fibrosis[43,44]. Cardiac-gated MRI image data demonstrate significantly larger left ventricular end-diastolic volume and end-systolic volume in the N-cad CKO group. Both ejection fraction and cardiac output are significantly reduced. These results are consistent with a decrease in force transmission due to loss of the cadherin/catenin adhesion complex at the ID. Using miniaturized electrocardiogram telemetry transmitters implanted in N-cad CKO mice, abrupt onset of spontaneous ventricular tachycardia was observed immediately prior to sudden death. The lethal arrhythmias were associated with decrease gap junction protein Cx43 and slow electrical conduction in the N-cad CKO mice. Relocalization and/or loss of Cx43 from the ID are often observed in human diseased myocardium[1]. In contrast, animals with half the normal level of N-cadherin show the normal heart histology and normal life span. However, the heterozygous mice exhibit an increased susceptibility to arrhythmia induced by electrical stimuli[25]. These mouse models demonstrate the critical role of N-cadherin in maintaining the ID structure, and suggest perturbation of the adhesive junctional complex may underlie the pathogenesis of cardiomyopathy.

Several groups have generated animal models of AC by manipulating plakoglobin expression in mice. Adult mice with inducible cardiac restricted deletion (CKO) of the *JUP* gene exhibit progressive loss of cardiac myocytes, DCM and cardiac dysfunction. Consistent with altered desmosome ultrastructure in plakoglobin CKO hearts, expression of desmosomal proteins are decreased at the ID. Focal areas of myocyte loss and replacement by fibrous tissue, along with patchy inflammatory infiltrates, are revealed in the myocardium of PG CKO. Animals with perinatal myocardial deletion of *JUP* gene exhibit early onset cardiomyopathy and severe ventricular arrhythmias[45]. Deletion of JUP in the developing heart before maturation of the ID likely explains the more severe phenotype compared to deletion in the adult heart[45]. Cardiomyopathy is also observed in mice overexpressing either wild-type (WT)[46] or truncated PG (*i.e.*, Naxos)[47,48] in the heart. In both models, PG accumulates in the nuclei of the cardiomyocytes. The molecular mechanisms involve activation of Hippo signal pathway, inhibition of Wnt/β-catenin target genes and enhanced adipocyte gene expression in the mutant PG mouse heart. Interestingly, it has been reported that heterozygous PG-null mice ex-

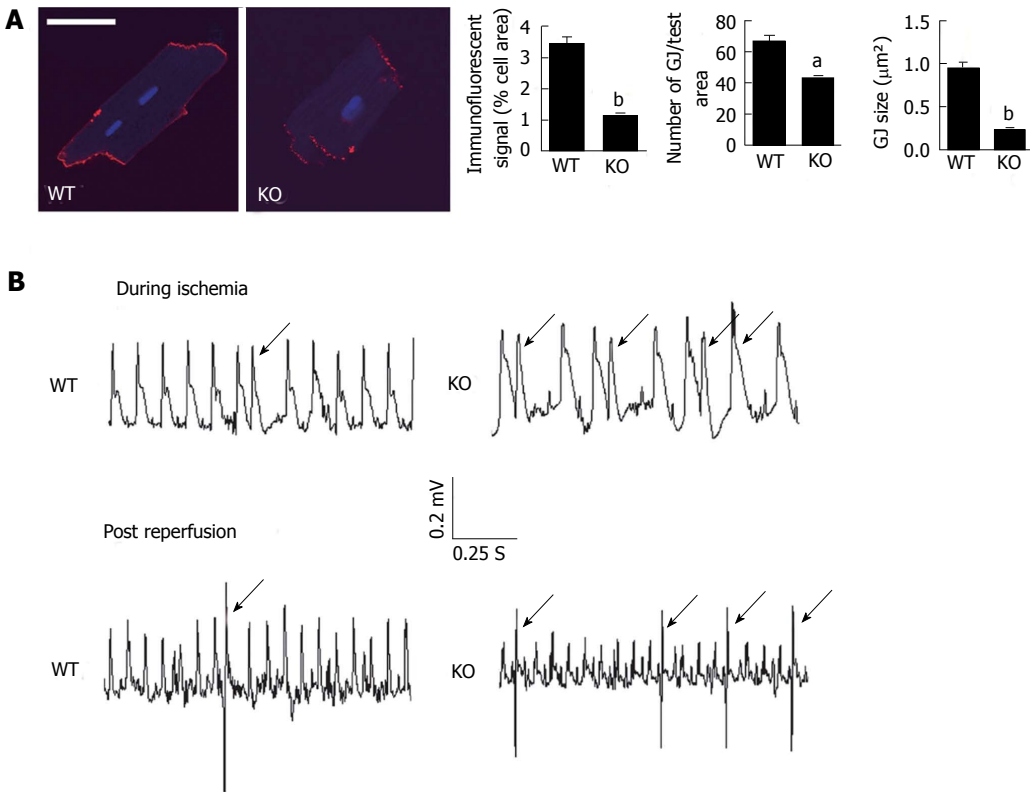

Figure 2 Loss of αT-catenin in the mouse heart leads to reduced expression of Cx43 and ventricular arrhythmia following acute ischemic injury. A: Adult cardiomyocytes isolated from wildtype (WT) and αT-catenin knockout (KO) hearts were immunostained for Cx43. Ten cardiomyocytes from each animal were examined for five or more contiguous pixels of high signal intensity. The amount of specific immunoreactive signal at intercalated disc (ID) for Cx43, the number of Cx43-containing plaques (gap junction, GJ) and their size (GJ size) were quantified and are shown in the panel at right. Scale bar; 50 um. The error bars represent the s.e.m. $^{a}P < 0.05$; $^{b}P < 0.01$; B: Representative telemetry ECGs of different patterns of premature ventricular contractions (PVCs, arrow) during ischemia-reperfusion (I-R) injury in WT and αT-catenin KO mice. Mice from WT and αT-catenin KO were subjected to ligation of the left anterior descending artery for 30 min and 7 d reperfusion. A miniaturized telemetry ECG transmitter was implanted before I-R.

hibit altered right ventricular contractility and arrhythmia without affecting myocardial structure at 10 mo of the age. Endurance exercise (*e.g.*, daily swimming) exacerbates disease progression in these mice[49] suggesting endurance exercise can enhance disease progression among the people suffering AC.

In contrast to PG, β-catenin is not required for maintaining the mechanical junctions in adult myocardium in physiologic conditions. The upregulation of PG and its ability to substitute for β-catenin in adherens junction are responsible for the lack of ID defects in β-catenin knockout mice[50]. However, compared to wild-type, β-catenin mutant mice are unable to respond to hypertrophy induced by hemodynamic stress, indicating β-catenin signaling is essential to pathological hypertrophic growth of cardiomyocytes[51,52]. In comparison, mice with overexpression of non-degradable or active form of β-catenin develop DCM, and premature death[53]. These data suggest that both localization and cellular signaling changes mediated by β-catenin can cause abnormal cardiac function as well as cardiomyopathy.

Alpha-catenins are key cytoplasmic molecules thought to be indispensable for maintenance of tissue morphogenesis. αE-catenin is ubiquitously expressed in all tissue. Ablation of αE-catenin expression specifically in the mouse heart results in progressive DCM, defects in right ventricle, and reduced expression of cytoskeleton protein, vinculin in ID region. Similar to the human ventricular rupture patients mentioned above, these mice exhibit increased susceptibility to ventricular free wall rupture after myocardial infarction[54]. αT-catenin is a recently identified novel member of the α-catenin family with restricted expression in heart and also the only α-catenin in the adherens junction that interacts with the desmosomal protein PKP2 (Figure 1)[14-16,55]. Germline deletion of αT-catenin in mice alters PKP2 distribution without affecting other junctional components of the area composita. Phenotypically, these mice exhibit early onset DCM, cardiac dysfunction, and gap junction remodeling. Our study suggested that disruption of the area composita in the αT-catenin KO hearts weakens the actin and the desmin cytoskeletal networks that results in a reorganization of the cytoskeleton and leads to alteration of expression and cellular distribution of Cx43 and gap junction remodeling (Figure 2)[56]. Furthermore, the diminished levels of gap junctional Cx43 in the ID of αT-catenin-ablated cardiomyocytes, as well as the reduced number and size of Cx43-containing gap junction plaques in αT-catenin-KO cardiomyocytes in vitro and *in vivo*, may lead to an increased incidence of arrhythmias. In response to acute ischemic injury, the αT-catenin mutant mice exhibit increased ventricular arrhythmia[56]. Importantly,

although reperfusion is essential to prevent irreversible cellular injury and preserve ventricular function, reperfusion and the attendant recovery from ischemia can cause ventricular arrhythmias, cellular injury, and SCD. In this regard, it is important to emphasize the increased susceptibility to ventricular arrhythmia observed after the first 24 h of reperfusion in αT-catenin KO animals in comparison with WT (Figure 2)[56]. Taken together, these data demonstrate that alterations in either αE- or αT-catenin can cause DCM. Because of the unique interaction with desmosomal PKP2, αT-catenin may play more important role than αE-catenin in maintaining area composita structure and function. The identification of αT-catenin not αE-catenin mutations in AC patients provides further evidence for a unique role of αT-catenin in the pathogenesis of AC.

In the mouse, there are two homologous genes of mXinα and mXinβ. Mice with germline deletion of mXinα exhibit HCM accompanied by disruption of the ID. Prolonged QT interval is detected from ex vivo isolated mXinα mutant mouse heart, suggesting loss of mXinα perturbing conduction system of the cardiac muscle cells[23].

Role of desmosome-associated proteins in animal models of cardiomyopathy

DSP is a major desmosomal component, and indispensable for the linkage of the desmosomal cadherins to cytoskeletal filament network. Mice with cardiac restricted deletion of DSP in perinatal heart exhibit a high incidence of embryonic lethality with malformation of heart structure. In contrast, heterozygous DSP knockout mice are viable and display AC-like phenotype[57]. Histology analysis shows enlarged ventricles, poorly organized myocytes with large area of fibrosis, and excess accumulation of fat droplet in the myocardium. Echocardiography demonstrates the thinning wall, increased end-diastolic and end-systolic dimension and reduced systolic function. Further study demonstrates that DSP deficiency results in nuclear translocalization of plakoglobin and reduction of β-catenin-mediated Wnt signaling thus enhancing adipogenic gene expression[57]. Transgenic mice with cardiac-restricted overexpression of the AC-associated DSP mutation (R283H) exhibit increased cardiomyocyte apoptosis, cardiac fibrosis, and lipid accumulation, along with ventricular enlargement and cardiac dysfunction in both ventricles[58].

Recently a transgenic mouse model overexpressing the human AC-associated mutation N266S in DSG2 has been generated. The DSG2-N266S transgene mice exhibit a biventricular cardiomyopathy with aneurysms, ventricular arrhythmias, and sudden death. Histological study demonstrates pronounced myocardial damage, coagulative necrosis, massive neutrophil infiltrate, and calcification[59].

Heterozygous mutations in PKP2 are the most common mutations in AC patients. However, transgenic mice with overexpression of PKP2 AC-associated mutations have not been generated. Constitutive knockout of PKP2 in mice leads to embryonic lethality due to ventricular free wall rupture[12]. Interestingly, heterozygous PKP2 mice without histological or gross anatomical abnormalities in hearts exhibit impaired ventricular conduction, altered electrocardiographic parameters and arrhythmic death when treated with sodium channel blocker[60]. These results suggest a possible cross talk between desmosome and sodium channel complex, and sodium current dysfunction may contribute to arrhythmogenesis in PKP2-deficient hearts.

CONCLUSION

Genetic mutations account for a significant percentage of cardiomyopathies, and are a leading cause of congestive heart failure. Thanks to advanced study on structure and function of human genes and widely available genetic screening for mutated genes, genetic cardiomyopathy is now more commonly diagnosed. The primary role of adhesive junctional complexes is providing mechanical attachment between muscle cells by linking cellular membrane to cytoskeleton filaments. Mutations in genes encoding adherens junctional or desmosomal proteins disrupt either cell-cell adhesion, or membrane-actin/intermediate filament interaction, or both, thus affecting contractility and cell-cell communication. With respect to the latter, decrease in conduction velocity can lead to reentry, causing ventricular arrhythmia and sudden cardiac death. The underlying mechanisms may include adhesion proteins influence connexon trafficking, channel assembly, and/or stability at the ID. Reduced amount and organization of Cx43-containing gap junction plaques likely play a fundamental role in the increased incidence of arrhythmias. Moreover, perturbation of the normal cellular distribution of junctional proteins between the membrane verses the cytosol may alter signaling pathways, such as pathogenic activation of the Hippo pathway, suppression of the canonical Wnt signaling, leading to enhanced cell death, replacement of fibrotic adipocyte, and cardiac dysfunction.

Treatment of cardiomyopathy depends on the etiology, the severity of symptoms, complications, and age of the patient. Treatment may include lifestyle changes, medicines, surgery, and implanted devices to correct arrhythmias. Because of the crucial role of adhesive junctional complexes in the pathogenesis of cardiomyopathy, identifying specific protein interactions mediated by cell adhesive proteins may provide novel therapeutic strategies to prevent, attenuate and possibly reverse the disease phenotype.

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REVIEW

Autoantibodies to apolipoprotein A-1 as a biomarker of cardiovascular autoimmunity

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Abstract

Immune-driven inflammation plays an important part in atherogenesis and is therefore believed to be key to the development of cardiovascular disease (CVD), which is currently the leading cause of death in the Western world. By fulfilling some of the Koch postulates, atherogenesis has even been proposed to be considered as an autoimmune disease, raising the hope that CVD could be prevented by immunomodulation. Nevertheless, the role of the immune system and autoimmune reactions in atherosclerosis appear to be a double edged-sword, with both pro-atherogenic and anti-atherogenic attributes. Hence, if immunomodulation is to become a therapeutic option for atherosclerosis and CVD, it will be crucial to correctly identify patients who might benefit from targeted suppression of deleterious autoimmune responses. This could be achieved, for example, by the detection of disease-associated autoantibodies. In this work, we will review the currently available clinical, *in vitro*, and animal studies dedicated to autoantibodies against apolipoprotein A-1 (anti-apoA-1 IgG), the major proteic fraction of high density lipoprotein. Current clinical studies indicate that high levels of anti-apoA-1 IgG are associated with a worse cardiovascular prognosis. In addition, *in vitro* and animal studies indicate a pro-inflammatory and pro-atherogenic role, supporting the hypothesis that these autoantibodies may play a direct causal role in CVD, and furthermore that they could potentially represent a therapeutic target for CVD in the future.

Key words: Autoantibodies; Cardiovascular disease; Atherosclerosis; Apolipoprotein A-1; Autoimmunity; Biomarkers

Core tip: This review provides a comprehensive and critical analysis of the most recent basic research articles and clinical trials on the role of autoantibodies to apolipoprotein A-1 as biomarkers and potential mediators of cardiovascular diseases (CVD). Evidence from both *in vitro* and *in vivo* studies showed that anti-apolipoprotein A-1 IgG might have critical pro-atherosclerotic activities by activating immune cells to release pro-inflammatory mediators and proteases. In addition, these autoantibodies might increase heart rate and arrhythmias both in humans and animal models. These studies suggest a causal role of anti-apolipoprotein A-1 immunoglobulins of G class in CVD, indicating that those autoantibodies could potentially represent an emerging therapeutic target to better fight CVD.

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INTRODUCTION

Current epidemiology of cardiovascular diseases and preventive strategies

Despite increasing public awareness and major therapeutic progress, cardiovascular disease (CVD) remains the leading cause of morbidity and mortality worldwide. In the United States, CVD prevalence in the general population is expected to reach 40%, with direct related costs set to reach 800 billion dollars per year in the next two decades[1].

In Europe, CVD causes 47% of all deaths (Figure 1), accounting for 4 million fatalities each year, and costing 196 billion euros a year. Roughly half of these costs (54%) have been attributed to direct health care costs, and the other half (46%) to indirect losses (Heart Network: www.ehnheart.org).

Because the disease progresses asymptomatically, the first indication that an individual has atherosclerosis is often a severe cardiovascular event. According to statistics obtained in the United States during the last two decades, the first indicator of atherosclerosis for 30%-50% of patients was an acute, and in many cases fatal, myocardial infarction (MI)[2]. Current guidelines address this problem by identifying high-risk individuals according to the cumulative presence of different Framingham risk factors (smoking, obesity, diabetes, dyslipidemia, and hypertension), with the decision to go forward into preventive treatment made according to the estimated risk. Based upon these clinically-based cardiovascular (CV) risk stratification tools, individuals identified as at-risk for atherosclerosis and CVD are subjected to treatment that directly addresses the established risk factors, combining lifestyle modification (*e.g.*, smoking, exercise, diet) with anti-platelet therapy (aspirin), and medication to reduce both blood pressure (anti-hypertensive agents) and levels of circulating cholesterol (statins).

While this strategy has undoubtedly made some impact, current CV risk stratification tools only have the power to segregate very high-risk individuals from very low-risk individuals, and lack sensitivity and specificity in persons deemed to be at intermediate risk[3]. As a consequence, up to 60% of CV events occur in primary prevention (*i.e.*, in patients with asymptomatic CVD), affecting subjects deemed at low or intermediate risk of CVD (false negative)[4,5]. At the same time, other patients are unnecessarily given lifelong prevention medication (false positives) (reviewed in[2,6]).

