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FIELD OF VISION

Turning scar into muscle

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Abstract

After the demonstration that somatic cells could be reprogrammed to a pluripotent state, exciting new prospects were opened for the cardiac regeneration field. It did not take long for the development of strategies to convert somatic cells directly into cardiomyocytes. Despite the intrinsic difficulties of cell reprogramming, such as low efficiency, the therapeutic possibilities created by the ability to turn scar into muscle are enormous. Here, we discuss some of the major advances and strategies used in direct cardiac reprogramming and examine discrepancies and concerns that still need to be resolved in the field.

Key words: Direct reprogramming; Cardiomyocytes; Induced pluripotent stem cells; Cardiac regeneration; microRNAs

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INVITED COMMENTARY ON HOT ARTICLES

Every cardiologist's dream come true - to transform scar into muscle - seemed closer to becoming real when, in 2010, Ieda *et al*[1] at the Gladstone Institute in California published in the journal *Cell* about the direct conversion of fibroblasts derived from neonatal hearts and tail-tips of mice into cardiomyocyte-like cells using three transcription factors. The group followed the strategy devised by the seminal work of Takahashi *et al*[2], who demonstrated for the first time that somatic cells could be reprogrammed to a pluripotent state through the overexpression of 4 transcription factors. In their original work, Takahashi *et al*[2] used an antibiotic selection gene system to investigate, out of 24 candidates, which transcription factors were critical for the generation of induced pluripotent stem (iPS) cells. Using a similar strategy, Ieda *et al*[1] tested 14 cardiac transcription factors and, by serially withdrawing each factor, they came across a combination of three factors that were necessary and sufficient to induce the reprogramming of fibroblasts into what they called induced-cardiomyocytes (iCM) both *in vitro* and *in vivo*. These three factors were Gata4, Mef2c and Tbx5 (GMT), well known transcription factors activated during cardiogenesis. Major advances that led to this exciting discovery are summarized in Table 1.

The findings of Ieda *et al*[1] were based on the expression of the enhanced green fluorescent protein (eGFP) driven by the α-myosin heavy chain (α-MHC) gene promoter. Therefore, if a fibroblast was converted to an iCM by retroviral transduction with the factors, the cell would become green. Fluorescent activated cell sorting would then allow identification and purification of the iCM. While transducing 14 factors induced only 1.7% of the fibroblasts to become green, GMT transduction

Table 1 Advances in cell reprogramming

Year	Major advances	Ref.
2006	First report to demonstrate that it was possible to reprogram mouse somatic cells to a pluripotent state through the overexpression of 4 transcription factors	[2]
2007	Reprogramming of human cells to a pluripotent state was achieved	[6,7]
2008	First evidence of direct reprogramming of somatic cells from the same organ (pancreatic exocrine cells to beta-cells) without a pluripotency step	[8]
2010	First report of direct reprogramming of somatic cells into an unrelated tissue (tail-tip fibroblasts to neurons)	[9]
2010	Direct reprogramming of somatic cells to cardiomyocytes through the overexpression of 3 cardiac transcription factors was reported	[1]
2012	*In vivo* direct reprogramming of fibroblasts to cardiomyocytes was demonstrated through the injection of viral vectors into infarcted myocardium	[3,4]
2012	First report of direct reprogramming of somatic cells to cardiomyocytes by microRNAs	[5]

resulted in 20% of the cells becoming green. However, expression of eGFP meant that the α-MHC gene promoter was being activated in the transduced cells, not that they had become true cardiomyocytes. Using a second screening round, now examining the expression of cardiac troponin T (cTnT), Ieda *et al*[1] found that only 30% of the green cells were also positive for this cardiac marker. Furthermore, when they compared global gene expression profiles, iCM displayed similar, but not identical, gene expression to neonatal cardiomyocytes. But even though not all reprogrammed cells expressed genes or proteins characteristic of neonatal cardiomyocytes, in those that did, approximately 30% showed intracellular calcium concentration oscillations and fired action potentials after 4-5 wk in culture. In the end, about 1%-2% of the reprogrammed cells were beating iCM. Clearly, the method needed improvement and robustness before one could envisage its application as therapy. In addition, independent confirmation by other laboratories was in order.

The first confirmation that direct reprogramming to a cardiac lineage was possible came from Efe *et al*[10] in 2011. Using the conventional Yamanaka iPS factors (in fact c-Myc was dispensable) plus small molecules that inhibited the Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway, they were able to show conversion of mouse embryonic fibroblasts into atrial-like cells. This method relied on starting reprogramming into a pluripotent state, just to cut it short by changing culture conditions, and driving a cardiogenic program by using the JAK-STAT inhibitors followed by exposure to BMP4. As mentioned above, all reprogrammed cells were atrial-like, expressing the atrial isoform of myosin light chain and firing action potentials typical of atrial or pacemaker cells. Although clearly distinct from the Ieda strategy[1], Efe *et al*[10] reassured us that turning fibroblasts into cardiac-like cells without passing through the pluripotent state was possible.