For this reason, strong calls have been made to exploit existing knowledge and technology to improve the sensitivity and specificity of risk stratification approaches used to guide preventive therapy[2]. To be effective as public health measures, new approaches would have to be not only sensitive and specific, but also low-cost, non-invasive and adaptable to scale-up and commercialization for widespread use[2,6]. While solutions involving imaging technologies such as ultrasound, chest computed tomography (CT) and magnetic resonance imaging have been proposed[2], their implementation at population level in primary care is currently difficult to envisage mainly for economic reasons, and also because of health hazards related to radiation exposure.

As a more viable alternative strategy with respect to costs and health issues, much attention has been drawn to CV biomarkers that allow, on the basis of a simple blood sample measurement, to quantify either the amount of myocardial necrosis, such as cardiac troponins[7], the degree of myocardial stretch, such as natriuretic peptides[8], or the amount of systemic inflammation, such as high sensitive C-reactive protein (hs-CRP)[9], to only quote the "usual suspects" in the field. The complete list of candidate CV biomarkers is much longer, reflecting the numerous studies published in the field (Figure 2), but only a few of these candidates, notably those shown to be causally involved in the disease, are likely to make their way into clinical practice. For this reason it is hoped that improved knowledge of the pathogenesis of atherosclerosis will lead to the identification and validation of biomarkers for atherosclerosis and CVD, enabling the development of new risk stratification approaches[6].

Pathogenesis of atherosclerosis and cardiovascular disease

CVD is causally linked to atherosclerosis, the swelling of artery walls due to the formation of plaque lesions. Plaques are made up of leukocytes, smooth muscle cells and lipid deposits, with the surface of the plaque in contact with the arterial lumen covered with a fibrous connective tissue cap. Although atherosclerosis accumulates gradually and asymptomatically from childhood, it is accelerated by a number of established risk factors, including Framingham risk factors. Atherosclerotic plaques may remain stable as they grow, gradually reducing arterial blood flow as the lumen becomes increasingly obstructed, or may become prone to rupture. When plaque rupture occurs, the highly thrombogenic interior of the plaque is revealed, leading to atherothrombosis. The resulting ischemia is what causes CVD morbidity and mortality. Depending on the location of the affected artery the outcome can be myocardial infarction, stroke, or peripheral artery disease[4].

Atherosclerosis as an immune-mediated disease

Evidence linking high blood cholesterol to atherosclerosis, together with the presence of lipid deposits within atherosclerotic plaques led to the prevailing view that atherosclerosis was a lipid-related disease. This view was held until the 1990's, when a series of discoveries led to a paradigm shift in the understanding of atherosclerosis, shifting emphasis from lipid metabolism and transport to inflammation[10-12]. Inflammatory responses are now believed to underlie all of the key steps in atherosclerotic

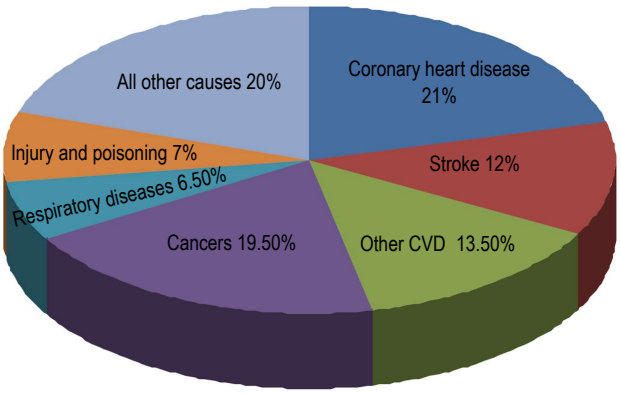

Figure 1 Deaths by cause in Europe for the latest available year, and by gender. Adapted from European Heart Network (www.ehnheart.org). CVD: cardiovascular disease.

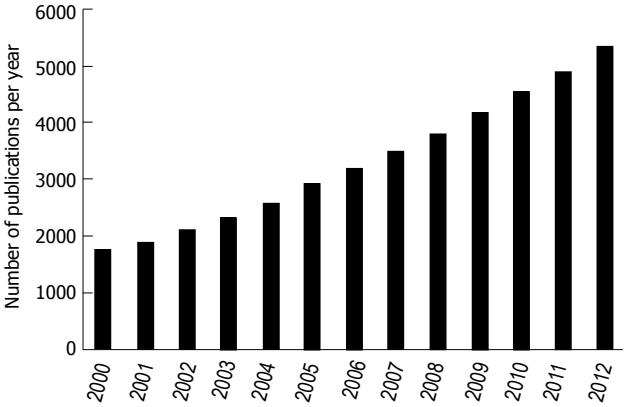

Figure 2 Annual evolution of publications on cardiac biomarkers since 2000. This graphic represents the number of publications per year indexed and retrieved in Pubmed between 2000 and 2012 when the key words "cardiovascular biomarkers" are entered. Entry date: 22nd of January 2014.

pathogenesis, from the initial modification of healthy arterial endothelium to thrombus formation at the site of plaque rupture.

According to this current paradigm (reviewed in[2,10-12]), atherosclerosis is initiated by inflammatory activation of arterial wall endothelial cells, allowing adhesion of circulating leukocytes. Expression of inflammatory chemokines leads to the migration of these leukocytes, predominantly circulating monocytes, across the endothelium and into the tunica intima. At this site the monocytes mature, acquiring a macrophage phenotype and the capacity to ingest native and modified low-density lipoprotein (LDL) particles that exit the blood and permeate the activated arterial endothelium. Following extensive lipid ingestion, these macrophages become "foam cells", which are the main constituents of an early atherosclerotic lesion. Foam cells release a broad range of cytokines and serve to amplify the inflammatory response, as well as inducing the proliferation of resident smooth muscle cells and promoting local angiogenesis. Chronic inflammation leads to the formation of an advanced atherosclerotic plaque, comprising a mass of foam cells surrounding a "necrotic core" of lipids released by dead and dying cells, capped by a fibrous layer made up of smooth muscle cells and extracellular matrix. Inflammatory responses also play a key role in atherothrombosis, which is recognized to account for up to 80% of acute CV manifestations[13]. Inflammation influences the local extracellular matrix composition through a complex interplay between different matrix-metalloproteinases (MMPs) determining the propensity of the fibrous cap to rupture[14-17]. Furthermore, a pro-inflammatory microenvironment also promotes thrombus formation *via* the activation of coagulation factors, leading to acute vessel occlusion[6].

Detailed analysis of the content of atherosclerotic plaques, together with the advent of a wide range of genetically modified mouse strains, has enabled further elucidation of the inflammatory pathogenesis of atherosclerosis[18]. The identification of autoantibodies as well as autoreactive T cells in atherosclerotic plaques[19], and the correlation established in clinical studies between their detection and disease severity provided a clear indication that adaptive immunity plays a role in atherosclerosis (reviewed in[20]). This role was underlined in a number of studies in which ApoE$^{-/-}$ knockout mice, which are predisposed to hypercholesterolemia and atherosclerosis, were crossed with different mouse strains deficient in specific arms of the adaptive immune system. These studies revealed a key pro-atherogenic role for the Th1 subset of CD4 T cells, and an anti-atherogenic role for the regulatory T cell subset (reviewed in[20]), as well as both pro- and anti-atherogenic roles for different B cell subsets[21]. In addition, they highlighted the importance in atherogenesis of signaling through pattern recognition receptors (PRR) of the innate immune system, such as Toll-like receptors (TLR) (reviewed in[18,20]).

Atherosclerosis as an autoimmune disease?

Grounded on the fact that atherogenesis fulfills several of "Koch" postulates (Table 1), atherosclerosis has even been proposed to be of autoimmune etiology[22,23]. This hypothesis is based on the following evidence. Firstly, atherosclerotic plaques are infiltrated by both T cells and antibodies specific for various autoantigens[20], patients suffering from autoimmune disease, such as systemic lupus erythematosus (SLE), anti-phospholipid syndrome (APS) and rheumatoid arthritis (RA) display an increased CV risk, independently of traditional CV risk factors[24-26]. Secondly, as reviewed elsewhere, in patients without autoimmune diseases, but established CVD, levels of antibodies directed against various and numerous endogenous epitopes, such as modified LDL, heat-shock proteins (HSP), and cardiolipin, have been shown to independently predict CV outcome[27]. Thirdly, *in vivo* and *in vitro* evidence demonstrated that some autoantibodies might directly influence atherogenesis and atherosclerotic plaque vulnerability, mostly by activating innate immune receptors, thereby supporting a causal role of humoral autoimmunity in atherosclerosis[28-31].

Nevertheless, the relationship between autoantibodies

Table 1 Koch posultates applied to the role of autoimmunity in atherosclerosis

Basic Koch postulates	Koch postulates transposed to the role of autoimmunity in atherosclerosis	Koch postulates met ?
Pathogens must be detected in the diseased host at every stage of the disease	Autoantibodies and auto-reactive T cells can be detected in atherosclerotic plaques and serum of patients in primary or secondary prevention of CVD	Yes
Pathogens must be isolated from the diseased host and grown in culture	Autoreactive T-cells can be isolated and cultivated from diseased host presenting experimental atherosclerosis	Yes
When inocculated in healthy animals, the pathogens from pure culture must induce the disease	Passive or active immunization drastically affect the course of atherogenesis in animal models	Yes
The pathogen must be re-isolated from the diseased animal and must correspond to the primary pathogen in pure culture	Protective autoantibodies of expected specificity can be isolated from animals exposed to active immunization	Partly

To establish a causality link between a mircoorganism and an infection, the four Koch postulates must be fulfilled. When applied to the role of autoimmunity in atherosclerosis, the Koch postulates support a causal role between autoimmunity, atherosclerosis and cardiovascular disease (CVD). Adapted from references[20-28].

and CVD is debated, because some of them have been shown to be anti-atherogenic, while others act as pro-atherogenic molecules[27,28]. The reason for such duality is still elusive and will not be further discussed in the present work.

Another unresolved question concerns the mechanisms by which tolerance is broken to generate autoimmunity. Certain lines of evidence point to pathogen molecular mimicry, *i.e.,* cross-reactivity between microbial antigens and components of host structures, including modified LDL and HSP[32,33]. In addition, modification of proteins by oxidation can generate new epitopes that are recognized as non-self by the adaptive immune system[32]. However, the presence of a non-self-epitope is not normally sufficient to drive an autoimmune response, since in order to effectively prime T cells, antigen presenting cells must concomitantly receive "danger signals" through their PRR. In the case of pathogen molecular mimicry, the PRR ligands are provided by the pathogen in the form of pathogen-associated molecular patterns (PAMPs). In the absence of a pathogen, "sterile inflammation" can be induced when antigen presenting cells are stimulated *via* their PRR by an analogous set of structures called damage-associated molecular patterns (DAMPs), which are typically released by stressed or necrotic cells[31-33].

Hence, both pathogen molecular mimicry, as a consequence of infection with, *e.g.*, *Chlamydia pneumoniae* or *Helicobacter pylori*, or DAMP-mediated sterile inflammation represent mechanisms by which autoantibodies targeting antigens implicated in atherosclerosis can emerge (reviewed in[12]).

Autoantibodies as CV risk stratification tools?

As mentioned previously, there is a clear need for new biomarkers to improve current CV risk stratification[6,34]. Driven by the paradigm shift of atherogenesis moving from a lipid-centered to inflammatory-centered etiology, the quest for new potential cardiovascular risk markers to better assess global cardiovascular vulnerability was principally oriented on inflammatory biomarkers, including autoantibodies[27-29].

Among the advantages identified for some autoantibodies is that they meet the current benchmark specifications requested for novel CV biomarkers[35,36]. Firstly, their association with CV outcomes has not only been shown to be independent of traditional CV risk factors (reviewed in[27,28]), but could also provide incremental predictive information over current CV risk stratification tools. Secondly, the stability provided by their long half-life place them as good candidates for long-term prognosis when compared to biomarkers with a shorter half-life. Thirdly, their measurement is typically simple, accurate, robust, and achievable at moderate costs.

Autoantibodies as potential therapeutic targets?

Providing that some autoantibodies have been shown to modulate atherogenic processes in antagonistic ways (reviewed in[27,28]), attempts to induce atheroprotective immunity through active immunization raised the hope that vaccination against different specific antigens (wide variety of modified LDL, HSP, *etc.*) could lead to lifelong protection against atherosclerosis and CVD. This hypothesis is currently under active investigation in humans[37,38].

On the other hand, neutralizing the deleterious effects of pro-atherogenic autoantibodies represents another interesting therapeutic modality which could currently be achieved through passive immunization with intravenous immunoglobulins (IVIG). In this respect, data concerning IVIG administration in humans after MI yield rather contradictory results[38,39], and although data restricted to animal models do support an anti-atherogenic role of IVIG[40-43], the costs related to IVIG therapy may well prohibit widespread administration of IVIG to all MI or CVD patients in the long-term, even if it proves to be effective. One solution might be to identify specific autoantibodies that could then be selectively neutralized by anti-idiotypic molecules rather than IVIG. Accordingly, an approach based on the detection of specific autoantibodies would enable the identification of a subset of CVD patients that could benefit either from immunomodulation (passive or active immunization) or from a specific mimetic peptide-based therapy. Such a strategy could represent an affordable step forward toward personalized medicine in the field of CVD, allowing a more targeted therapeutic intervention.

HIGH-DENSITY LIPOPROTEIN, APOLIPOPROTEIN A-1, AND ITS RELATED AUTOANTIBODIES

Human apolipoprotein A-1 (apoA-1) is a 28-kD protein with 243 amino acid residues encoded by the apolipoprotein multigene superfamily located on chromosome 11q23[44]. The protein is synthesized as a 24 amino-acid-longer prepro sequence of apoA-1, primarily by hepatocytes in the liver and also by enterocytes. Mature apoA-1 constitutes the principal protein fraction of high density lipoprotein (HDL) whose protective role in the CV system derives, to a great extent, from the inverse association of HDL-cholesterol and apoA-1 plasma concentrations with the risk of myocardial infarction. The atheroprotective role of HDL in the cardiovascular system has been attributed to the pleiotropic effects of HDL, including reverse cholesterol transport from resident arterial wall macrophages to the biliary tract for elimination, vasodilatation, anti-thrombotic, anti-coagulant and anti-inflammatory effects[45,46]. Mirroring those versatile properties, mass spectrometry analyses revealed that HDL encompasses very heterogeneous macromolecular complexes of lipids and proteins. Only one third of the up to 80 different proteins identified in HDL is dedicated to lipid transport. The remaining proteins being either acute-phase proteins, proteases, anti-oxidant, anti-thrombotic enzymes or proteins involved in complement regulation[45,46].

In addition to being the principal protein fraction of HDL and a limiting factor for HDL formation, apoA-1 *per se* has many of the HDL-related atheroprotective properties, such as inhibition of immune cell trans-endothelial migration, inhibition of monocyte activation, inhibition of cytokine production induced by T-cell contact, inhibition of lipid peroxidation, and interference with innate immune receptors pro-inflammatory signaling[45]. There is also a growing body of evidence indicating that both acute and chronic inflammatory conditions induce post-translational modifications of apoA-1 transforming HDL and apoA-1 into pro-inflammatory molecules[46]. Furthermore, as reviewed in the next paragraphs, recent data suggest that that humoral autoimmunity to apoA-1 and HDL could be new possible biomarkers for CVD, and possibly a mediator of inflammation, atherosclerosis, and CVD.

ANTI-APOA-1 IGG IN AUTOIMMUNE DISEASES

Anti-apoA-1 IgG in SLE and APS patients

In 1995, using early phage display technology, Merrill *et al*[47] reported that sera derived from SLE patients were immunoreactive against a protein displaying 82% DNA sequence homology with human apoA-1, followed by the confirmation that those sera were indeed reactive to human apoA-1 when coated on gamma-irradiated enzyme-linked immunosorbent assay plates. Further understanding of anti-apoA-1 autoantibody architecture was provided by the same group in 1998 who reported that high levels of anti-apoA-1 IgG were retrieved in a significant subset of SLE (32.5%) and primary APS patients (22.9%)[35]. Those autoantibodies were found to be associated with the presence of anti-beta2glycoprotoein I (β_2GPI) antibodies, and to display an optimal affinity for mature HDLs[48]. In 2001, Abe and colleagues characterized six different monoclonal anti-apoA-1 antibodies (derived from two SLE patients) displaying a low specificity, as reflected by their broad cross-reactivity to single strand DNA, thrombin, cardiolipin (CL), and to HDL[48,49]. Because of the latter observation, anti-apoA-1 IgG were considered a possible subgroup of anti-HDL antibodies[49]. The first insight regarding the potential pathogenicity of this class of autoantibodies in atherogenesis was demonstrated in 2003 by Delgado Alves and colleagues, who showed an inverse correlation between anti-HDL IgG and paraoxonase-1 (PON-1) activity, and with the total antioxidant capacity of the corresponding sera[50]. More specifically, those initial results suggested that anti-HDL, and later anti-apoA-1 IgG[51], could be related to atherogenesis, through HDL dysfunction[52,53], whose pathophysiological role in atherogenesis was starting to be recognized[54].

Anti-apoA-1 IgG in rheumatoid arthritis patients

In 2010, we demonstrated in a case-control study that anti-apoA-1 IgG levels were higher in patients suffering from rheumatoid arthritis (RA) than in matched-controls (17% *vs* 2%, $P = 0.01$)[55]. In this study, those autoantibodies were associated with higher oxidized LDL levels and were significantly associated with anamnestic CVD. Nevertheless, no association was found with the RA disease activity score[55]. Concomitantly, in a longitudinal prospective study which will be described in detail in the following paragraph entitled "anti-apoA-1 IgG as independent predictors of CV risk", we confirmed that those autoantibodies were predictive of CVD in RA patients, and were associated with a pro-inflammatory cytokine profile[56,57].