In 2012, new data on direct reprogramming into cardiac-like cells appeared in the literature and, as has been frequently the case for stem cells and cell therapies in the heart, reports were controversial. If we direct ourselves firstly towards the transcription factor methodology, Chen *et al*[11], using the lentiviral vectors provided by Srivstava's group, were unable to convert cardiac or tail-tip fibroblasts into iCM. Using three distinct myocardial lineage reporter cells (α-MHC, Nkx2.5 and cTnT promoters coupled to Cre recombinase), they were not able to detect reporter activation in the first two lines (α-MHC and Nkx2.5) in spite of significant overexpression of the GMT factors. In contrast, the cTnT reporter showed robust activation, with 35% of the fibroblasts transduced with GMT expressing the reporter protein (eGFP) and showing over 200-fold higher message levels for cTnT. Nonetheless, expression of other cardiac marker genes was only minimally elevated, indicating a very inefficient reprogramming. In order to discard the absence of cardiac reprogramming due to the lack of an appropriate niche, Chen *et al*[11] transplanted cardiac fibroblasts transduced with GMT into the hearts of infarcted mice. These fibroblasts were obtained from transgenic animals that constitutively expressed luciferase and eGFP, thus allowing the *in vivo* tracking of the cells. Much to their surprise, the authors found that GMT transduced cells survived less than non-transduced ones and, in their majority, expressed fibroblast markers, indicating that conversion to a cardiac-like phenotype was not attained.

In contrast to the results just described, Qian *et al*[3] reported in *Nature* the successful *in vivo* reprogramming of endogenous cardiac fibroblasts to cardiomyocyte-like cells after GMT transduction. It should be noted that the use of a retroviral system to infect the hearts resulted in transduction of the three factors (GMT) to dividing cells only. Therefore, the experiments were performed in mice subject to left anterior descending (LAD) artery ligation, where non-muscle cells, especially fibroblasts, proliferate intensely to form a scar. Using transgenic mice in which the periostin promoter drove either β-galactosidase (β-gal) or enhanced yellow fluorescent protein (eYFP), the authors were able to track the cells that were converted into iCM after GMT. Since periostin is preferentially expressed in fibroblasts and these comprise about 50% of the heart cells, the fibroblasts converted to iCM could be identified by either blue (β-gal) or yellow (eYFP) staining. Performing additional staining for cardiac markers, such as α-actinin, tropomyosin, α-MHC and cTnT, Qian *et al*[3] showed that approximately 35% of the myocytes in the border/infarct zone were double-labeled by cardiac markers and β-gal, indicating their origin as fibroblasts that turned into iCM. The iCM isolated from the border region of infarcted hearts were shown to couple to native cardiomyocytes, to propagate calcium waves, and to fire action potentials and contract after stimulation. Furthermore, in mice that were injected with GMT after acute myocardial infarc-

tion, ejection fraction, stroke volume, cardiac output and scar size were significantly improved when compared to infarcted animals in which only dsRed (a dye) was injected. The authors thus concluded that the improvement in cardiac function was a consequence of the formation of new cardiomyocyte-like cells, the iCM. Of note, the percentage of iCM formed from the total GMT-infected population remained at 12%, even after using adjuvant molecules (in their report, thymosin β4).

In support of Qian *et al*[3] findings, both *in vivo* and *in vitro*, Song *et al*[4] reported in *Nature* the reprogramming of adult tail-tip and cardiac fibroblasts using four transcription factors, namely Gata4, Mef2c, Tbx5 and Hand2 (GMTH). In these experiments, using the three Srivastava's factors (GMT) induced 2.9% iCM and the addition of Hand2 increased that percentage of successful reprogramming to 9.2%. As in the Ieda *et al*[1] paper, the reprogrammed fibroblasts were shown to exhibit different degrees of reprogramming with variable expression of cardiac proteins and distinct levels of sarcomere organization. Using the same approach of targeting dividing cells with retroviral vectors, Song *et al*[4] were able to show that iCM were generated in hearts of mice subject to LAD ligation after intracardiac injection of GMTH. iCM isolated from these hearts and identified by lineage tracing exhibited calcium transients, fired action potentials and contracted (with different degrees of maturity). As described by Qian *et al*[3], the injection of GMTH into infarcted hearts led to significant increases in stroke volume and ejection fraction compared with infarcted animals injected with eGFP retroviruses alone. Scar area was also significantly decreased in the GMTH-treated animals.

A different approach was taken by Jayawardena *et al*[5] using a combinatorial strategy to identify microRNAs (miRs) able to reprogram fibroblasts into cardiac-like cells *in vitro* and *in vivo*. Using reverse transcription polymerase chain reaction for identification of cardiac gene up-regulation, a cardiac promoter (α-MHC) coupled to a reporter gene [cyan fluorescent protein (CFP)], and transgenic mice allowing lineage tracing of cardiac fibroblasts, they transiently transfected distinct combinations of 6 synthetic miRs based on their role in cardiac development into cardiac fibroblasts. The combination of miR-1, -133, -208 and -499 was able to induce reprogramming of the fibroblasts to cardiac-like phenotype. As with the transcription factors described above, the degree of reprogramming seemed to be variable and partial. Addition of an inhibitor of JAK I increased reprogramming efficiency 3-fold, reaching up to 27% of cells expressing the cyan fluorescence in fibroblasts derived from α-MHC-CFP hearts. Injection of a lentivirus with the four miRs into the hearts of double-transgenic mice expressing red tomato under control of the FSP promoter after LAD ligation allowed identification of double-labeled cells, expressing the red tomato protein and cTnT. Quantification of labeled cells in the infarcted hearts indicated up to 1% of reprogramming when using the four miRs, but functional evaluation was not performed in these experiments.

Taken together, these results open exciting new therapeutic possibilities for therapy of cardiac diseases; however, several concerns remain unresolved. First, it seems to be generally accepted that efficiency of *in vitro* direct reprogramming into the cardiac lineage is low. In this context, it needs to be sorted out whether the discrepancies in Ieda's and Chen's work can be explained by differences in cell types, reporter systems, transduction efficiency, levels of GMT expression or in methods for evaluating reprogramming. In addition, as has been demonstrated for iPS cells[12], transcription factor stoichiometry can be critical for the reprogramming process and may be very difficult to control. Nonetheless, it is also important to point out that, even though many strategies could be used to improve reprogramming efficiency *in vitro*, such as the addition of small molecules and the combination of different approaches (transcription factors and miRs), it may be an inherently inefficient process due to the low turnover of cardiomyocytes[13]. In the case of *in vivo* experiments, the consequences of partial reprogramming will have to be assessed from a safety standpoint and experiments in larger animal models will be required. Additionally, the retroviral approach in myocardial infarction models might overcome the *in vitro* efficiency concern, but it raises other issues, especially related to the potential risks of vector integration into DNA. It would be ideal if efficient non-integrating methods of reprogramming could be developed for therapeutic purposes. Indeed, these are thrilling times for the cardiac regeneration field.

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Coronary atherosclerosis: Significance of autophagic armour

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Abstract

Autophagy is a lysosomal degradation pathway of cellular components such as organelles and long-lived proteins. Though a protective role for autophagy has been established in various patho-physiologic conditions such as cancer, neurodegeneration, aging and heart failure, a growing body of evidence now reveals a protective role for autophagy in atherosclerosis, mainly by removing oxidatively damaged organelles and proteins and also by promoting cholesterol egress from the lipid-laden cells. Recent studies by Razani *et al* and Liao *et al* unravel novel pathways that might be involved in autophagic protection and in this commentary we highlight the importance of autophagy in atherosclerosis in the light of these two recent papers.

Key words: Atherosclerosis; Autophagy; Inflammasome; Reactive oxygen species; Apoptosis; Efferocytosis

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INVITED COMMENTARY ON HOT ARTICLES

Atherosclerosis still remains as the major etiological factor for the world's largest killing disorder, i.e., coronary artery disease. Despite several novel advances at the therapeutic front, the treatment of atherosclerosis still remains elusive. In the recent past, the focus has shifted to the prevention of its occurrence rather that its cure. Universally acknowledged as a lifestyle disorder, studies in animal models show that calorie restriction is anti-atherogenic[1]. The direct cellular response for calorie restriction or fasting has been elucidated as "autophagy". Autophagy (literally meaning "self-eating") is a lysosomal pathway of cellular digestion of damaged or excess organelles and misfolded protein aggregates as well as invading micro-organisms[2]. Depending upon the mode of cargo delivery to lysosomes, three different forms of autophagy have been described: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA). While in microautophagy and CMA, cargo is delivered directly into the lysosomes through invaginations in the lysosomal membrane and a membrane translocation complex respectively, macroautophagy on the other hand involves sequestration of cytoplasmic components in a double membrane vesicle called the autophagosome, which subsequently fuses with the lysosomes to form an autophagolysosome resulting in the degradation of components *via* lysosomal hydrolases. Macroautophagy (referred to as "autophagy" hereafter) is an evolutionary conserved, highly regulated and dynamic pathway regulated by autophagy-related (*ATG*) genes

and *ATG* null mice are used as *in vivo* model systems to study autophagic functions and regulation[2]. Although autophagy has been implicated in the pathogenesis of neurodegenerative disorders, cancers and heart failure[3], the role of autophagy in atherosclerosis is a burgeoning area of research. Autophagy has been observed in macrophages, smooth muscle cells and vascular endothelial cells and it protects the cells against oxidative stress by degrading the damaged intracellular material[4]. A special kind of autophagy, termed as "lipophagy", also helps in cholesterol egress from lipid-laden cells to high density lipoprotein (HDL) *via* lysosomal lipases[5].