ANTI-APOA-1 IGG IN OTHER NON-AUTOIMMUNE POPULATIONS

If high levels of anti-apoA-1 IgG are initially described as raised in patients with autoimmune diseases associated with an increased risk of CVD, high levels of those autoantibodies can also be detected in patients without autoimmune disease, but with CVD, such as acute coronary syndrome[58-62], and severe carotid stenosis[63,64].

In addition, the existence of elevated levels of anti-apoA-1 IgG was demonstrated in patients with periodontitis[65], and patients under hemodialysis[66], two clinical conditions known to be associated with increased CVD risk[67,68]. Finally, the existence of high levels of anti-apoA-1 IgG was recently reported in obese, but other-

wise healthy subjects[69].

In those different settings the prevalence of a high titer of anti-apoA-1 IgG varies between 10% and 20%, against 0% to 6.5% in healthy blood donors or controls[56,59,65]. The clinical relevance of such findings will be discussed later.

ANTI-APOA-1 IGG AS A MARKER AND POSSIBLE MEDIATOR OF INFLAMMATION AND ATHEROGENESIS

Anti-apoA-1 IgG are associated with a pro-inflammatory and pro-atherogenic cytokine profile in humans

Most studies published to date have reported significant associations between high levels of anti-apoA-1 IgG levels and markers of oxidative stress, inflammation and endothelial dysfunction related to atherogenesis and atherosclerotic plaque rupture.

In SLE patients, anti-apoA-1 IgG levels were found to be positively correlated with nitric oxide ($r = 0.37$, $P = 0.007$), inversely related to PON-1 activity ($r = -0.31$, $P = 0.006$), and the total anti-oxidant capacity of the sera ($r = -0.47$, $P < 0.0001$) suggesting that those autoantibodies could interfere with the anti-oxidant properties of HDL, giving rise to a pro-oxidative micro-environment facilitating atherogenesis[70]. Similarly, RA patients tested positive for those autoantibodies were shown in two different studies to have higher plasma levels of oxLDL levels[55,56], considered a major player in all stages of atherogenesis[2,10,11]. Furthermore, RA patients tested positive for anti-apoA-1 antibodies were found to have higher levels of interleukin-8 (IL-8) and MMP-9[55], two inflammatory mediators known to be associated with atherogenesis, and atherosclerotic plaque vulnerability in humans[14,71].

In a retrospective study involving MI patients, we reported a positive association between anti-apoA-1 IgG and serum amyloid A (SAA) protein levels ($r = 0.76$, $P = 0.006$), a multifunctional protein located at the crossroad of inflammation and cholesterol homeostasis[58].

Subsequently, in a prospective cohort study involving MI patients ($n = 127$), we noted the same relationship between anti-apoA-1 IgG and oxLDL levels as had been documented in RA patients[59]. MI patients considered as positive for anti-apoA-1 IgG had significantly higher median levels of oxLDL when compared to patients tested negative for those autoantibodies (226.5 U/L *vs* 47.7 U/L, $P < 0.0001$), and a positive correlation between oxLDL and anti-apoA-1 IgG was observed (Spearman $r = 0.28$, $P < 0.05$)[59]. On the other hand, no association with PON-1 activity was observed in this study.

In a prospective study enrolling 221 MI patients, we demonstrated that patients tested positive for anti-apoA-1 antibodies had higher circulating levels of IL-6, TNF-α, and MMP-9, and lower MMP-3 levels[72], a cytokine constellation known to be associated with increased atherosclerotic plaque vulnerability, and worse CV prognosis[73,74]. This increase in MMP-9 levels retrieved in anti-apoA-1 IgG positive patients was associated with an increase in MMP-9 activity[63].

Furthermore, in our periodontitis study, we observed a positive correlation between anti-apoA-1 IgG and ADMA levels (Spearman; $r = 0.20$, $P = 0.02$)[65], a marker of endothelial-dependent dysfunction with strong CV prognostic value[75,76].

Among other associations observed between anti-apoA-1 IgG CV relevant prognostic features was an association with basal heart rate. In one of our prospective MI cohort studies[60], we demonstrated that when compared to those tested negative for anti-apoA-1 IgG, patients tested positive for those antibodies had a higher basal heart rate upon discharge, a well-established CV prognostic feature after MI[77-79]. The possible impact of those autoantibodies on nervous autonomic dysfunction will be presented in the paragraph entitled "Anti-apoA-1 IgG elicits a positive chronotropic effect on cardiomyocytes".

In conclusion, the most consistent associations observed so far between anti-apoA-1 IgG and CV-relevant markers of inflammation concern mostly oxLDLs and MMP-9. Although no causal link can be inferred based on such statistical associations, they were nevertheless instrumental in orienting the subsequent *in vitro* and animal studies described in the next paragraph.

Anti-apoA-1 IgG as active mediators of atherosclerosis and atherosclerotic plaque vulnerability in vitro and in vivo

Experiments carried out in cellular and animal models indicated that certain autoantibodies contribute directly to the induction of atherogenesis and atherosclerotic plaque vulnerability through their capacity to signal through innate immune receptors, notably TLR-2 ([29-31]; reviewed in[27]). By analogy, we investigated whether anti-apoA-1 autoantibodies could act through innate immune receptors signaling to elicit a pro-inflammatory response.

In this respect, we recently showed that lipopolysaccharide-free anti-apoA-1 IgG dose-dependently induced the production of a range pro-inflammatory cytokines, such as IL-8, MMP-9, IL-6, TNF-α, and MCP-1 in human monocyte-derived macrophages[55,63,72], and that this process was mediated by the TLR2/CD14 complex[72]. In addition, our *in silico* modeling studies revealed evidence of structural homology between apoA-1 and part of the extracellular domain of TLR2, suggesting a molecular mechanism for this cross-reactivity[72]. Our current understanding on how anti-apoA-1 IgG promotes sterile inflammation through the activation of TLR2/CD14 complex is summarized in Figure 3.

Anti-apoA-1 IgG elicits a positive chronotropic effect on cardiomyocytes

We have recently demonstrated that there is a positive association between levels of anti-apoA-1 IgG and resting heart rate following myocardial infarction, a well-established parameter for CVD prognosis in secondary prevention[60,79]. In the same study, we showed that in the presence of aldosterone, anti-apoA-1 IgG elicits a

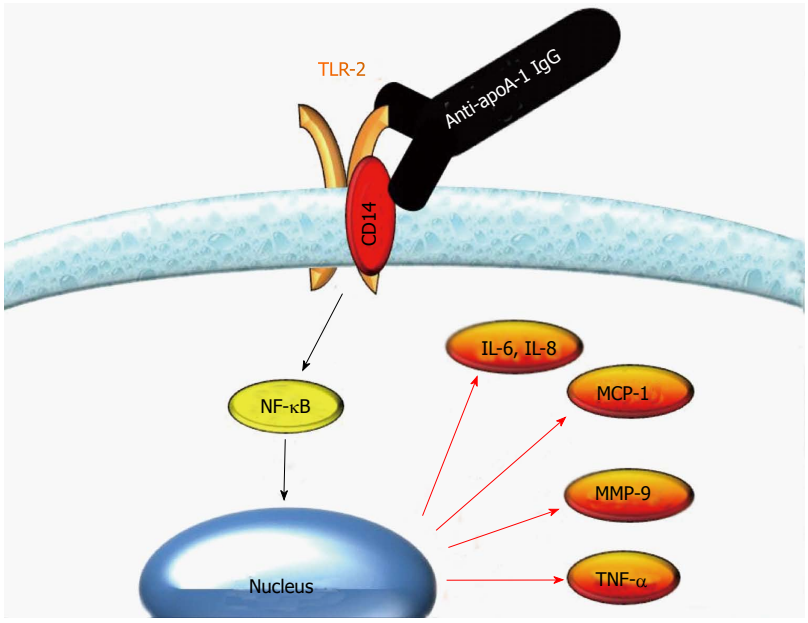

Figure 3 Autoantibodies against apolipoprotein A-1 IgG elicit a pro-inflammatory response through Toll-like receptor 2/CD14 complex on human macrophages. Autoantibodies against apolipoprotein A-1 (anti-apoA-1) IgG specifically bind to Toll-like receptor (TLR)2 due to conformational homology between apoA-1 and TLR2. In the presence of CD14, the binding of anti-apoA-1 IgG to TLR2 induces a nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)-dependent production of pro-inflammatory cytokines. MMP-9: Matrix-metalloproteinases; IL-8: Interleukin-8.

dose-dependent increase in the spontaneous contraction rate of neonatal rat ventricular cardiomyocytes[60]. Using patch-clamp electrophysiology combined with a pharmacological approach, we subsequently showed that this positive chronotropic effect was mediated by L-type calcium channel activation, itself induced by the concomitant activation of both the mineralocorticoid receptor-dependent phosphatidyl 3-kinase pathway and the protein kinase A pathway[80]. In support of an activation mechanism involving aldosterone and antibody, we demonstrated that the chronotropic effect can be abrogated by addition of eplerenone, an aldosterone antagonist, and by intravenous immunoglobulins[60,80]. Hence, there is compelling evidence in support of a role for anti-apoA-1 IgG in the induction of a positive chronotropic effect in cardiomyocytes, but further work will be required to define (1) whether this is a direct or indirect effect; and (2) if anti-apoA-1 IgG acts directly on cardiomyocytes, which receptor does it engage to activate the protein kinase A pathway (Figure 4)? At the present time, there is no indication suggesting that this chronotropic effect could be mediated by interference with the activity of the autonomous nervous system; further work will be required to address this question.

Anti-apoA-1 IgG induces atherosclerosis and death in apoE$^{-/-}$ mice

Animal studies that we have performed provided direct evidence that anti-apoA-1 IgG was sufficient to induce atherosclerosis. Passive immunization of atherosclerosis-prone apoE$^{-/-}$ mice with anti-apoA-1 IgG increased both atherosclerotic lesion size and histological features of atherosclerotic plaque vulnerability[63]. In a lupus-prone mouse model, Srivastava and colleagues demonstrated that the presence of anti-apoA-1 antibodies was associated with a decrease in the anti-oxidant properties of HDL which inferred a decrease in PON-1 activity, leading to an increase in pro-inflammatory reactive oxygen species[81]. These results support the hypothesis that anti-apoA-1 IgG and HDL dysfunction are two related phenomena. Although a causal link between anti-apoA-1 IgG and HDL dysfunction remains elusive, these results are consistent with clinical observations reported previously[49-52].

ANTI-APOA-1 IGG AS INDEPENDENT PREDICTORS OF CV RISK

In 2010, we demonstrated that anti-apoA-1 IgG positivity assessed in samples taken within the first 24 h of patient admission for MI was a significant and independent predictor of MACE during 1 year follow-up[60]. The presence of high anti-apoA-1 IgG levels on admission increased the subsequent risk of MACE by 4-fold, independently of Framingham risk factors [adjusted OR = 4.3, 95%CI: 1.46-12.6, $P = 0.007$][60]. Cox regression analysis demonstrated that for each arbitrary unit increase in anti-apoA-1 IgG, there was a concomitant 3% increase in MACE risk ($P = 0.0003$). All 221 patients tested negative for anti-nuclear antibodies and no association with any other autoantibodies (rheumatoid factor, anti-β2GPI and anti-cardiolipin antibodies) was observed[60].

These findings were extended in an ancillary study derived from the same cohort of patients aimed at comparing, in a "head to head" fashion, the prognostic accuracies of other autoantibodies described as potentially relevant for CV event prediction. Among those, we measured antibodies to β2GPI domain I and IV, cardiolipin, heat-shock protein 60 (anti-HSP-60), and phosphorylcholine (anti-PC IgM)[61]. In this study, autoantibodies to apoA-1 were found to be the only autoantibodies to significantly predict subsequent MACE occurrence, although a non-significant trend was observed for anti-cardiolipin ($P = 0.05$), and anti-HSP60 antibodies $P = 0.07$). In this study, the prognostic accuracy measured by the area under the curve (AUC) was rather modest (AUC: 0.65, $P = 0.007$)[61], and of the same order of magnitude as the 10-year global

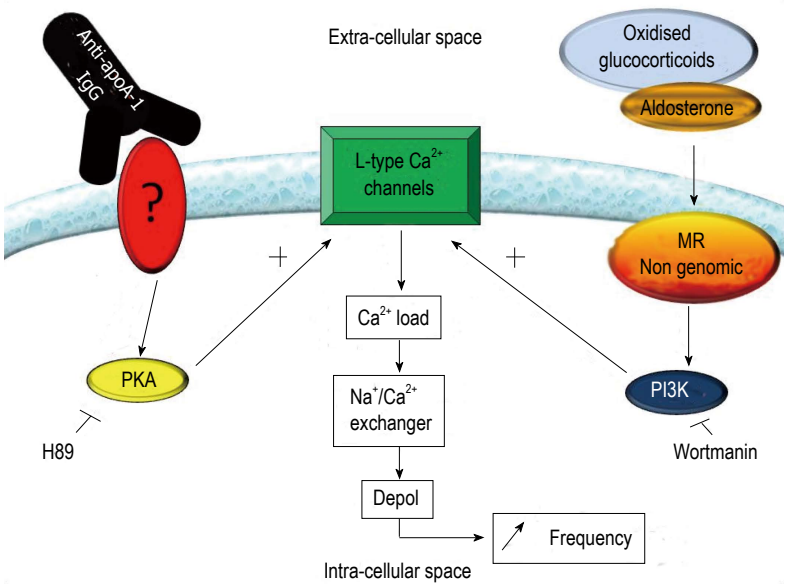

Figure 4 Current understanding of the mechanism by which autoantibodies against apolipoprotein A-1 IgG elicit chronotropic responses in nenonatal rat cardiomyocytes. Stimulation of the mineralocorticoid receptor (MR), either by aldosterone or oxidized glucocorticoids, induces the downstream activation of PI3K, which in turn activates L-type calcium channels. Anti-apoA-1 IgG has been shown to sensitize the L-type calcium channel in a protein kinase A (PKA)-dependent manner. The PI3K and PKA activated pathways alone are not sufficient to induce an increase in basal contraction rate, when simultaneously activated L-type calcium channels are activated, leading to an increase in intracellular Ca^{2+}. This signal is amplified by the Na^{+}/Ca^{2+} exchanger, leading to an increase of the prepotential slope of the cells, which ultimately translates into an increased contraction rate.

Framingham risk score. Risk analyses demonstrated that anti-apoA-1 IgG positivity increased the risk of MACE by 4-fold, independently of the 10-year global Framingham risk score (adjusted hazard ratio = 3.8, $P = 0.002$)[61]. Those preliminary results pointed to anti-apoA-1 IgG as a promising humoral autoimmune candidate for MACE prediction in secondary prevention settings.

Furthermore, in a single center prospective study involving 138 patients presenting to the emergency room with acute chest pain, we demonstrated that anti-apoA-1 IgG values assessed on the first sample available had a relatively good diagnostic accuracy for non-ST elevation myocardial infarction (NSTEMI) with an AUC of 0.75 ($P < 0.0001$) that could be increased up to 0.88 when combined with anti-PC IgM and the NSTEMI-TIMI score to generate a clinical antibody ratio (CABR) score[62]. Also, anti-apoA-1 IgG was found to be a good predictor (AUC = 0.80, $P < 0.0001$) of subsequent troponin I elevation when the first sample tested negative, which was the secondary endpoint of this study. Risk analyses indicated that in the presence of high anti-apoA-1 IgG levels, the risk of subsequent NSTEMI diagnosis was increased by 6-fold after the adjustment for NSTEMI-TIMI score (OR: 6.4, 95%CI: 1.72-24.2). At the pre-specified cut-off, this test displayed an interesting negative predictive value of 88% and 95% for the primary and secondary study endpoints, respectively. To summarize, in ACS patients, the predictive accuracy according to ROC curve analysis revealed AUC values ranging between 0.65 and 0.75[61,62]. If these AUC values are relatively modest (they should ideally be above 0.80[36]), they are still in the same range as those reported for the Framingham risk score, which currently determines patient management[3].

Furthermore, we demonstrated that anti-apoA-1 IgG were also predictors of MACE at one-year after elective surgery for severe carotid stenosis with an AUC of 0.74 (95%CI: 0.59-0.90, $P = 0.01$)[64], and that its combined used with myeloperoxidase could improve the predictive accuracy of the model[64]. In this study, high levels of anti-apoA-1 IgG were associated with a 5-fold increase in MACE during follow-up (exact OR = 5.29, 95%CI: 1.08-34.02, $P = 0.04$), which remained significant after adjustment for the 10-year Framingham risk score according to conventional logistic regression, but not when the exact logistic regression model was applied[64].

In a longitudinal prospective study involving 133 RA patients followed-up for a median duration of 9 years[56], we demonstrated that high levels of anti-apoA-1 IgG was associated with a 4-fold increase in MACE during follow-up, independently of Framingham risk factors and RA disease duration (HR = 4.2, 95%CI: 1.5-12.1). In this study, ROC curve analyses indicated that those autoantibodies were the strongest predictors of MACE with an AUC of 0.73 ($P = 0.0008$), a specificity of 50%, and a sensitivity of 90% at the predefined cut-off[56]. In addition to their independency of traditional CV risk factors to predict poor CV outcome, we also demonstrated that anti-apoA-1 IgG provides incremental prognostic information over traditional cardiovascular risk factors in ACS, in severe carotid stenosis, and in RA patients. When compared to current risk stratification tools (NSTEMI-TIMI score in acute chest pain patients, or the 10-year global Framingham risk score in ACS, RA, or severe carotid stenosis patients), it significantly improved the patient risk reclassification with significant integrated discrimination index values ranging between 1.8% and 175%[57,62,64].