Recently, two papers published in the April 4 2012 issue of *Cell Metabolism* add new dimensions to our understanding of the role that autophagy plays in regulating atherosclerotic plaque development. Although it is well established that crosstalk between lipid metabolism and inflammation orchestrates the development of atherosclerotic lesions[6], Razani *et al*[7] provide yet another link between the two processes by elucidating that autophagy prevents cholesterol crystal-induced inflammasome activation. On the other hand, Liao *et al*[8] provide evidence that autophagy prevents macrophage apoptosis and defective efferocytosis, both of which characterize advanced plaques and are associated with necrotic cores. Using various animal models, the authors unambiguously establish the anti-inflammatory and anti-apoptotic role of autophagy in atherosclerosis.

Both these authors, utilizing animal models with different genetic backgrounds and taking p62/SQSTM1 as a marker, have shown that autophagy becomes dysfunctional as the lesion development progresses. Razani *et al*[7] fed *ApoE*-null mice and their wild type counterparts a western diet (WD) for 2 mo and observed a dramatic increase in the p62 levels in atherosclerotic aortas, which increased with increasing age of the *ApoE*$^{-/-}$ mice and was consistent with the findings of Liao *et al*[8] who also found increasing levels of p62 in macrophage-rich areas of lesion in WD-fed *LDLR*$^{-/-}$ mice with increasing age. However, this increase in p62 expression levels was not due to an increase in p62 mRNA levels[7,8]. Considering the protective role of autophagy in cell survival and maintenance of cellular homeostasis, complete autophagic deficiency would be incompatible with life and Razani *et al*[7] show that haploinsufficiency of autophagy is inadequate to accelerate atheroma progression. This implies that initially the autophagy is functional and becomes severely compromised with the plaque progression. This is also reflected by the similar protein levels of Beclin-1 (which is involved in autophagosome nucleation) in WD-fed *ApoE* null and wild type mice[7]. It is important in this context to mention here that high fat diet or chronic lipid challenge decreases the fusion between autophagosomes and lysosomes[9], which might explain why, despite the initiation of autophagy and formation of autophagosomes, the autophagic flux through lysosomes becomes defective as the plaque progresses and lipid content increases. Further, Singh *et al*[5] have shown that autophagy also plays a significant role in the reverse cholesterol transport by hydrolysis of intracellular lipid droplets of foam cells *via* lysosomal lipases (lipophagy) and promoting their egress through the ATP-binding cassette A1 to HDL. Thus, defective lipophagy increases intracellular lipid content and progressively leads to dysfunctional autophagy forming a vicious circle.

What are the consequences of this defective autophagy? As we have seen from the cytoprotective role of basal autophagy in various cells, it is apparent that in the absence of autophagy apoptosis would increase. Liao *et al*[8] have also shown that *ATG5* null mice show increased apoptosis upon exposure to oxidative and endoplasmic reticulum stress. Furthermore, they have shown that inhibition of autophagy results in increased activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity resulting in increased reactive oxygen species (ROS) production, which may damage mitochondria and other organelles including lysosomal membrane destabilization[8]. Due to a defect in the autophagy, damaged mitochondria release more ROS and cytochrome c, and the spillage of lysosomal hydrolases due to lysosomal rupture would ultimately result in apoptosis of macrophages. Further worsening the situation is the observation that autophagy inhibition also reduces phagocytic clearance of apoptotic macrophages, i.e., efferocytosis, ultimately resulting in a necrotic core[8].

Another consequence of this increased ROS, release of mitochondrial DNA and cathepsins, is activation of inflammasomes. Inflammasomes are multimeric protein complexes involving pattern recognition receptors (PRRs) that participate in inflammatory signaling. The nucleotide-binding domain leucine-rich repeat containing (NLR) family, pyrin domain containing 3 (NLRP3) inflammasome is the best-characterized inflammasome of all, and is responsible for caspase-1 mediated cleavage and secretion of pro-inflammatory cytokines interleukin (IL)-1β and IL-18[10]. The activation of the NLRP3 inflammasome is a two-step process. The first step, also called the "priming step", involves activation of nuclear factor kappa B (NF-κB) by PRRs or by cytokine receptors resulting in the increased expression of pro-IL-1β and NLRP3 itself[11]. The second step involves the assembly and activation of the NLRP3 inflammasome by various exogenous and endogenous stimuli such as pore-forming toxins[12], crystalline substances (silica, asbestos and monosodium urate crystals)[13,14], extracellular ATP[12] (released by dying cells), peptide aggregates and various other microbial stimuli[15]. The activators of both these steps are present in atherosclerotic plaques. Whereas the priming signal can be provided by modified low density lipoprotein (LDL) which activates NF-κB *via* toll-like receptor (TLR)4/6 and various scavenger receptors (CD36 and SR-A), cholesterol crystals can activate the NLRP3 inflammasome. Moreover, oxidized LDL (ox-LDL) found in early atherosclerotic lesions can provide both the signals. However, since none of the activators of the NLRP3 inflammasome has been shown to inter-