Anti-apoA-1 IgG as a biomarker predictive of atherosclerosis and atherosclerotic plaque vulnerability

Of clinical relevance, we have also demonstrated that anti-apoA-1 IgG is also detectable in a proportion of healthy subjects without autoimmune disease and CVD (0%-6.5%), albeit at lower levels than seen in patient cohorts[56,59,65]. Significantly, in a small case-control study on healthy subjects[69], we demonstrated that anti-apoA-1 IgG levels in the obese subgroup were raised to levels previously described in CVD patients, with high levels of anti-apoA-1 IgG being a significant predictor of coro-

nary artery calcifications visualized by chest computed tomography. Because coronary artery calcifications are a major predictor of subsequent cardiovascular events in asymptomatic subjects[82], the results of this preliminary study suggest that anti-apoA-1 IgG may be a valuable biomarker for use in primary prevention to screen for the presence of coronary artery lesions. Indeed in this setting, anti-apoA-1 IgG testing had a negative predictive value of 94% to detect the presence of coronary artery calcification, with an AUC of 0.83[69]. Similarly, we demonstrated in patients with periodontitis younger than 50 years old that anti-apoA-1 IgG was the only predictor of a pathological ankle brachial index[65], a measure used to detect peripheral artery disease and known to reflect the global atherosclerosis burden[83,84].

Extending those results, we also reported that the presence of anti-apoA-1 antibodies in patients with severe carotid stenosis was associated with histological features of atherosclerotic plaque vulnerability determined on surgical biopsy specimens[63]. Indeed, in this study, we demonstrated that circulating levels of anti-apoA-1 IgG were positively correlated with intraplaque macrophages (r = 0.33, P = 0.002), MMP-9 expression (r = 0.43, P = 0.0001) and neutrophils (r = 0.42, P = 0.0001), and inversely correlated with total collagen content (r = -0.29, P = 0.008). Furthermore, patients deemed as positive for anti-apoA-1 IgG had significantly higher levels of macrophages, MMP-9 expression and neutrophils within their atherosclerotic lesions, and lower levels of total collagen when compared to patients tested negative for those autoantibodies[63]. Interestingly, those findings were mimicked in apoE$^{-/-}$ mice exposed to passive immunization with anti-apoA-1 IgG when compared to the CTL group[63]. Taken together those results indicate that assessing anti-apoA-1 IgG levels could not only be a possible biomarker of atherosclerosis, but could also be used to detect the presence of atherosclerotic plaque vulnerability. Because assessing atherosclerotic plaque vulnerability is currently an unmet clinical need, the possibility of using anti-apoA-1 IgG detection as a simple and affordable surrogate biomarker of atherosclerotic plaque fragility is of patent clinical interest.

FUTURE PERSPECTIVES

Because current *in vitro* and *in vivo* results indicate that anti-apoA-1 IgG could well be active mediators of atherogenesis, those autoantibodies may represent an emergent therapeutic target. In other words, we speculate that measuring circulating levels of anti-apoA-1 IgG would enable the identification of a subset of patients who would benefit from specific therapy aimed at reversing the deleterious effect of those autoantibodies. In this respect, we have demonstrated that the chronotropic effect of those autoantibodies could be reversed by existing therapeutic compounds such as IVIG and eplerenone, a selective MR antagonist[80].

In parallel, we will pursue our work aimed at defining the exact CV-relevant epitope(s) targeted by those autoantibodies. Once determined, those epitopes could be useful both for the detection of anti-apoA-1 IgG by occupying binding sites, and for neutralizing the pathogenic effects of the antibodies (pro-arrythmogenic and pro-inflammatory effects), which hopefully would translate in a reduction of atherogenesis-related complications in humans.

CONCLUSION

To summarize, recent studies demonstrate that IgG autoantibodies against apoA-1 are raised in many diseases associated with a high cardiovascular risk, such as SLE, ACS, RA, severe carotid stenosis, and end-stage renal disease. To date, high levels of anti-apoA-1 IgG have been shown to be an independent prognostic marker of poor CV outcome in MI, RA and carotid stenosis patients, to display clinically relevant properties for NSTEMI diagnosis in acute chest pain patients, to be associated with atherosclerotic plaque vulnerability in patients with severe carotid stenosis, and to predict coronary artery lesions in obese, but otherwise healthy subjects. In most studies reported so far, high levels of anti-apoA-1 IgG are associated with a pro-inflammatory cytokine profile, and in SLE/APS, those autoantibodies have been shown to be associated with the presence of dysfunctional HDLs.

Concomitantly, *in vitro* data tend to indicate that anti-apoA-1 IgG are active modulators of atherogenesis by (1) promoting a sterile inflammation through the TLR2/CD14 complex; and (2) eliciting specific neutrophil chemotaxis. Furthermore, *in vitro* experiments suggest that those autoantibodies could act as pro-arrhythmogenic molecules through an aldosterone-dependent L-type calcium channel activation that can be reversed using existing therapeutic compounds. In parallel, work in mouse models demonstrated that passive immunization with anti-apoA-1 IgG increases atherogenesis, atherosclerotic plaque vulnerability, death rate, and decreases the anti-oxidant properties of HDL by inhibiting PON-1 activity. The preliminary clinical results need to be replicated in larger multicenter cohorts and further basic science studies will be required to gain a better understanding of the pathophysiological involvement of anti-apoA-1 IgG in atherogenesis. Nevertheless, the current converging *in vitro* and animal observations lend weight to the hypothesis that anti-apoA-1 IgG are active mediators of atherogenesis rather than innocent bystanders. Hence, these autoantibodies, could in the future, represent a new possible therapeutic target, whose deleterious effect could be abrogated by therapeutic synthetic apoA-1 mimetic peptides. In this context, anti-apoA-1 IgG appears to be a promising biomarker of pathological cardiovascular autoimmunity, allowing the identification of a subset of CVD patients who could benefit from specific immunomodulation in the future, substantially contributing to the development of personalized medicine in the field of CVD.

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ORIGINAL ARTICLE

Elevated blood pressure: Our family's fault? The genetics of essential hypertension

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Abstract

AIM: To provide an updated review on current genetic aspects possibly affecting essential hypertension (EH), and to further elucidate their role in EH.

METHODS: We searched for genetic and epigenetic factors in major studies associated with EH between Jan 2008-Oct 2013 using PubMed. We limited our search to reviews that discussed mostly human studies, and were accessible through the university online resource. We found 11 genome wide association studies (GWAS), as well as five methylation and three miRNA studies that fit our search criteria. A distinction was not made between genes with protective effects or negative effects, as this article is only meant to be a summary of genes associated with any aspect of EH.

RESULTS: We found 130 genes from the studies that met our inclusion/exclusion criteria. Of note, genes with multiple study references include: *STK39*, *CYP17A1*, *MTHFR-NPPA*, *MTHFR-NPPB*, *ATP2B1*, *CSK*, *ZNF652*, *UMOD*, *CACNB2*, *PLEKHA7*, *SH2B3*, *TBX3-TBX5*, *ULK4*, *CSK-ULK3*, *CYP1A2*, *NT5C2*, *CYP171A*, *PLCD3*, *SH2B3*, *ATXN2*, *CACNB2*, *PLEKHA7*, *SH2B3*, *TBX3-TBX5*, *ULK4*, and *HFE*. The following genes overlapped between the genetic studies and epigenetic studies: *WNK4* and *BDKRB2*. Several of the identified genes were found to have functions associated with EH. Many epigenetic factors were also correlated with EH. Of the epigenetic factors, there were no articles discussing siRNA and its effects on EH that met the search criteria, thus the topic was not included in this review. Among the miRNA targets found to be associated with EH, many of the genes involved were also identified in the GWAS studies.

CONCLUSION: Genetic hypertension risk algorithms could be developed in the future but may be of limited benefit due to the multi-factorial nature of EH. With emerging technologies, like next-generation sequencing, more direct causal relationships between genetic and epigenetic factors affecting EH will likely be discovered creating a tremendous potential for personalized medicine using pharmacogenomics.

Key words: Essential hypertension; Epigenomics; Genome-wide association study; Genes; MicroRNAs

Core tip: Essential hypertension (EH) is considered a multifactorial disease, indicating that many genetic, epigenetic, and environmental influences affect the initiation and continuance of the disease. Our goal is to provide an updated report on current genetic aspects possibly affecting EH by elucidating genetic factors' role in EH. We found 130 genes meeting our inclusion/exclusion criteria. To our knowledge, this is the first review to discuss both genetic and epigenetic factors associated with EH in one article. With emerging technologies, more direct causal relationships between

genetic and epigenetic factors with EH will likely be discovered, creating tremendous potential for personalized medicine using pharmacogenomics.

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INTRODUCTION

Approximately 1 in 3 American adults, or about 67 million people, have hypertension (HTN)[1]. According to the American Heart Association, the majority of Americans who have had first heart attacks, first strokes, or chronic heart failure had underlying HTN, a known risk factor for each condition[2]. HTN costs the United States approximately $47.5 billion annually in direct medical costs and roughly $3.5 billion annually in lost economic productivity[3].

Essential hypertension (EH), the most common form of HTN[4], is defined as an elevation in blood pressure of unknown cause and increases the risks for cerebral, cardiac, and renal complications[5]. EH is thought to be a multifactorial disease, indicating that many factors affect the initiation and continuance of the disease[6]. From a genetic perspective, many single nucleotide polymorphisms (SNPs), genes and epigenetic factors are associated with EH. This suggests that people with these hereditary factors might have a genetic predisposition to having high blood pressure. Additionally, since EH has idiopathic origins, environmental factors may also play an important role in the cause of the disease. Weight gain and dietary factors appear to have a major role in causing EH due to impaired renal function, though the mechanisms are not well understood[7].

There has been some discussion on the common disease, common variant (CDCV) and common disease, rare variant (CDRV) hypotheses and their relation to complex diseases, such as EH[8]. The CDCV hypothesis predicts that there are common disease-producing alleles/variants that are found in all human populations with a particular phenotype for a certain disease. However, insufficient data has led to scientists challenging the validity of this hypothesis and its compatibility with many diseases[9]. Meanwhile, the CDRV hypothesis predicts that diseases with genetic predispositions may not be found commonly in the diseased human population[10]. One study argued that with human lineage, diseases were more likely to favor multiple rare variations contributing to disease, rather than common variations contributing to disease[11]. This is because common variations might have external factors that would have eliminated these genes from the population, while rare variants are new, contributing to disease[12].

The purpose of this article is to provide an updated report on the current genetic aspects that could affect EH, and to further elucidate the role of genetic factors in EH. This includes summarizing genome-wide association studies (GWAS), as well as studies that identified genes with specific physiological functions. We also summarize current knowledge of the epigenetics in EH and/or HTN.

MATERIALS AND METHODS

Since genetic factors that influence EH in the literature are broad, we looked at specific categories of genetic factors and their influence on EH. Genetic marker studies were chosen since these studies looked specifically at what genes were involved with EH, and if any had specific physiologic effects. As epigenetics has become an emerging field of interest in genetics, DNA modification related to EH is also included, specifically focusing on DNA methylation and RNA regulation studies. It is important to note that a distinction was not made between genes with protective effects or negative effects, as this article is only meant to be a summary of genes associated with any aspect of EH.

For the search criteria, specific keywords used for each category of genetic and epigenetic factors are listed below in Figure 1.

Inclusion criteria

Reviews were selected if there was a primary focus on the genes and genetic factors associated with EH. Additionally, reviews between Jan 2008-Oct 2013 were chosen to obtain the most current information. Reviews were selected that discussed human studies, with little if any focus on animal studies. Reviews were also included if there was discussion of non-European populations since EH affects many ethnicities. Lastly, the results reported from the selected reviews were limited to reviews that discussed cohorts in populations greater than 1000 individuals. Cohorts with populations > 1000 people were chosen to reduce selection bias within the primary studies, and to ensure that the genes found could apply to large populations. From the articles that were selected to be in the study, the authors identified if the genes had known pathways related to EH.

For epigenetic factors associated with EH, we included articles that discussed various epigenetic modifications and their physiologic effects, as well as specific techniques such as methylation. If the studies had relevant animal data, this was included due to the fact that there is limited epigenetic information in human studies. Articles that discussed miRNA and the association with EH were also included to ensure a more thorough gathering of data. No articles for siRNA met our search criteria. Therefore, a discussion on siRNA as it relates to EH is not provided in this article.

Exclusion criteria

Reviews were excluded if the reviews involved rare types of HTN and/or were too detailed on EH physiology. While EH physiology is important, it does not contribute to the purpose of this paper in understanding the genetic

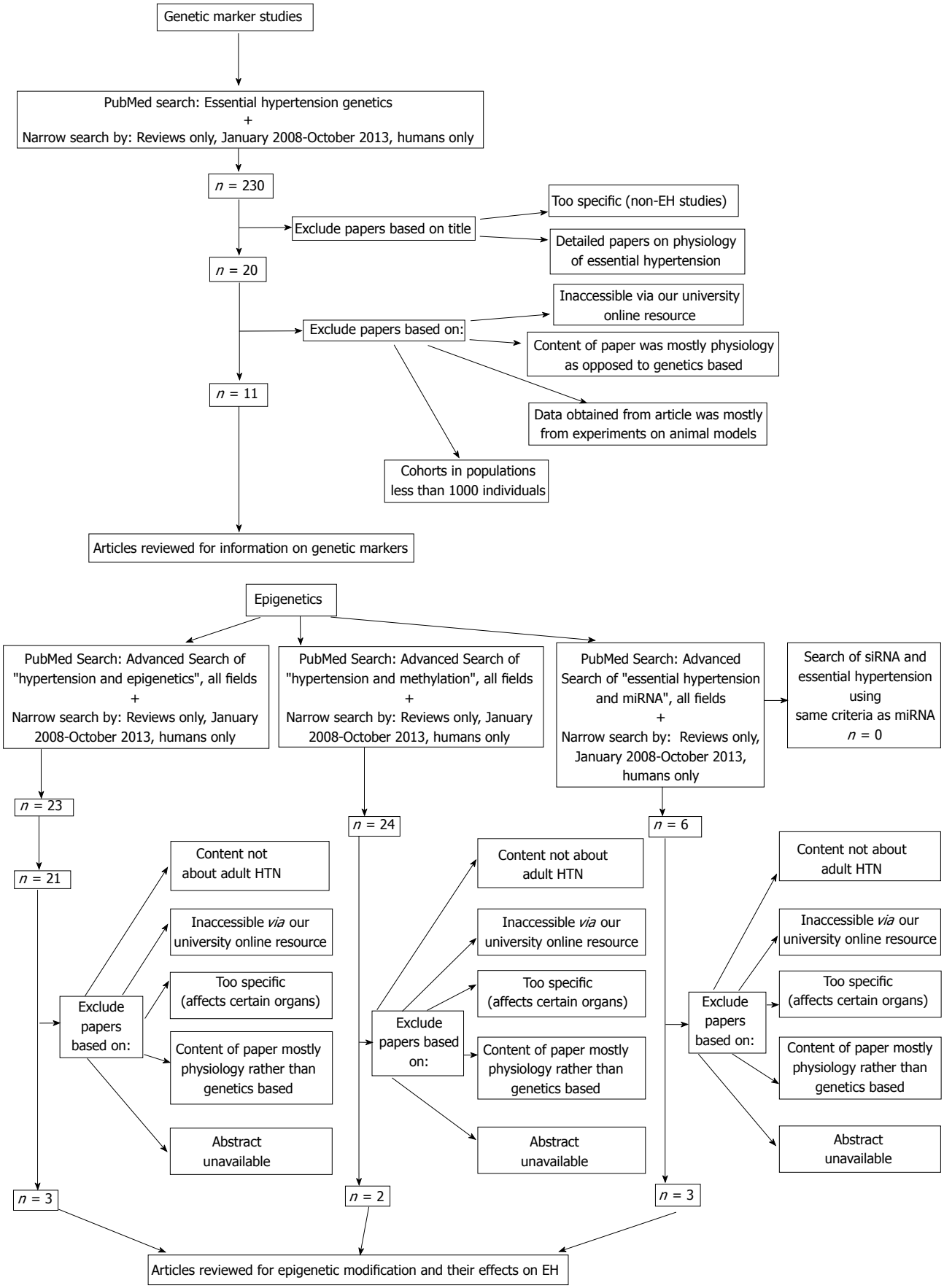

Figure 1 Search methodology for genetic and epigenetic factors associated with essential hypertension. Visual Understanding Environment v.3.2.1 (Tufts University) was used to produce the images. HTN: Hypertension.