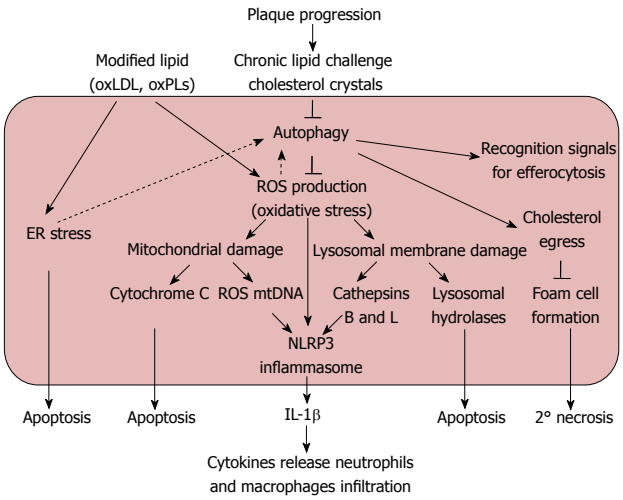
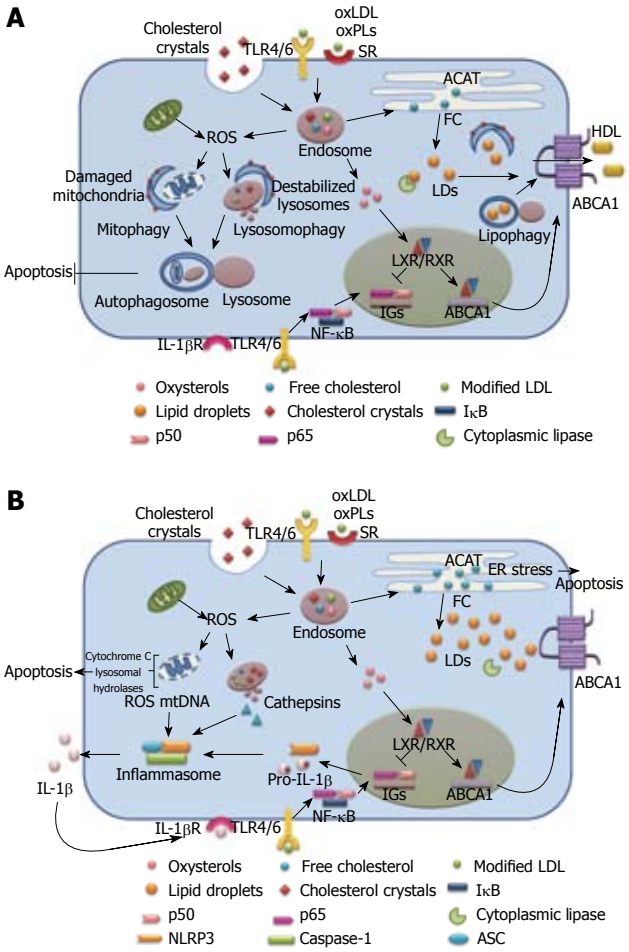


Figure 1 Proposed pathways elucidating the possible role of autophagy in atherosclerosis. ER: Endoplasmic reticulum; IL-1β: Interleukin-1β;oxLDL: Oxidized low density lipoprotein; oxPLs: Oxidized phospholipids; ROS: Reactive oxygen species; NLRP3: Nucleotide-binding domain leucine-rich repeat containing (NLR) family, pyrin domain containing 3.



Figures 2 Schematic depiction of the relationship between autophagy, cholesterol metabolism, inflammasome activation and atherogenesis in (A) early lesions when autophagy is functional, and (B) advanced lesion where autophagy is compromised. ABCA1: ATP-binding cassette A1; ACAT: Acylcoenzyme A cholesterol acyltransferase; ASC: Apoptosis-associated speck-like protein; ER: Endoplasmic reticulum; FC: Free cholesterol; HDL: High density lipoprotein; IGs: Inflammatory genes; IL-1β: Interleukin-1β; IL-1βR: Interleukin-1β receptor; LD: Lipid droplets; LXR: Liver X receptor; NF-κB: Nuclear factor kappa B; NLRP3: Nucleotide-binding domain leucine-rich repeat containing (NLR) family, pyrin domain containing 3; oxLDL: Oxidized low density lipoprotein; oxPLs: Oxidized phospholipids; ROS: Reactive oxygen species; RXR: Retinoic X receptor; SR: Scavenger receptor; TLR: Toll-like receptor.

act directly with NLRP3, this suggests that a common upstream signaling event is involved in its activation[10]. A number of hypotheses have been put forward to explain the assembly and activation of the NLRP3 inflammasome. One of the suggested mechanisms is that NLRP3 senses intracellular ROS. Earlier NADPH oxidases were thought to be responsible for NLRP3 activation but recently ROS produced in mitochondria have also been implicated in NLRP3 activation[13,16,17]. Cathepsins B and L released upon lysosomal membrane destabilization due to cholesterol crystals or ROS have also been shown to activate NLRP3[18]. In fact, cathepsin B- and L-deficient mouse macrophages display reduced secretion of IL-1β when exposed to cholesterol crystals[19] and increased levels of cathepsins B and L co-localizing with macrophage markers are found in atherosclerotic plaques as compared to normal healthy tissue[20,21]. NLRP3 activation through any of the above stated pathways ultimately leads to an increase in pro-inflammatory IL-1β secretion that promotes secretion of many other chemokines and cytokines and also induces expression of endothelial adhesion molecules[22] resulting in infiltration of neutrophils which not only themselves result in chronic inflammation and increased risk of thrombosis, but also trigger intimal recruitment of monocytes[23].

We agree with the authors in proposing a pathway for the observed protective effects of autophagy in atherosclerosis (Figures 1 and 2), noting the following points: (1) oxLDL results in oxidative and endoplasmic reticulum stress through the activation of CD36 as well as the TLR2/6 pathway[23]; (2) This oxidative stress has the inherent capacity to induce cellular autophagy; (3) As the atherosclerotic lesion progresses, the increased intracellular lipid accumulation results in dysfunctional autophagy; (4) Inhibition of autophagy results in the increased activity of NADPH oxidase leading to the production of ROS that results in mitochondrial damage and lysosomal membrane rupture; (5) In the absence of autophagic rescue, the accumulation of damaged mitochondria and lysosomes initiates an inflammatory response by activating the NLRP3 inflammasome as the lesion progresses into an advanced stage; (6) The co-operation between macrophage apoptosis induced by ROS overproduction and defective efferocytosis (due to deficient autophagy) results in atherosclerotic plaque necrosis, which in turn can precipitate acute athero-thrombotic events; and (7) Although Razani *et al*[7] and Liao *et al*[8] have made appreciable efforts towards unraveling the role of autophagy in atherosclerosis, the role of this phenomenon in plaque development is far from clear. Consequently, there is a need to explore the mechanism that governs the crosstalk between autophagy and apoptosis

within the cells of the arterial wall and how this crosstalk is responsible for the initiation and regression of atherosclerotic lesions.

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REVIEW

Overview of resistant hypertension: A glimpse of the cardiologist's current standpoint

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Abstract

Hypertension is a major risk factor for cardiovascular disease, resulting in increased incidence of cerebrovascular events, ischaemic heart disease, heart failure, and renal impairment. Thus, it is one of the most important preventable causes of premature morbidity and mortality. Despite current knowledge on the management of hypertension and the availability of several effective antihypertensive medications, uncontrolled hypertension remains a common and challenging clinical problem. Resistant hypertension is a complex condition with multiple contributing factors and overlapping comorbidities. Although there is limited hard evidence regarding resistant hypertension, our understanding of this condition has improved recently. This article will present an overview of resistant hypertension and highlight recent publications about this topic.

Key words: Hypertension; Resistant hypertension; Management

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INTRODUCTION

Resistant hypertension is defined as high blood pressure (BP) in spite of appropriate lifestyle interventions and administration of three different antihypertensive drugs at optimal dose amounts, which should include a diuretic agent[1,2]. The goal systolic BP is usually defined as < 140 mmHg and < 90 mmHg for diastolic BP; however, current views on cardiovascular risk suggest lower targets for high-risk patients (e.g., those with diabetes mellitus, renal dysfunction). By this definition, subjects who achieve adequate BP control with optimal doses of 4 or more antihypertensive medications are considered to have resistant hypertension. This group of patients controlled with ⩾ 4 drugs accounts for 30% of the resistant hypertension cases[3], and should be considered as a separate category in future studies (as they seem to represent a more benign phenotype), to better characterize the most severe forms of truly resistant hypertension. Although arbitrary, the above definition focuses on a subset of hypertensive patients with a higher cardiovascular risk and potentially reversible causes of hyper-

tension that should be thoroughly pursued. Prognosis among patients with resistant hypertension compared with those who are non-resistant, though expectedly worse, is not well established in the literature[4,5]. Resistant hypertension pathophysiology needs a greater understanding, as does its incidence and prognosis. Whether the worse cardiovascular outcome is related to a resistant-to-treatment phenotype or linked to BP control also needs to be better understood. Knowing the patterns of medication class use, the importance of medication adherence, and the efficacy of certain therapy interventions (change in medication class, increase in drug dose, novel antihypertensive treatments) in a population with resistant hypertension would help to guide future interventions and improve management of these challenging patients.

PREVALENCE

The prevalence of resistant hypertension, though not uncommon, is not well established and has been defined as a priority area in future studies[2]. There is no specifically designed research concerning resistant hypertension, and our knowledge mainly derives from cross-sectional analyses and some recent clinical trials. However, failure to apply a uniform definition of resistant hypertension, very selective populations, restricted treatment regimens, and the inability to exclude pseudoresistant hypertension has limited the interpretation of results. The 2003 to 2008 National Health and Nutrition Examination Survey included > 15 000 unselected adults in the United States, and found resistant hypertension criteria to be met in 8.9% of all individuals with hypertension, and in 12.8% of the hypertensive drug-treated population[5]. Another large survey in Spanish hypertensive patients found similar results, with 12.2% incidence of resistant hypertension in treated patients[6]. The work by Daugherty *et al*[5] has found that 16% of the patients were taking ⩾ 3 medications, once they had excluded poor adherence to treatment regimens. Furthermore, this study has shown that 1 in 50 cases in whom antihypertensive treatment is started will develop resistant hypertension within 1.5 years[4]. Nonetheless, in a progressively older population, with an increasing incidence of comorbidities related to resistant hypertension such as diabetes, obesity and renal impairment, the prevalence of resistant-to-treatment hypertension is much more likely to increase.