Table 1 Genetic associations with essential hypertension according to cohort

Cohort	Genes
Framingham offspring cohort	*CCL20-WDR69, CDH13, TGFBR2,* ***STK39***
Amish cohort	***STK39***
AGEN	*NPR3,* ***CYP17A1****, FGF5,* ***MTHFR****,* ***NPPA****,* ***NPPB****,* ***ATP2B1****,* ***CSK****,* ***ZNF652***
BP-extremes	***UMOD***
BRIGHT	*BCAT1*
CARe	*c21orf91, GPR98 and ARRDC3*
CBPgen	***CYP17A1****,* ***CACNB2****,* ***PLEKHA7****,* ***SH2B3****,* ***TBX3****,* ***TBX 4****,* ***TBX5****,* ***ULK4***
CHARGE	*CPLX3,* ***PLEKHA7****,* ***TBX3****,* ***UMOD****,* ***CYP17A1****,* ***CSK-ULK3****,* ***CYP1A2****,* ***NT5C2****,* ***CYP171A****,* ***PLCD3****,* ***SH2B3-ATXN2****,* ***CACNB2****,* ***SH2B3****,* ***TBX3****,* ***TBX4****,* ***TBX5****,* ***ULK4****, c10orf107, BLK-GATA4, CASZ1, FGF5,* ***MTHFR****,* ***NPPA****,* ***NPPB****,* ***ATP2B1****,* ***CSK***
FHS	*ANKMY, FOXD3*
GBPgen	***UMOD****,* ***CSK-ULK3****,* ***CYP1A2****,* ***NT5C2****,* ***CYP171A****,* ***PLCD3****,* ***SH2B3-ATXN2****, ATXN2, c10orf107, GNAS-EDN3,* ***MECOM (MDS1 locus)****, FGF5,* ***MTHFR****,* ***NPPA****,* ***NPPB****,* ***ATP2B1****,* ***CSK****,* ***ZNF652***
GENE-centric	*SOX6, AGT, LSP1-TNNT3,* ***MTHFR****,* ***NPPA****,* ***NPPB****,* ***ATP2B1****,* ***HFE***
Health2	***ATP2B1***
HUFS	*IPO7, MYLIP, PMS1, SLC24A4, YWHAZ, CACANA1H*
Hypergenes	*NOS3*
ICBP	*ADAMTS-8, ADM, BAT2-BAT5, CHIC2-PDGFRA1, EBF1, FES, FIGN, FLJ32810-TMEM133, GOSR2, GUCY1A3-GUCY1B3, JAG1, MOV10, NOV, NPR3-c5orf23, PIK3CG, PLCE1, SLC39A8, SLC4A7, NPR3,* ***CYP17A1****, CACNB2,* ***PLEKHA7****, SH2B3, TBX3-TBX5, ULK4, GNAS-EDN3,* ***MECOM (MDS1 locus)****, FGF5,* ***MTHFR****,* ***NPPA****,* ***NPPB****,* ***ATP2B1****,* ***CSK****,* ***ZNF652****,* ***HFE***
KARE	***ATP2B1***
KORA S3	*CCNG1*
Suita study	*CCBE1*
WGHS	*BLK-GATA4, CASZ1*
Study reference not mentioned in article	*ADD1, ADD2, ADRB1, ADRB2, APOB, CACNA1A, CACNA1C, CLCNKB, CYBA, CYP11B2, CYP2C8, EDN1, EDNRA, GNB3, SCNN1A, SCNN1B, SCNN1G, SGK1, KCNJ1, ACE, ADRB2, AGT, APLNR, BDKRB2, CAPN13, CYP11B2, CYP19A, GNB3, MMP3*

Bolded genes are ones are found in multiple cohorts. The genes are identified and listed according to their respective cohorts, with a separate category to identify genes without specific references in any of the articles reviewed. Specific locations for the genes are provided where possible. Novel genes are identified, as are genes associated with physical properties.

basis for EH. Additionally, reviews were eliminated if the articles were inaccessible or the reviews focused on animal models. Genome-wide linkage studies were also exxcluded, as there was no consistency in the results for genetic markers associated with EH. Also, articles were excluded if their abstracts were unavailable. Lastly, articles were excluded if there was no access available through the library at the University of Hawaii medical school.

RESULTS

Genetic marker studies

A total of 11 genetic marker studies (genome-wide association studies) are found to contain relevant information with regards to gene associations with EH. Many of the studies identify genes within cohorts, and there are some genes identified in multiple cohorts. These can be found from references[12-21], identified in Table 1. Furthermore, some of the genes have specific phenotypic effects, or associate with other genes and/or proteins related to EH. Some of the genes found have no known function, or the authors do not list the function. These can be found in references[12-21], identified in Table 2. Genes listed with hyphens include all of the genes found inclusive of, and between, the genetic range listed.

Table 1 demonstrates the numerous amount of genes found to affect populations greater than 1000 individuals. There are several cohorts identified, each with multiple genes that are associated with EH. Also, there are some genes that are repeated in different cohorts, indicating that different populations have some genes in common with respect to EH.

Tables 1 and 2 contain the meta-analysis of two large studies with European subjects, Cohorts for Heart and Aging Research in Genetic Epidemiology Consortium and GlobalBPGen[12], which reveal fourteen loci that reached genome-wide significance. These are thought to account for 1.5% of the observed variance in blood pressure[12]. Many of the related genes have now been matched to physiologic functions (see "Known Pathway", rows 1-6) that play a role in blood pressure (BP) regulation. Further studies were done on subjects of non-European descent, including African American, Japanese, Korean, and Han Chinese populations, which are listed as "Non-European Genes". Table 2 specifically identifies the genes with known pathways related to EH regulation. Table 2 lists genes without a current known pathway to explain their influence on EH regulation.

Epigenetics and EH

Tables 3 and 4 identify many correlations between DNA and histone modifications, as well as miRNA-gene interactions and their effect on EH. Many of the genes identified were also identified through GWAS, indicating a possible mechanism for how the identified genes affect EH. It is important to note that the authors found no articles that discussed siRNA and its association with EH after conducting the literature search, thus the epigenetic section does not include siRNA.

DISCUSSION

To our knowledge, this is the first review to discuss both genetic and epigenetic factors associated with EH in one article. As one can see, many genetic factors are involved with EH. There are many genes from genetic marker studies that are found to have some association with EH, as seen in Table 2. Some genes do have known physiologic pathway associated with EH, however, many do not. Our literature review herein denotes 129 genes. Of note, genes/gene regions with multiple study references

Table 2 Genes with their identified physiological pathway and genes identified with their associated physiological functions related to essential hypertension

Genes	Pathway related to EH
NOS3	RAAS pathway[22]
SH2B3	Endothelial cell function[17]
AGT	Renal electrolyte balance[17]
NPPA	Control of extracellular fluid volume and electrolyte homeostasis[23]
NPPB	Involved in vasorelaxation and inhibition of renin and aldosterone[24]
NPR3	Involved with regulating blood volume and pressure, pulmonary hypertension, and cardiac function[25]
UMOD	Constitutive inhibitor of calcium crystallization in renal fluids[26]
CYP17A1	Involved with steroid/aldosterone synthesis. Enzyme dysfunction leads to increased levels of mineralocorticoid activating hormones[17]
ATP2B1	Codes for enzymes that have a critical role in intracellular calcium homeostasis[27]
CACNB2	Encodes for a subunit of a voltage-dependent calcium channel protein that is a member of the voltage-gated calcium channel superfamily[28]
SLC24A4	Encodes for a member of the potassium-dependent sodium/calcium exchanger protein family[29]
YWHAZ	Protein interacts with insulin receptor substrate 1 protein, suggesting a role in regulating insulin sensitivity[30]
ADAMTS-8	Enzyme encoded by the gene disrupts angiogenesis *in vivo*[31]
ADM	Protein encoded by gene may function as a hormone in circulation control[32]
c5 site between *SUB1* and *NPR3*	SNP associated with SBP NPR3 encodes natriuretic peptide receptor C/guanylate cyclase C for natriuretic peptide clearance[33-35] Also found relationship with DBP
CACANA1H	Codes for α1 subunit of voltage-dependent calcium channel for heart contractions and associated with SBP in African Americans[36]
ENPEP	Facilitates production of angiotensin Ⅱ in RAAS pathway and associated with SBP and DBP[33]
ADD1 and *ACE*	ADD1 codes for α-adducin protein that interacts with sodium channel of Na/K co-transporter and Na/K ATPase[37] Angiotensin converting enzyme produces angiotensin-converting enzyme which converts angiotensin Ⅰ to angiotensin Ⅱ in RAAS pathway[38]
ADD2	β-adducin is a cytoskeletal actin-binding protein implicated in glomerular lesions[39]
CYP11B2	Contributes to aldosterone synthesis in RAAS pathway[40]
AGT	Encodes angiotensinogen in RAAS pathway[41]
LOC344371 and *RASGRP3*	Activation decreases vascular responsiveness to endothelin-1 and angiotensin Ⅱ in rats[41]
EDN3	Endothelin-3 involved in vasoconstriction[42]
BCAT1	Associated with salt sensitivity[43]
CASZ1	Zinc-finger transcription factor that is associated with DBP[33]
ADRB2	Ion channel involved with regulation of vasoconstriction[12]
CYP11B2	Enzymatic defects results in decreased aldosterone and increased salt-wasting[12,17]
MMP3	Gene variants affect arterial stiffness and endothelial function[44]
NR3C2	Involved with aldosterone signaling[12]
SCNN1B	C terminus deletion leads to reduced ENaC clearance and increased ENaC activity[12]
APLNR	Mediator of cardiovascular disease[45]
BDKRB2	Involved in catecholamine synthesis[46]
MTHFS	Involved with catecholamine binding[47]
SOX6	Required in transcription for maintenance of cardiac and skeletal muscle cells[17]
CACNA1A	Involved with regulating SBP[48]
CCNG1	Involved with regulation of SBP and DBP and is component of regulating hypertension[15]
CPLX3	Involved with regulating DBP[15]
CSK	Cytoplasmic tyrosine kinase involved with angiotensin Ⅱ-dependent vascular smooth muscle cell contraction[17]
CACNA1C	Regulates calcium influx after depolarization[49]
CLCNKB	Involved in renal salt absorption[50]
EDN1	Endothelin-1 involved in vasoconstriction[51]
EDNRA	Endothelin receptor type A involved in vasoconstriction[52]
KCNJ1	Potassium channel involved with potassium homeostasis[53]
SCNN1A	Involved with renal sodium regulation[54]
SCNN1B	Involved with renal sodium regulation[55]
SCNN1G	Involved with renal sodium regulation[56]
SGK1	Activation of certain potassium, sodium and chloride channels, playing a role in cellular stress response[57]
SLC12A1	Cotransporter involved in sodium and chloride reabsorption in the distal convoluted tubule[58]
SLC12A3	Cotransporter involved in sodium and chloride reabsorption in the loop of Henle[59]
TNNT3	Involved in calcium-induced muscle contraction[60]
WNK1	Kinase involved with sodium and chloride transport[61]
WNK4	Kinase regulates balance between sodium chloride and potassium reabsorption in kidneys[62]
GOSR2	Interacts with target-localized SNAREs, allowing angiotensinogen to move between Golgi compartments, possibly leading to vasoconstriction[63]
GUCY1B3	Receptor for nitric oxide involved with vasodilation[64]
ATXN2	Possible association with regulation of GFR[65]
SLC4A7	Possible transporter of sodium and bicarbonate ions[66]
CDH13	Regulates endothelial cell growth[67]
Identifier information	Gene
Non-European genes	*NPR3*, *IPO7*, *MYLIP*, *PMS1*, *SLC24A4*, *TBX3*, *YWHAZ*, *FIGN-GRB14*, *ALDH2*, c5 site between *SUB1* and *NPR3*, *CACANA1H*, *SNP* upstream of *CCBE1*, *ENPEP*, *ST7L-CAPZA1*
Gene-gene interaction	*ADD1* and *ACE*, *ADD1* and *ADD2*, *ADD1* and *CYP11B2*, *AGT* and *ACE*, *c20q12*, *IMPG1*, *LOC344371* and *RASGRP3*, *PCDH15*, *NPR3-c5orf23*, *CSK-ULK3*, *BAT2-BAT5*, *BLK-GATA4*, *GNAS-EDN3*
Gene-environment interaction	Body Mass Index: *ADD1*, *ADRB2*, *CAPN13*, *CYP11B2*, *CYP19A1*, *MMP3* Black, Male: *AGT* Level of physical activity: *GNB3*, *NR3C2*, *SCNN1B*, *APLNR*, *BDKRB2* Oral contraceptive use: *COL25A1* Preterm birth: *MTHFS*
Unknown function/ function could not be determined	*GNAS-EDN3*, *NPR3-c5orf23*, *BLK-GATA4*, *ST7L-CAPZA1*, *CSK-ULK3*, *FIGN-GRB14*, *c10orf107*, *c21orf91*, *LSP1-TNNT3*, *GNAS-EDN3*, *BAT2*, *IPO7*, *MYLIP*, *PMS1*, *TBX3*, *TBX4*, *TBX5*, *ANKMY*, *BAT2*, *BAT3*, *BAT4*, *BAT5*, *ALDH2*, SNP upstream of *CCBE1*, *BCAT1*, *PCDH15*, *c20q12*, *IMPG1*, *CAPN13*, *CYP19A1*, *GNB3*, *COL25A1*, *PCDH15*, *IMPG1*, c5 site between *SUB1* and *NPR3*, *CHIC2-PDGRA1*, *APOB*, *HFE*, *CYPBA*, *CYP1A2*, *CYP2C8*, *EBF1*, *FES*, *FGF5*, *FIGN*, *FLJ32810*, *GNB3*, *LSP1*, *NOS3*, *TMEM133*, *FOXD3*, *GPR98*, *ARRDC3*, *GUCY1A3*, *JAG1*, *MECOM* (MD1 locus), *MOV10*, *NOV*, *NPR3-c5orf23*, *NT5C2-CYP171A*, *PIK3CG*, *PLCD3*, *PLCE1*, *PLEKHA7*, *RPL6-PTPN11-ALDH2*, *SLC39A8*, *ULK4*, *ZNF652*, *CCL20*, *WDR69*, *TGFBR2*, *STK39*

Only genes with pathways related to EH were identified. Genes identified with their associated physiological functions associated with EH. If there were genes that coded for proteins, but these proteins were not found to affect EH, then it was listed as unknown function or the function could not be determined. Genes with hyphens indicate genome wide association studies associated genomic regionsin, in which the genetic pathway could not be determined and properly evaluated for its involvement with EH. EH: Essential hypertension; RAAS: Renin-angiotensin-aldosterone system; SBP: Stolic blood pressure; DBP: Diastolic blood pressure.

Table 3 DNA methylation and histone modification associated with essential hypertension

Ref.	Study	Subjects	Results	Site of modification and type
Smolarek *et al*[68]		Humans	5-mC significantly higher in healthy subjects than entire group of patients with EH	N/A
Wang *et al*[69]		Humans	Increased methylation levels observed at 2-CpG sites in comparison with normotensive controls	*SULF1*: Methylation
Liang *et al*[70]		Humans	Regulation of renal sodium reabsorption β-2 adrenergic stimulation → inhibition of histone deacetylase-8 in kidney → increased histone acetylation and decreased genetic transcription of WNK4 caused increased blood pressure 11β-hydroxysteroid dehydrogenase type 2–converts active glucocorticoids to inactive glucocorticoids Promoter methylation of *HSD11B2* gene decreased expression of renal 11β-hydroxysteroid dehydrogenase type 2 affects regulation of volume and BP homeostasis ENaCα-epithelial sodium channel–affects Na+ reabsorption in the distal nephron Proposed mechanism: Methylation of Lys79 of histone H3 suppresses ENaCα transcription ACE1-Angiotensin-converting enzyme ACE1-up-regulated in association with increased binding of histone 3 acetylation (H3Ac) and 4th lysine trimethylation (H3K4me3) and in association with decreased binding of histone ninth lysine residue demethylation (H3K9me2)	*WNK4*: Decreased transcription and increased histone acetylation *HSD11B2*: Promoter methylation ENaCα: Methylation of Lys79 of histone H3 *H3K4me3*: Histone 3 acetylation and 4th lysine trimethylation. *H3K9me2*: Decreased binding of histone 9th lysine residue demethylation
Udali *et al*[71]	Friso *et al*[72]	Humans	11β-hydroxysteroid dehydrogenase 2 methylation at HSD11B2 promoter in DNA of PBMCs of hypertensive patients inversely related to enzyme function Promoter methylation of HSD11B2 gene plays a role in HTN	*HSD11B2*: Methylation in promoter region
	Lee *et al*[73]	Rats	Na^+-K^+-2 Cl^- cotransporter 1 (NKCC1) Methylation status of NKCC1 promoter–elevated in hearts of spontaneously hypertensive rats SHRs–significant hypomethylation of NKCC1 associated with increase in gene expression contributing to HTN	*NKCC1*: Methylation in promoter region *NKCC1*: Hypomethylation in promoter region
	Riviere *et al*[74]	Human endothelial cell lines and rats *in vivo*	Somatic angiotensin-converting enzyme (= ACE1) Promoter methylation levels: Higher levels of methylation associated with transcriptional repression Therefore hypomethylation of promoter region of sACE could contribute to HTN	*sACE*: Methylation in promoter region
Millis[75]		Human	Methyl CpG binding protein-2 (MECP-2) Methylates and thereby silences the expression of the norepinephrine transporter gene Phenyl-ethanolamine N-methyltransferase (PMNT)–converts Norepinephrine into Epinephrine Also mimics gene-silencing actions of MECP-2 Leads to increased synaptic levels of catecholamines (increased Epinephrine release and decreased Norepinephrine reuptake) CTGF Lysine methyltransferase that methylates the histone H3K79 site of nucleosomes that inhibits the expression of CTGF (in the cells of the collecting ducts)	*MECP-2*: Methylation *PMNT*: Methylation *H3K79*: Methylation of histone site of nucleosomes

As demonstrated in Table 3, many of the genes identified undergo methylation. If the reviews discuss results from individual studies, then the separate studies are placed in the second column. The results are listed based on the gene/site of modification, along with a description of what occurs as a result of the modification. The last column provides a summary of the gene/site of modification and the type of modification that occurs at that particular site. CTGF: Connective tissue growth factor.

include: *STK39*, *CYP17A1*, *MTHFR-NPPA*, *MTHFR-NPPB*, *ATP2B1*, *CSK*, *ZNF652*, *UMOD*, *CACNB2*, *PLEKHA7*, *SH2B3*, *TBX3-TBX5*, *ULK4*, *CSK-ULK3*, *CYP1A2*, *NT5C2-CYP171A*, *PLCD3*, *SH2B3-ATXN2*, *CACNB2*, *PLEKHA7*, *SH2B3*, *TBX3-TBX5*, *ULK4* and *HFE*. The following genes overlap between the genetic studies and epigenetic studies: *WNK4* and *BDKRB2*. While *WNK4* and *BDKRB2* are found in both genetic and epigenetic studies, it appears that *WNK4* (kinase regulates balance between sodium chloride and potassium reabsorption in kidneys), and *BDKRB2* (involved in catecholamine synthesis) may be associated with EH through interactions with miRNA.