PSEUDORESISTANCE

Inaccurate measurement of BP

A poor BP measurement technique can often be misleading. The most common errors are an inappropriate cuff size, incorrect patient and arm positions, and inadequate premeasurement at rest. Moreover, marked arterial stiffness which does not compress with pressure should be seen as a potential cause of pseudohypertension, especially in the elderly or in those with other treatment symptoms.

Poor adherence to therapeutic plans

Non-adherence to lifestyle interventions and/or antihypertensive therapy is a major cause of uncontrolled hypertension. About half of the patients who were prescribed an antihypertensive drug had stopped taking it within one year[7]. Factors that influence adherence include patient demographics, side effects of medications, convenience of drug dosing, cost and number of medications, as well as patients' knowledge, beliefs and attitudes about hypertension. A study on resistant hypertension highlighted the importance of patients' compliance by reporting a BP reduction to normotensive values in about 30% of the participants, attributable merely to patient self-perception of being monitored and without any changes in medication regimen[8]. Nonetheless, physician failure to comply with guidelines and actively pursue adequate BP in their patients is, also, a significant contributor to the lack of BP control. The latter may be illustrated in a recent clinical study which has found that resistant hypertension patients were taking less than 50% of the recommended maximal daily doses of their antihypertensive medications at follow-up[9]. Moreover, patients already on 3 or more antihypertensive medications are at an increased risk for poor treatment adherence and clinical inertia (because of the potential adverse effects of multiple drug combinations or physician scepticism about the benefit of intensifying therapy).

White-coat effect

Most patients have higher BP levels when measured by the physician than when assessed out of the office. This "white-coat" effect is often more pronounced in patients with severe hypertension. Moreover, 20%-30% of patients with apparent resistant hypertension referred for ambulatory BP monitoring in fact have normal BP readings[10]. It is of note that patients with white-coat hypertension have less severe target organ damage and cardiovascular risk compared to those with persistent elevated BP readings in ambulatory monitoring.

RESISTANT HYPERTENSION

Resistant hypertension should not be confused with uncontrolled hypertension, which includes pseudoresistance, inadequate therapeutic regimen, as well as true resistant-to-treatment hypertension. Thus, resistant hypertension is a diagnosis of exclusion that should be carefully established using a stepwise patient evaluation, according to the American Heart Association state of the art manuscript on resistant hypertension[2].

In the presence of uncontrolled hypertension, the patient's evaluation should begin with a correct BP measurement to avoid false high readings, and then confirmed by out-of-office BP monitoring to exclude white-coat hypertension. Patient compliance assessment is of utmost importance. The next step, after pseudoresistance