Prior to GWAS, studies were somewhat successful in isolating genes associated with rare monogenic forms of hypertension that are inherited in a classic Mendelian fashion. The introduction of GWAS has made it possible to identify novel loci that could not be predicted physi-

Table 4 MiRNA targets associated with essential hypertension

Ref.	Subjects	Results	miRNA targets
Xu *et al*[76]	Human plasma	hcmv-miR-UL112; miR-605; miR-623; let-7e;miR-516b; miR-600; kshv-miR-K12-6-3p; miR-602; miR-1252 miR-296-5p; miR-133b; miR-30 d; miR-625*; miR-1236; miR-518b; miR-1227; miR-664; miR-615-5p; miR-18b*; miR-1249; miR-324-3p; ebv-miRBART17-3p; miR-634; ebvmiR-miRBART19-5p; miR-486-5p; kshvmiR-K12-10a; kshv-miR-K12-10b	INF-1 is direct target of hmcv-miR-UL112 Indicates link between CMV infection and EH
Batkai *et al*[77]	Human	Endothelial miRNA	
		miR-126	SPRED-1; PIK3 regulatory subunit-2; VCAM-1; CXCL12;
		miR-217	
		miR-122	RhoB
		miR-21	SirT1
		miR-24	SLC7A1
		miR-27b, -130a, -210, -378, -17–92, let-7f	Nitric oxide pathway Hypoxia-induced mechanism
		miR-15, -16, -20a, -20b, -24, -221, -222	Pro-angiogenic Anti-angiogenic
		Renal miRNA	
		miR-29b	Fibrotic pathway; collagen genes; *Mmp2*; *Itgb1*
		miR-200a, miR-200b,	
		miR-141, miR-429, miR-205, miR-192	Biomarkers of nephrosclerosis
		miRNA targeting RAAS	
		miR-155	*AGTR1*
		miR-526b and -578	*AVPR1A*
		miR-34a, and -34c	*BDKRB2*
		miR-765	*TBXA2R*
		miR-383	*NR3C2*
		miR-9	*NFATc3*
		miR-124 and miR-135a	*NR3C2*
		miRNA targeting smooth muscle cells	
		miR-143 and miR-145	Actin stress fibers;
		miR-21	ACE; KLF5;
		miR-21, -26b, -98, and -1826	myocardin; MRTF-B; calmodulin kinase II-δ
		miR-221 and -222	PTEN; Bcl-2; cGMP signaling
		miRNA in other etiologic factors	Nitric oxide and ANP pathway
			p27(Kip1), p57(Kip2) and/or c-kit
		miR-296-5p, let-7e, hcmv-miR-UL112	Association with hypertension
		hcmv-miR-UL1	IRF-1
		miR-637	*ATP6V0A1*, chromaffin granule function
Fung *et al*[78]	Human	miR-155	Suppress expression of *AGTR1*

Table 4 demonstrates how miRNAs affect different aspects of blood pressure regulation. Also, there appears to be a link between cytomegalovirus (CMV) infections and essential hypertension; miRNA has been identified as a possible mediator of this connection. The asterisk identified for some miRNAs[78] are not defined in the original article, but are assumed to be a part of the proper notation for that miRNA. EH: Essential hypertension.

ologically, using non-family cohorts.

This review shows that no Mendelian variants or epigenetic factors are consistently associated with EH in the large cohort studies examined. Furthermore, it was not possible for the authors to correlate the epigenetic factors associated with the pathways identified, as there were no clear relationships between EH and the individual genes. Therefore, it can be inferred that EH follows multifactorial inheritance and insinuates that it follows the CDRV genetic hypothesis. In regards to identifying rare variants, GWAS is used for polymorphism detection, and is not set up to identify SNPs with low mean allele frequencies (MAFs) (low MAFs are usually under 1%, and sometimes even as high as 5%). Therefore, other techniques will need to be used to identify rare variants. Next-generation sequencing has revolutionized our ability to sequence thousands of genes at one time in a cost-effective manner. Using full exome or full genome sequencing of EH cohorts, next-generation sequencing will help to identify rare, as well as low-MAF, variants associated with regulating blood pressure[12]. This will likely show the exact genetic factors responsible for EH instead of mere associations which have been the mainstay of our genetic search using GWAS. Similar high throughput techniques will likely also improve our identification of epigenetic regulators.

Insufficient evidence was found in this study to pursue single site genetic marker or epigenetic testing to provide a simple genetic risk assessment for EH. Genetic algorithms comprised of information from multiple genes and epigenetic factors, along with family history and environmental variables, could potentially be developed to provide a genetic risk assessment for EH. However, it will be difficult to know what to do with this data, since preventative factors such as exercise and a healthy diet would be recommended to anyone at any level of personal and/or family history risk for EH. A similar concept was examined in a recent publication evaluating genetic testing and type 2 diabetes[79]. The evaluation of genomic applications in practice and prevention (EGAPP) consortium recommend against using genetic diabetic markers for risk assessments since it would be of limited benefit[79]. Additionally, for cardiovascular morbidity, current non-genetic algorithms already exist[80,81] that assess the risk of heart disease using a patient's medical profile.

Although risk assessments may be difficult, pharmacogenomic utility may be found by studying risk alleles in individuals and treating their HTN in a personalized manner based on the pathway affected to obtain optimal blood pressure control[13].

To our knowledge, this is the first review to discuss both genetic and epigenetic factors associated with EH in one article. Insufficient evidence was found in this review to pursue any one single genetic test to provide a genetic risk assessment for EH.

In conclusion, while there exist genetic and epigenetic associations that play a role in EH, there are still no well-established cause-and-effect relationships for the development of EH. With emerging technologies, such as next-generation sequencing, a more direct relationship may be established between genetic and epigenetic factors and EH. Extensive algorithms for EH will likely need to be developed to incorporate these genetic risk factors, in concert with a patient's personal risk factors. However, the utility of this approach will need to be proven. There is a large potential for personalized medicine through pharmacogenomics that will come from our better understanding of the genetic factors and pathways involved in EH.

ACKNOWLEDGMENTS

The authors would like to thank all of the patients who participated in the studies that were cited throughout this article. Without them, we would not be able to further our collective knowledge.

COMMENTS

Background

Essential hypertension (EH) is thought to be a multifactorial disease, meaning that environmental and genetic factors affect the initiation and continuation of the disease. While there have been several publications discussing the genetic factors involved with EH, to date there has been no single publication that has discussed both genetic and epigenetic factors in one article.

Research frontiers

While EH is thought to be a multifactorial disease, several genetic factors have been associated with EH. In the area of genetic and epigenetic factors associated with EH, their remains a need to review the most updated information regarding genetic and epigenetic factors and discuss both in one article.

Innovations and breakthroughs

Previously, scientists would have to refer to Genome-wide association studies and epigenetic studies to understand how genetic and epigenetic factors are associated with EH. This is the first review article to discuss both genetic and epigenetic factors in one article. Also, this article discusses the most current up-to-date literature, providing a more recent understanding of genetic factors associated with EH.

Applications

Next-generation sequencing will allow scientists to analyze thousands of genes in a cost-effective manner. Using full exome or full genome sequencing of EH cohorts, next-generation sequencing will help to identify rare, as well as, low-mean allele frequency variants associated with regulating blood pressure. This will be useful in the growing field of pharmacogenomics, where medical regimens are being tailored to individuals based on specific genetic polymorphisms. This will help to personalize treatment regimens and improve the care given to patients with EH.

Terminology

Essential hypertension is a form of hypertension that has no known cause, but is responsible for most cases of hypertension. Genome-wide association studies look at the whole genome of populations of individuals who suffer from a specific condition to see if these individuals have any genes that differ from the general population without the condition in question. Pharmacogenomics is an emerging field where scientists and doctors use someone's genetic code to determine appropriate doses for medications to ensure fewer side effects and the best possible therapy.

Peer review

The present study appears well conducted for design and contents. Inclusion criteria and exclusion criteria are reasonable.

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SYSTEMATIC REVIEW

Heart and lung, a dangerous liaison-Tako-tsubo cardiomyopathy and respiratory diseases: A systematic review

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Abstract

AIM: To investigate the possible association between Tako-tsubo cardiomyopathy (TTC)-a reversible clinical condition mimicking an acute myocardial infarction characterized by multifactorial pathophysiologic mechanisms- and respiratory system diseases.

METHODS: We systematically searched PubMed and EMBASE medical information sources, to identify the different triggering causes, limiting our search to articles in English. The search keywords were: "tako-tsubo cardiomyopathy", "takotsubo", "takotsubo cardiomyopathy", "broken heart syndrome", "stress-induced cardiomyopathy", "apical ballooning syndrome", and "ampulla cardiomyopathy in combination with respiratory diseases, lung, pulmonary disease. For each kind of disease, we registered: author, year and country of study, patient sex, age, concurring situation, and outcome.

RESULTS: Out of a total of 1725 articles found, we selected 37 papers reporting a total of 38 patients. As expected, most patients were women (81.6%), mean age was 65 ± 10 years. Outcome was favorable in 100% of cases, and all the patients have been discharged uneventfully in a few days.

CONCLUSION: An association between respiratory diseases and TTC is likely to exist. Patients with severe respiratory diseases, due to the high dosages of β2-agonists used or to the need of invasive procedures, are highly exposed to the risk of developing TTC.

Key words: Tako-tsubo cardiomyopathy; Stress cardiomyopathy; Respiratory diseases; Lung; Chronic obstructive pulmonary disease; Asthma

Core tip: This is the first study evaluating the association between respiratory diseases and Tako-Tsubo cardiomyopathy (TTC). Patients with severe respiratory diseases, due to the high dosages of β2-agonists used or to the need of invasive procedures, are highly ex-

posed to the risk of developing TTC. Thus, in these patients a certain caution should maintained, along with a special alertness in suspecting and recognizing this particular disease.

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INTRODUCTION

Tako-tsubo cardiomyopathy (broken heart syndrome)

Tako-Tsubo cardiomyopathy (TTC) is a reversible clinical condition mimicking an acute myocardial infarction (AMI)[1]. The original Japanese term "tako-tsubo" indicates the particular shape of the end-systolic left ventricle in ventriculography resembling that of the round-bottom and narrow-neck pot used for trapping octopuses[2]. Other terms have been used to define this cardiac entity, *i.e.*, "apical ballooning", "acute stress cardiomyopathy" or "broken heart". Typical presentation involves chest pain and/or dyspnea, transient ST-segment elevation on the electrocardiogram (ECG), and a modest increase in cardiac troponin[3].

The Mayo clinic diagnostic criteria include: (1) transient hypokinesis, akinesis or dyskinesis in the left ventricular mid segments with or without apical involvement; regional wall motion abnormalities that extend beyond a single epicardial vascular distribution; and, frequently but not always, a stressful trigger; (2) absence of obstructive coronary disease or angiographic evidence of acute plaque rupture; (3) new ECG abnormalities (ST-segment elevation and/or T-wave inversion) or modest elevation in cardiac troponin and, and (4) absence of myocarditis or pheochromocytoma[1].

Although TTC is still underdiagnosed, the current prevalence estimate is approximately 1% to 3% (even 6% to 9% in women) of all acute coronary syndromes[4]. The mean age ranges from around 60 to 75 years, both in men and women[5], but its occurrence is much more likely (approximately 90%) in postmenopausal women[6]. After a first finding on a large cohort of patients in Italy[7], a precise temporal periodicity has been reported, characterized by highest occurrence peaks during morning hours and summer months[8-9]. Interestingly, quite similar to AMI, Monday seems to be a critical day for onset[10].

Even if TTC is frequently characterized by dramatic clinical presentation and urgent presentation to the Emergency Department, the prognosis is generally favorable, with a rapid short-term improvement of left ventricle systolic function[11]. According to several studies, in-hospital mortality rates range from 0% to 8%[4], with higher mortality rates for males than females[12].

Multifactorial pathophysiologic mechanisms are likely to be involved, but the most accepted pathogenic hypothesis considers a rapid elevation of circulating catecholamine, triggered by emotional and/or physical stress, as a key mechanism[13-14]. In fact, the major determinants of sympathetically mediated myocardial reversible dysfunction in patients with TTC include all the direct effects of catecholamines upon the myocardium, i.e. cellular damage, contraction band necrosis, defects in perfusion, altered cellular metabolism, and negative inotropic effects of epinephrine *via* stimulation of the cardioprotective β_2-adrenergic receptors-G_i signaling pathway[15]. It has been recently shown that the apical ventricular region has a greater β_2:β_1 adrenoceptor ratio, with a higher responsiveness and vulnerability to sympathetic stimulation[16]. Again, the different occurrence of wall motion abnormalities could be explained by interindividual anatomical differences in the distribution of β-adrenergic receptors[17,18].

Broken heart and broken lung: Is there a relationship?

The clinical onset of TTC is usually preceded by an emotional and/or physical stress with a similar distribution in approximately two-thirds of the patients. A long list of stressors has been reported, and this is continuously updated. Men seem to be more prone to physical stress and women to emotional stress[19]. Among emotional stressors, for example, death or severe illness of a family member, receiving bad news, financial loss, move to a new residence, natural disasters, dispute or litigation, car accident, assault, surprise party, public speaking, and so on[3]. Among physical stressors, surgery, cardiovascular procedures, medications and illicit drugs, and medical conditions, including gastroenterologic, endocrine, hematologic, renal, infectious, and neurologic diseases[3]. Thus, we aimed to more-in-depth investigate the relationships between TTC and respiratory diseases.

MATERIALS AND METHODS

We systematically searched PubMed and EMBASE medical information sources, to identify the different triggering causes, limiting our search to articles in English. The search keywords were: "TTC", "takotsubo", "takotsubo cardiomyopathy", "broken heart syndrome", "stress-induced cardiomyopathy", "apical ballooning syndrome" and "ampulla cardiomyopathy in combination with respiratory diseases, lung, pulmonary disease. Further papers were sought by means of manual search of secondary sources, including references from primary articles. For each kind of disease, we collected a set of data, including author, year of publication, country where the study was performed, and patient sex, age, concurring situation, and outcome.

RESULTS

Out of a total of 1725 articles found (1341 with the precise MeSH term (Takotsubo cardiomyopathy), we se-

Table 1 Respiratory symptoms or diseases and Tako-tsubo cardiomyopathy: Synopsis of published case reports

Symptom/disease	Gender, age (yr)	Concurring condition	TTC outcome	Country	Ref.
COPD					
Dyspnea	Female, 57	COPD Unexpected death of her son	Favorable	United States	Pezzo *et al*[26]
Dyspnea	Female, 51	COPD, Hypothyroidism Financial problems	Favorable	Poland	Bilan *et al*[27]
Status asthmaticus	Female, 66	COPD with multiple hospitalizations, heavy smoker	Favorable	United States	Rennyson *et al*[28]
Exacerbation	Female, 63	Multiple admissions	Favorable	United States	Makam *et al*[24]
Exacerbation	Male, 52	Financial unavailability to buy his drugs	Favorable	Spain	Pham *et al*[29]
Exacerbation	Female, 62	(1) COPD exacerbation (2) Family dispute (3) Acute thrombosis of aortobifemoral prosthesis	Favorable	Germany	Sager *et al*[30]
Exacerbation	Female, 68	COPD β2 agonist abuse	Favorable	Brazil	Salemi *et al*[21]
Exacerbation	Female, 63 Male, 59	Severe longstanding COPD, heavy smoker Ex-smoker, COPD Salbutamol abuse	Favorable	New Zealand	White *et al*[22]
Exacerbation	Female, 76	COPD β2 agonist abuse	Favorable	United States	Mendoza *et al*[23]
Exacerbation	Female, 63	multiple exacerbations with noninvasive ventilation	Favorable	United States	Laktikova *et al*[25]
Asthma					
Bronchial asthma	Female, 74	Jet lag, 3 cups of coffee, 1-h sauna	Favorable	Taiwan	Chang *et al*[37]
Allergic rhinitis	Female, 84	Nasal decongestant abuse	Favorable	Brazil	Wang *et al*[34]
Status asthmaticus	Female, 46	Ketamine + epinephrine administration	Favorable	United States	Osuorji *et al*[33]
Bronchial asthma	Male, 72	Acute asthmatic attack	Favorable	Italy	Pontillo *et al*[31]
Suspected intractable bronchial asthma	Female, 62	Relapsing polichondritis	Favorable	Japan	Sato *et al*[35]
Allergic asthma	Male, 70	Allergy Cephalosporin use	Favorable	Italy	Santoro *et al*[36]
Asthma	Male, 53	Cocaine	Favorable	United States	Sarkar *et al*[38]
Status asthmaticus	Male, 50	b2 agonist abuse	Favorable	United States	Salahuddin *et al*[32]
Pulmonary embolism					
Pulmonary embolism	Female, 79	Long distance travel Popliteal vein thrombosis	Favorable	United States	Challa *et al*[42]
Pulmonary embolism	Female, 65	Pyelonephritis Emotional stress	Favorable	Italy	Fedele *et al*[41]
Malignancies. invasive procedures/surgery					
Cardiopulmonary bypass	Female	Mitral valve plasty	Favorable	Japan	Itoh *et al*[48]
Rigid bronchoscopy for debridment	Male, 77	Esophageal carcinoma + central airways invasion	Favorable	United States	Guerrero *et al*[50]
Intubation	Female, age not given	Parathyroid surgery (canceled)	Favorable	United States	Mueller *et al*[49]
Bronchoalveolar lavage	Male, 68	Fever and cough productive of sputum, history of tuberculosis	Favorable	South Korea	Ok *et al*[51]
Lung transplantation	Female, 55	End-stage lung fibrosis	Favorable	France	Michel-Cherqui *et al*[47]
Squamous carcinoma	Male, 51	Pulmonary resection	Favorable	South Korea	Lee *et al*[44]
Non-small cell lung cancer	Male, 72	Pulmonary resection	Favorable	Japan	Toyooka *et al*[45]
Lung adenocarcinoma	Male, 59	Heavy smoker, first diagnosis of malignancy with multiple metastases	Favorable	Turkey	Kepez *et al*[46]
Miscellaneous					
Cough	Female, 82	Bad coughing "pill went down the wrong way"	Favorable	United States	Butman *et al*[59]
Dyspnea	Female, 51	Diving (examination)	Favorable	France	Chenaitia *et al*[58]
S. pneumoniae pneumonia	Female, 65	Sepsis	Favorable	Australia	Geng *et al*[55]
Pulmonary edema	Female, 73	Frightening episode	Favorable	Northern Ireland	Daly *et al*[56]
Pulmonary edema	Female, 59	Motor-vehicle collision	Favorable	United States	Ritchie *et al*[57]
Pneumothorax	Female, 64	COPD	Favorable	United States	Kumar *et al*[52]
Pulmonary hypertension	Female, 69	Initiation of intravenous trepostinil	Favorable	United States	Cork *et al*[54]
Pulmonary hypertension	Female, 81	Right ventricular involvement	Favorable	Italy	Citro *et al*[53]
Smoking and "Venus"	Male, 81	Adulterous sexual intercourse	Favorable	Italy	Brunetti *et al*[60]

COPD: Chronic obstructive pulmonary disease. TTC: Tako-Tsubo cardiomyopathy.

lected 37 papers reporting a total of 38 patients (Table 1). As expected, most patients were women (n = 31, 81.6%),

mean age was 65 ± 10 years. Outcome was favorable in 100% of cases, and all the patients have been discharged uneventfully in a few days. As for country of origin, 15 studies (40.5%) were conducted in the United States, 5 (13.5%) in Italy, 3 (8.1%) in Japan, 2 (5.4%) each in Brazil, Korea and France, and one (2.7%) each in Poland, Spain, Northern Ireland, Germany, Turkey, Australia, Taiwan, and New Zealand.

Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD)is relatively frequently associated with TTC. In a retrospective analysis of a large cohort of approximately 17000 patients with diagnostic angiographies in Hamburg, Germany, Hertting *et al*[20], out of the 32 cases of TTC found that 14 (44%) had COPD or asthma. Since 72% of these patients were taking β-mimetics, the authors postulated that this kind of medication could have acted as preconditioning factor for the cardiomyopathy or aggravated the sympathetic nervous system stress. In fact, several other cases of TTC in patients with β_2-stimulators abuse have been reported[21-23]. Multiple admissions for COPD exacerbations may act as a trigger[24-25], alone or in combination with emotional stressors, *i.e.*, unexpected death of a son[26], severe financial problems[27-29], or family dispute[30].

Asthma

Similarly to COPD, acute asthmatic attack may trigger TTC[31], and pharmacological treatments may potentiate such an effect[32-33]. Abuse of nasal decongestants in the course of allergic rhinitis has also been reported[34]. TTC episodes have also been described in the case of relapsing polychondritis with symptoms of intractable bronchial asthma[35], allergic asthma secondary to cephalosporin use[36], abuse of coffee to cope with jet lag[37], and also concomitant abuse of cocaine[38]. In the latter case, the TTC cardiotoxic effect could have been potentiated by catecholamines[39].

Pulmonary embolism

Arterial systemic embolization represents frequent complication during TTC. Mitsuma *et al*[40] studied the clinical characteristics and complications of 21 consecutive patients with TTC in Japan. Thromboembolism was found in 3 patients, 1 with ventricular thrombus and 2 with cardioembolic stroke. However, cases of pulmonary thromboembolism have been reported in elderly women as a consequence of acute pyelonephritis[41], and a popliteal vein thrombosis after a long distance travel[42].

Malignancies, invasive procedures and surgery

On the one hand, an association of TTC with malignancies has been hypothesized, potentially as a result of paraneoplastic phenomena[43]. On the other, surgery and invasive procedures represent severe physical stressors capable to trigger TTC onset. Several cases of TTC events in patients with lung malignancies undergoing pulmonary resection have been reported[44-46], and also after lung transplantation for end-stage fibrosis[47] or cardiopulmonary bypass[48]. Again, other cases were associated with intubation[49], debridement of central airways neoplastic invasion with rigid bronchoscopy[50], and even after a simple bronchoalveolar lavage[51].

Miscellaneous

Several other diseases or condition have been shown to trigger TTC. Among these, pneumothorax[52], pulmonary hypertension[53] also after attempt at treatment[54], pneumonia with sepsis[55], and pulmonary edema secondary to stressful events[56,57]. A TTC episode occurred after acute dyspnea secondary to the stress of scuba diving in a 51-year-old woman (at the third immersion, as her level-3 diving examination), has been reported[58]. Finally, 2 singular episodes of dyspnea occurred in ultraoctuagenarians, both of them triggering a TTC episode: A bad coughing since "pill went down the wrong way" in a 82-year-old lady[59], and a sudden dyspnea occurred in a 81-year-old man during an adulterous sexual intercourse with a young lady[60].

DISCUSSION

If the question is: "Does an association between respiratory diseases and TTC exist" the answer is yes. On the one hand, patients with severe respiratory diseases, such as asthma or COPD, are exposed to a high risk of developing TTC in the course of critical exacerbations, when they are also compelled to assume high dosages of β_2-agonists. On the other hand, patients with lung cancer are often exposed to invasive procedures, both diagnostic and surgical, that may be relevant in predisposed subjects. Patients with acute respiratory symptoms or diseases should always be approached with caution in the event of invasive procedures or surgery, keeping in mind the possible acute cardiologic complications.

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COMMENTS

Background

Tako-Tsubo cardiomyopathy (TTC) is a reversible clinical condition mimicking an acute myocardial infarction. Its onset is characterized by multifactorial pathophysiologic mechanisms, and stress may paly a crucial role.

Research frontiers

Patients with acute respiratory symptoms or diseases should be approached with caution in the event of invasive procedures or surgery, keeping in mind the possible acute cardiologic complications and the availability of managing abilities.

Innovations and breakthroughs

This is the first study evaluating the association between respiratory diseases and TTC.

Applications

More attention in either suspecting and recognizing TTC, and managing it.

Terminology

TTC is a reversible clinical condition mimicking an acute myocardial infarction. The original Japanese term "tako-tsubo" indicates the particular shape of the end-systolic left ventricle in ventriculography resembling that of the round-bottom and narrow-neck pot used for trapping octopuses.

Peer review

The authors have reviewed the association between respiratory diseases and Tako-Tsubo cardiomyopathy by searching case reports in world wide. They initially described general features of TTC, then discussed about relationship between TTC and respiratory disorders, including chronic obstructive pulmonary disease, asthma, pulmonary embolism, malignancies, invasive procedures, and miscellaneous. This article is well searched and summarized, and may provoke attention of TTC not only to cardiologists but also to pulmonologists and anesthesiologists.

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CASE REPORT

Long-lasting symptoms and diagnostics in a patient with unrecognized right sided heart failure: Why listening to the heart is so important

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Abstract

M Ebstein is usually diagnosed in early childhood or adolescence. The young woman in our case complainted of fatigue and shortness of breath, on physical examination she showed signs of heart failure with raised central venous pressure, palpable liver, an a cardiac murmer. Based on these findings she should have been referred to a cardiologist in an early stage after which transthoracic echocardiography resulted in the correct diagnosis. In this case the anomaly was missed for many years by different specialists and the patient was treated for liver disease, while she was suffering from liver congestion due to right-sided heart failure.

Key words: Ebstein; Congenital cardiology; Auscultation; Pregnancy; Liver

Core tip: The young woman in our case complainted of fatigue and shortness of breath, on physical examination she showed signs of heart failure with raised central venous pressure, palpable liver, an a cardiac murmer. Based on these findings she should have been referred to a cardiologist in an early stage after which transthoracic echocardiography resulted in the correct diagnosis.

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INTRODUCTION

The anamnesis and physical examination have an essential role in the work-up of patients. The current case shows clearly that with thorough examination of the patients'story and physical examination, the majority of medical tests could have been prevented and the correct diagnosis been found earlier.

CASE REPORT

Our patient was born in 1978 after an uncomplicated pregnancy. In childhood, she was seen by a paediatrician because of recurrent tonsillitis and iron deficient anaemia and at the age of seven she experienced an episode of jaundice with urobilinogen in her urine with no clear explanation at that time.

At the age of 13, she complained of headaches and fatigue which were correlated to sinusitis. For pain relief she took paracetamol (acetaminophen) and ibuprofen in high dosages. At age 22, she was analysed at the department of internal medicine because of jaundice. She still complained of fatigue and anorexia now. Thorough

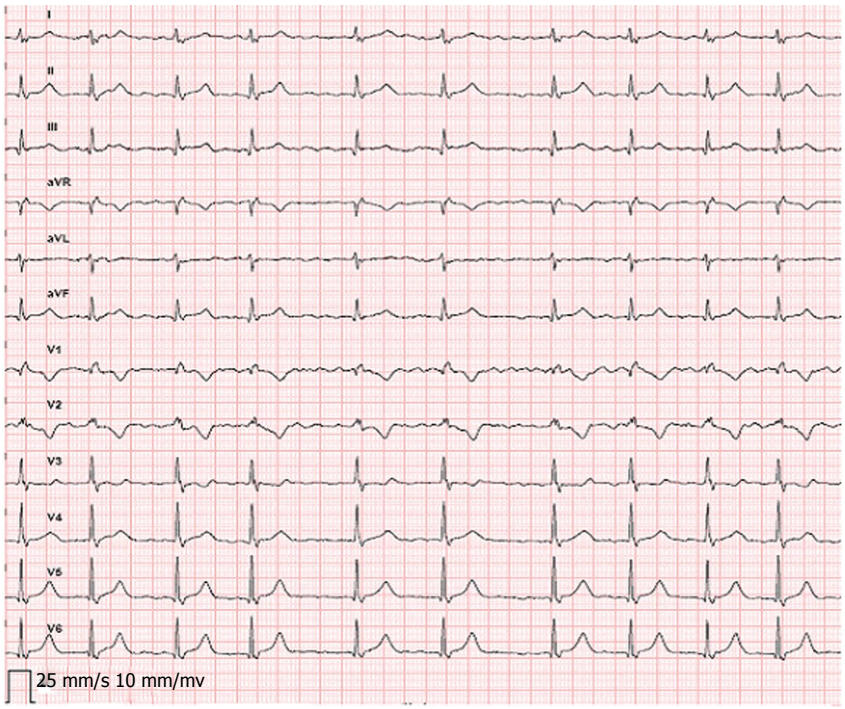

Figure 1 Electrocardiography showing atrial fibrillation, ventricular response 80 beats/min, intermediate axis, right bundle branch block.

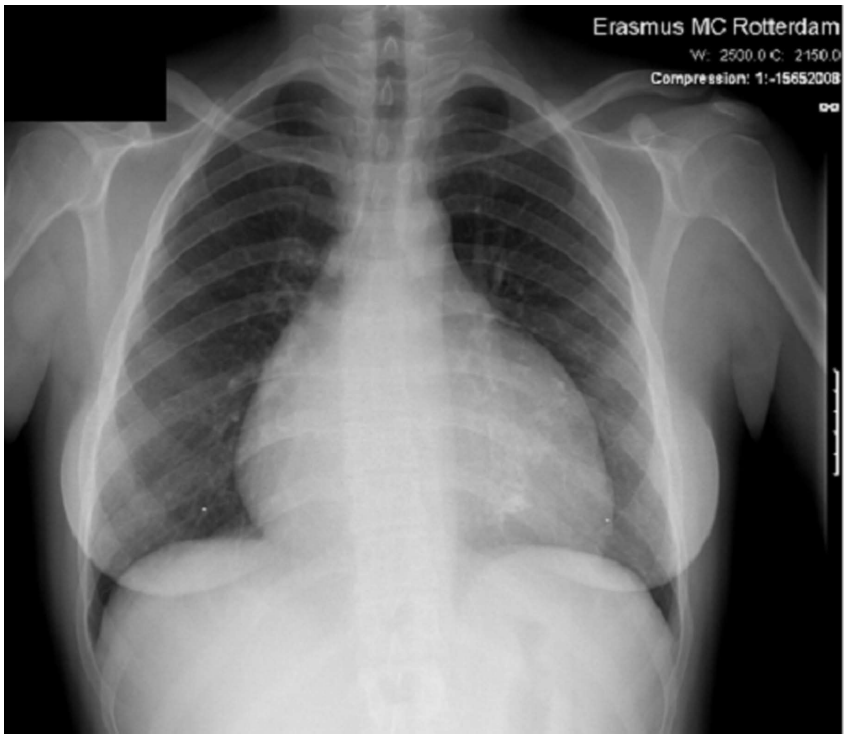

Figure 2 Chest X-ray showing marked cardiomegaly caused by right atrium enlargement, cor/thorax ratio 15.5/24.5.

screening revealed a solitary raised bilirubine, no serological evidence of viral infection, and no echographic abnormalities of the liver, gallbladder or biliary ducts. Two years later evaluation was repeated because of persisting elevated bilirubin levels. Due to headaches, she still used 3 g of paracetamol a day for years. The hyperbilirubinemia was now diagnosed as a toxic effect of this medication. After withdrawal of paracetamol, laboratory results did not improve and a liver biopsy was performed. This showed periportal fibrosis without cause. She was referred to the hepatologist for a second opinion and he found an irregular heart rate and referred her for cardiac consultation for the first time.

We saw a young woman with fatigue, shortness of breath and palpitations on minimal exercise. She could not work due to complaints. Additionally, several attempts to become pregnant were unsuccessful, which was severely stressful for her. Physical examination showed an icteric woman, irregular heart rate of 80 bpm with raised central venous pressure and 4 cm palpable liver. On cardiac auscultation a systolic murmur grade 2 out of 6 at the fourth intercostal space left and a soft diastolic

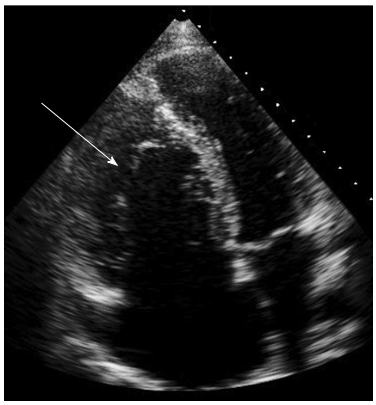

Figure 3 Echocardiography apical 4 chamber view showing the apically displaced tricuspid valve orifice, small right ventricle, large atrialized right ventricle and dilated right atrial.

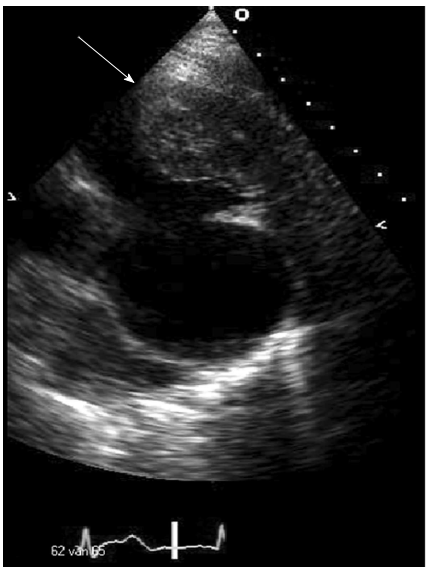

Figure 4 Echocardiography: Low right parasternal view visualizing a large mural thrombus in the large atrialized right ventricle and right atrial (arrow).

murmur were heard. The electrocardiography (Figure 1) revealed atrial fibrillation with slow ventricular response and a QRS-complex with right bundle branch block configuration. Chest X-ray showed cardiomegaly (Figure 2). Echocardiography revealed an enlarged right heart with apical displacement of the tricuspid valve (Figure 3). A large part of the right ventricle (RV) was atrialized and in the enlarged RA a large mural thrombus was found (Figure 4). The interatrial septum bulged out towards the compressed left atrium consistent with high RA pressures. Low velocity antegrade flow was found in the main pulmonary artery, in systole and diastole, indicating the RV was functioning as a conduit.

The diagnosis of the congenital defect, Morbus Ebstein, was made which was complicated by intracardiac thrombus. Anticoagulants were started and further analysis showed a perfusion defect in the right lung, consistent with recurrent small pulmonary emboli. After successful treatment of the cardiac thrombus, heart catheterisation was performed (pulmonary artery pressure 13/12; wedge 11 mmHg). As pulmonary hypertension was excluded, fortunately she could be referred for surgical correction. The tricuspid valve was repaired according to Chavaud, and a Carpentier ring was implanted. The atrialized RV was reduced and because of the small dimension of the remaining RV, a partial cavo-pulmonal shunt from superior caval vein to right pulmonary artery (Glenn shunt) was constructed. The post-operative course was uncomplicated and her condition improved considerably. Echocardiography post-operatively showed mild tricuspid-valve insufficiency. One year after surgery, she became pregnant gave birth to a healthy baby. Currently, seven years after surgery, our patient is doing reasonably well and is in NYHA class Ⅰ-Ⅱ.

DISCUSSION

Our patient experienced many complaints since childhood which could have been prevented if the correct diagnosis of a congenital heart disease was made earlier. Morbus Ebstein is a rare disorder with a prevalence of about 1 in 50000-200000 and was first described by Ebstein[1]. In 1866, he published post-mortem analyses of a nineteen-year-old man who presented with dyspnoea, palpitations, systolic murmur, cyanosis and eventually clinical features of heart failure. Obduction showed a deformation of the tricuspid valve with displacement of the effective tricuspid valve orifice towards the apex. Severity of this tricuspid anomaly can vary substantially and can be associated with other defects such as atrial septal defect or patent foramen ovale, which is present in 70%-80% of the patients[2]. Other associated defects are ventricular septal defects with or without pulmonary atresia, patent ductus arteriosus or aortic coarctation.

M Ebstein is usually diagnosed in early childhood or adolescence. In this case the anomaly was missed for many years by different specialists and the patient was treated for liver disease, while she was suffering from liver congestion due to right-sided heart failure. The frequent use of paracetamol had put the clinicians on the wrong trail in interpreting the elevated liver enzymes, also a liver biopt could have been prevented. All her symptoms, the fatigue, shortness of breath and her inability to become pregnant were due to her cardiac situation. The right tract was started after listening to the heart.

COMMENTS

Case characteristics

The young woman in this case complainted of fatigue and shortness of breath, on physical examination she showed signs of heart failure with raised central venous pressue, palpable liver, an a cardiac murmer.

Differential diagnosis

Initially the differential diagnosis of fatigue, jaundice and elevated liver enzymes is broad with primarily related to liver disease, medication effects but should incorporate a cardiac evaluation. With the progressive signs of right heart failure on physical examination, the suspicion of a cardiac diagnosis should have become stronger.

Laboratory diagnosis

The hyperbilirubinemia was now diagnosed as a toxic effect of this medication.

Imaging diagnosis

Echocardiography revealed an enlarged right heart with apical displacement of the tricuspid valv.

Treatment

The tricuspid valve was repaired according to Chavaud, and a Carpentier ring was implanted and the atrialized RV was reduced with a partial cavo-pulmonal shunt from superior caval vein to right pulmonary artery (Glenn shunt)

Term explanation

Ebstein is the name of the doctor who first described the anomaly.

Experiences and lessons

The current case underlines the importance of physical examination and maintaining a broad view to a patients'problem.

Peer review

The case report is well written and adresses common poblmes with diagnostics of Ebstein anomaly.

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CASE REPORT

Unreliability of aortic size index to predict risk of aortic dissection in a patient with Turner syndrome

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Abstract

Aortic size index (ASI) has been proposed as a reliable criterion to predict risk for aortic dissection in Turner syndrome with significant thresholds of 20-25 mm/m^2. We report a case of aortic arch dissection in a patient with Turner syndrome who, from the ASI thresholds proposed, was deemed to be at low risk of aortic dissection or rupture and was not eligible for prophylactic surgery. This case report strongly supports careful monitoring and surgical evaluation even when the ASI is < 20 mm/m^2 if other significant risk factors are present.

Key words: Aortic dissection; Aortic aneurysm; Turner syndrome

Core tip: Aortic size index (ASI) has been proposed as a reliable criterion to predict risk of aortic dissection in Turner syndrome. This case report emphasizes the need for careful monitoring and surgical evaluation of the patients even when the ASI is < 20 mm/m^2 if other significant risk factors are present.

Nijs J, Gelsomino S, Lucà F, Parise O, Maessen JG, La Meir M. Unreliability of aortic size index to predict risk of aortic dissection in a patient with Turner syndrome. *World J Cardiol* 2014; 6(5): 349-352 Available from: URL: http://www.wjgnet.com/1949-8462/full/v6/i5/349.htm DOI: http://dx.doi.org/10.4330/wjc.v6.i5.349

INTRODUCTION

Turner syndrome (TS) is a relatively common chromosomal disorder, caused by complete or partial X monosomy in some or all cells[1]. This abnormality is denoted medically as the 45,X karyotype as opposed to the usual 46,XX female karyotype. Many TS patients are actually mosaic, meaning that they have cells with more than one karyotype and occasionally there is mosaicism for cells containing Y chromosome material (Table 1)[2-4]. Short stature and gonadal dysgenesis are two of the characteristic clinical features of the syndrome, although many organ systems and tissues may also be affected to a lesser or greater extent. However, approximately 50% of karyotypically-proven, asymptomatic women with TS have evidence of abnormal cardiovascular development and most patients die from cardiovascular defects mainly involving the left ventricular outflow tract, left heart and/or aortic hypoplasia. Common congenital defects in surviving girls and adults with TS include bicuspid aortic valve (30%), aortic coarctation (12%) and partial anomalous pulmonary connection (18%)[5,6]. Nonetheless, the occurrence of aortic dilatation, dissection or rupture is one of major concerns in TS[7]. The annual incidence of aortic dissection or rupture is 15 cases/100000 for individuals < 20 years of age, 73-78 cases/100000 for women 20-40 years

Table 1 Chromosomal pattern based on karyotyping of women with Turner syndrome

Karyotype	Description	El-Mansoury *et al*[2] 2007 (*n* = 126)	Gravolt *et al*[3] 1996 (*n* = 304)	Hook *et al*[4] 1983 (*n* = 1043)
45,X	Monosomy X	48%	56%	58%
45,X/46,XX	Monosomy X mosaic with normal female sex chromosome complement	23%	17%	15%
46,X,i(Xq)	isochromosome X	13%	11%	15%
46,X,del(X)	deletion chromosome X	-	8%	6%
46,X,r(X)	ring chromosome X	3%	5%	2%
45,X/47,XXX	monosomy X mosaic with triple X chromosome complement	3%	3%	4%
45,X/46,XY	monosomy X mosaic with normal male sex chromosome complement	10%	-	-

old and 50/100000 for older women with TS[5].

Aortic root enlargement increases the risk of aortic dissection in TS although it is unclear whether such a life-threatening disease is always preceded by progressive dilatation as occurs in marfan syndrome (MS). However, despite connective disorders in which guidelines for monitoring of aortic root dimension and indications for surgical intervention are well established[8,9], reliable guidelines are lacking for TS and it is uncertain whether any cut-off value of aortic diameter can be used to identify Turner patients at high-risk of aortic dissection .

Furthermore, since body size is a major determinant of normal aortic dimensions, it may not be appropriate to apply standards derived from adult men to a syndrome more common in women and in which small body size is a main characteristic feature.

Aortic size index (ASI), which adjusts the aortic diameter to the body surface area[4], has been recently introduced as a reliable criterion to predict risk of aortic dissection in TS patients, but its usefulness in this clinical entity is still matter of debate.

We report a case of contained rupture of a dissected aortic arch in a patient with TS who, from the ASI thresholds proposed[9,10], was deemed to be at low risk of aortic dissection and was not eligible for prophylactic surgery.

CASE REPORT

A 23-year-old woman with TS (45,X karyotype), Graves-Basedow disease and systemic arterial hypertension treated with β-blockers, presented to our hospital facility

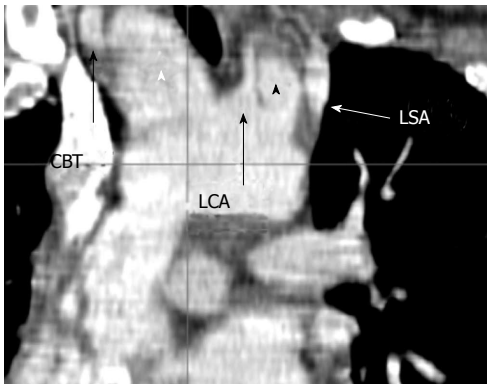

Figure 1 2D Coronal reformatted image. Digital multiplane reformatted image of the aortic arch, depicting the double barrel-shaped contained rupture of the aortic arch, in-between the common brachial trunk (CBT) and the left carotid artery (LCA) (white arrowhead) and LCA and the left subclavian artery (LSA) (black arrowhead).

because of fever unresponsive to antibiotics. She had experienced chest pain 1 mo previously which regressed spontaneously. She had no pain at hospital admission. Blood pressure was 110/82 mmHg.

The patient`s height and weight were 160 cm and 82 kg, respectively, with a body surface area of 1.85 m^2. TS was diagnosed at the age of 14 years after an evaluation for short stature and delay of pubertal development. Since then, the patient underwent yearly computed tomography (CT) which showed any aortic dilatation (the diameter of the ascending aorta at the latest scan before admission was 26 mm).

A CT scan at admission revealed a contained rupture of a dissected aortic arch with two false aneurysms between the common brachial trunk (CBT) and the left carotid artery (LCA), and between the LCA and left subclavian artery (LSA) (Figure 1). A peri-aortic hematoma (Figure 2) originating from the arch was present around the anterior aspect of the ascending aorta. The diameters of the aorta were =as follows: ascending aorta 26 mm, arch 30 mm and proximal descending aorta 19 mm. The ascending aortic size index was 14 mm/m^2. Echocardiography confirmed the diagnosis and revealed the presence of a bicuspid aortic valve and slight valve insufficiency.

A cardio-circulatory arrest with deep hypothermia was planned. After cannulation of the femoral vessels and the axillary artery trough a 10-mm graft (Vascutek, Terumo Ltd, Egham, United Kingdom) surgical access was gained through median sternotomy. The ascending aorta was resected and the arch inspected: a rupture was detected between the CBT and the LCA with the tear extending towards the LSA. Because of the hematoma, the CBT could not be encircled or clamped and antegrade cerebral perfusion was conducted *via* the LCA, until the CBT was reconstructed, after which selective antegrade cerebral perfusion *via* the axillary artery was added. Two 12-mm grafts (Gelsoft, Terumo Ltd, Egham, United Kingdom) were anastomosed to the LCA and LSA and a 14-mm graft (Gelsoft, Terumo Ltd, Egham, United Kingdom) was anastomosed to the CBT. A 28-mm graft

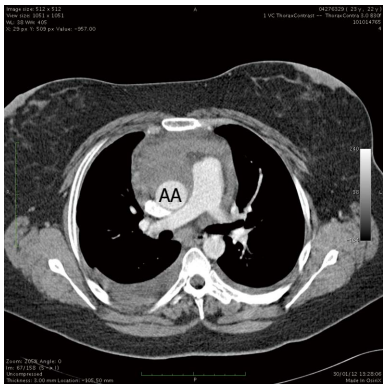

Figure 2 Axial plane computed tomography image of the ascending aorta. Axial image of the ascending aorta at the level of the pulmonary artery bifurcation. The ascending aorta is compressed in an oval shape due to the sub-adventitial spreading hematoma. AA: Ascending aorta.

(Gelweave Terumo Ltd, Egham United Kingdom) was anastomosed to the distal arch. Afterwards, the prosthesis was clamped and the distal body perfusion resumed through the femoral artery. The aortic valve was a "true" bicuspid valve with no raphe and 180° commissural orientation. The aortic root was normal and the effective height of the aortic valve was 9 mm. Therefore, there was no indication for valve and root replacement. After the proximal anastomosis was completed, the supra-aortic vessels were reimplanted on the ascending aorta prosthesis. Cardiopulmonary bypass time was 440 min, aortic cross-clamp time was 180 min and circulatory body arrest time was 20 min. The operation was routinely completed. After an uneventful course, the patient was transferred to the referring hospital on postoperative day 8. Pathologic examination of the aorta revealed very limited myxoid degeneration with no evidence of either fragmentation or separation of the elastic fibers.

DISCUSSION

In TS, it remains unclear whether aortic dissection is preceded by progressive dilatation as it is in connective disorders, and whether the thresholds employed in MS can be safely employed for TS. Nevertheless, a large proportion of these patients are small women and, for this reason, it is not correct to use standards derived from adult men in the general population and, for instance, an ascending aortic diameter even < 5 cm may represent, in these patients, a significant dilatation.

To overcome the body size issue, the ASI has been introduced which adjusts the aortic diameter to body surface area[10]. Davies *et al*[7] showed that patients with thoracic aortic aneurysms with ASI < 27.5 mm/m^2 are at low risk (approximately 4% per year), those with ASI between 27.5 and 42.4 mm/m^2 are at moderate risk (approximately 8% per year), and those above 42.5 mm/m^2 are at high risk (approximately 20% per year) of rupture, dissection, and death. Matura *et al*[8] employed this index in patients with TS demonstrating that subjects with ASI > 20 mm/m^2 require close cardiovascular surveillance and those with ASI ⩾ 25 mm/m^2 are at highest risk of aortic dissection.

We presented a case of a 23-year-old TS female with contained rupture of a dissected aortic arch. The ASI in our patient was 14 mm/m^2 at the level of the ascending aorta. Therefore, following current indications, there was no indication either for surgery or for close surveillance in this patient since the ASI was well below accepted thresholds. Hence, although recent studies[5] have confirmed that body surface area normalization is the most appropriate approach for determining aortic dilation in TS, in our experience ASI was unable to predict impending aortic dissection and rupture.

A recent study employing mathematical models of aortic disease in TS[11], showed that growth of the thoracic aorta is dynamic over time and risk factors such as aortic coarctation, bicuspid aortic valves, age, diastolic blood pressure, body surface area and antihypertensive treatment preferentially accelerated growth of the ascending aorta. Unfortunately, this model was not linked to aortic dissection and rupture. However other papers[3-5] report that bicuspid aortic valve, karyotype 45X, age 20-45 years, and hypertension are factors that confer an increased risk of dissection. All these features were present in the case reported therefore, in our opinion, when one or more of these factors are present, the risk of dissection should be taken into account even with ASI < 20 mm/m^2, and a close surveillance by a multidisciplinary team (cardiologists, radiologists, cardiac surgeons) should be recommended.

Although a CT scan with contrast is the most widely used diagnostic procedure, recent studies[12,13] have demonstrated that cardiac magnetic resonance imaging (CMRI) is an important tool for clinical care and it improves risk stratification of TS patients. Indeed, CMRI is outstanding for detection of the degree of aortic dilatation and coarctation that are not visible on echocardiography[14], but is limited by its high cost and poor tolerability due to claustrophobia and anxiety in some TS patients. Meanwhile, fast scan seeds, low radiation dose and increased anatomic coverage are improving the image quality of cardiac multidetector CT (MDCT) and reducing patient risks in children. Cardiac MDCT is also considered to effectively bridge the gaps among echocardiography and cardiac MRI in children with congenital heart disease. In addition, cardiac MDCT has better cost benefit compared with CMRI.

In conclusion, our experience emphasizes the need for careful monitoring and surgical evaluation of TS patients even when the ASI is small if other significant risk factors are present. Even though this is only a case report, it provides the idea and sounds the alarm that using only an ASI is not sufficient for risk stratification for aortic dissection in patients with TS.

Large prospective studies are needed for risk stratification for aortic dissection in TS in order to identify reliable thresholds to identify patients who may require referral for surgery before life-threatening complications occur.

ACKNOWLEDGMENTS

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COMMENTS

Case characteristics

A 23-year-old woman with Turner syndrome (TS).

Clinical diagnosis

Fever unresponsive to antibiotics, and chest pain.

Differential diagnosis

Other causes of chest pain, thoracic back pain.

Laboratory diagnosis

Blood, metabolic panel and liver function tests were within normal limits.

Imaging diagnosis

A computed tomography-scan at admission revealed a contained rupture of a dissected aortic arch with two false aneurysms between the common brachial trunk and the left carotid artery and the left carotid artery and the left subclavian artery, respectively. The diameters of the aorta were as follows: ascending aorta 26 mm, arch 30 mm and proximal descending aorta 19 mm.The ascending aortic size index was 14 mm/m^2.

Pathological diagnosis

Pathologic examination of the aorta revealed very limited myxoid degeneration with no evidence of either fragmentation or separation of the elastic fibers.

Treatment

The patient underwent aortic arch replacement and common brachial trunk, left carotid artery, and left subclavian artery replacement.

Related reports

Aortic root enlargement increases the risk of dissection in Turner syndrome but it is unclear whether aortic dissection is always preceded by progressive dilatation as occurs in Marfan syndrome. Nevertheless, a large proportion of these patients are small women and, for this reason, it is not correct to use standards derived from adult men in the general population and, for instance, an ascending aortic diameter even < 5 cm may represent, in these patients, a significant dilatation.

Term explanation

Aortic size index, which adjusts the aortic diameter to the body surface area, has been recently introduced as a reliable criterion to predict risk for aortic dissection in TS patients but its usefulness in this clinical entity is still a matter of debate.

Experiences and lessons

This case report emphasizes the need for careful monitoring and surgical evaluation of the patients even when the aortic size index is < 20 mm/m^2 if other significant risk factors are present.

Peer review

This is a potentially interesting case study that describes the limitation in using aortic size index to assess risk of aortic dissection in patients with Turner's syndrome.

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Acknowledgments

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