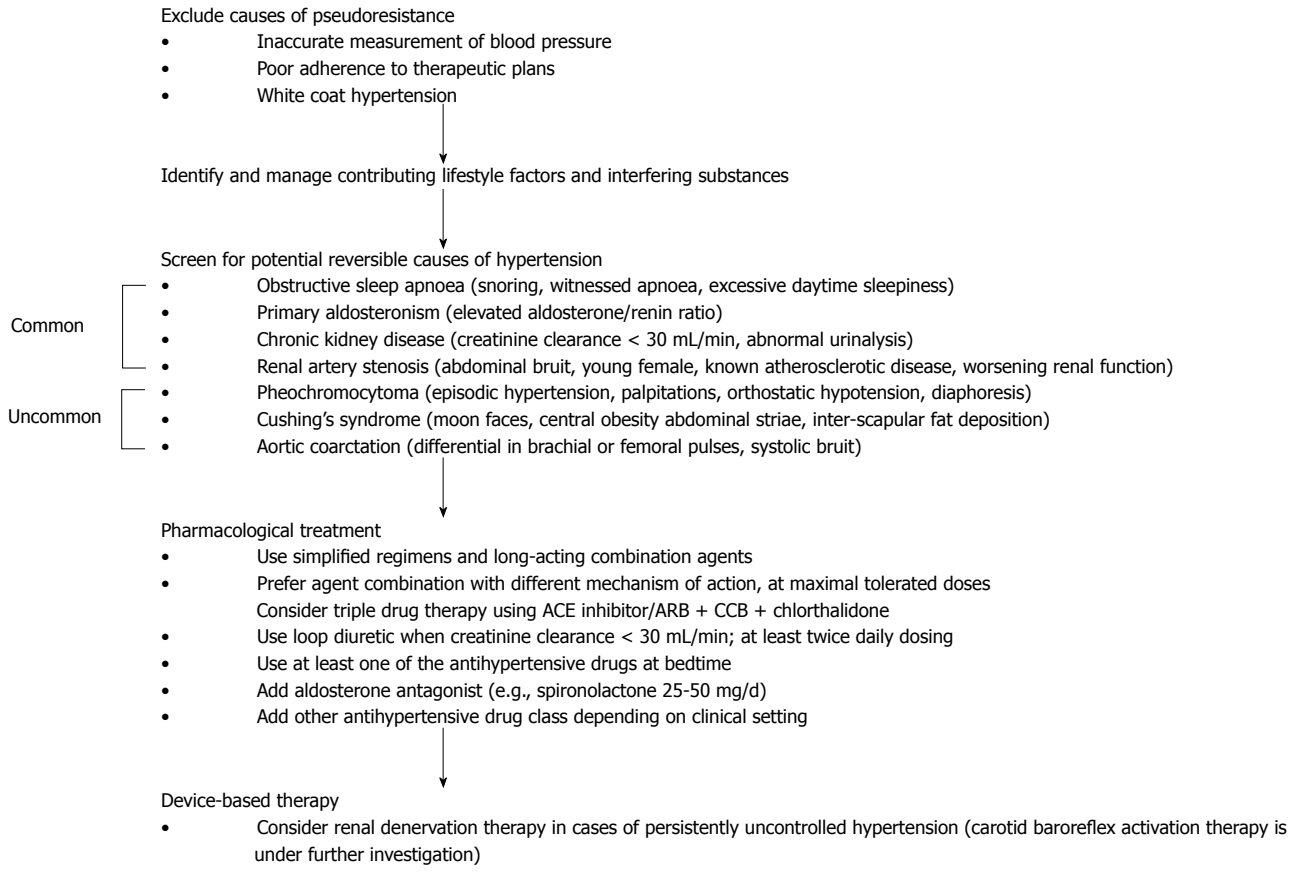

Figure 1 Diagnosis and management algorithm (adapted from Calhoun *et al*[2]). ACE: Angiotensin-converting enzyme; ARB: Angiotensin receptor blocker; CCB: Calcium channel blocker.

exclusion, is the identification and possibly reversing of contributing lifestyle factors. Obesity is a common feature in resistant hypertension patients and is related to poor antihypertensive treatment response. It has been reported that each 10% increase in weight is associated with a 6.5 mmHg increase in systolic BP[11]. Dietary salt intake can blunt the effects of the majority of antihypertensive drugs, especially in salt-sensitive patients such as the elderly and black race patients, and in renal impairment. Several pharmacological agents can significantly interfere with BP and/or antihypertensive effect of medications; hence interfering substances should be promptly discontinued or minimized. The recognition of other common contributing factors such as non-steroidal anti-inflammatory drug use, oral contraceptive hormones or progressive renal impairment is needed in order to achieve BP control in resistant-to-treatment patients.

Resistant hypertension is complex in nature and almost always multifactorial in aetiology. Although uncommon, the potential reversible causes of hypertension should always be carefully ruled out by medical history, physical examination, biochemical evaluation, and/or non-invasive imaging (Figure 1). Sleep apnoea is among the commonest secondary causes of hypertension. Its association with hypertension is due to the sustained increase in sympathetic nervous system activity, and its prevalence is approximately 85% of the true resistant-to-treatment patients[12]. However, continuous positive airway pressure has only shown a modest antihypertensive benefit[13,14]. Primary aldosteronism seems to be a much more common secondary cause of hypertension than previously recognized, with a reported prevalence of approximately 20% in resistant hypertension[15]. Recognition that most patients do not have low serum potassium levels, and a more effective screening using aldosterone and renin levels, has led to an improved detection of this condition. Renal parenchymal disease and renal artery stenosis are other common causes of secondary hypertension that should be assessed in both analytical and imaging modalities. Less common causes including aortic coarctation, Cushing syndrome, and pheochromocytoma are rarely seen (< 1%)[4].

The management of resistant hypertension is arduous and involves extensive testing, which should include the following: biochemical evaluation, including serum sodium, potassium, glucose, and creatinine (creatinine clearance); plasma aldosterone and renin; urinalysis; and 24-h urine collection to estimate dietary sodium, potassium, and aldosterone excretion. Testing for urinary or plasma metanephrines/catecholamines is indicated only when pheochromocytoma is suspected. Non-invasive imaging is mandatory when there is a suspicion of renal artery stenosis, adrenal adenoma/tumour, parenchymal renal disease or aortic coarctation.

GENETIC CONSIDERATIONS

High BP is a heritable trait influenced by several biological pathways and responsive to environmental stimuli. The determination of the genetic variants involved in hypertension would provide new insight into BP regulation, and there are several lines of evidence which point towards an important genetic contribution. Furthermore, it is reasonable to expect an even greater genetic role in severe phenotypes, as true resistant hypertension. To date, few variants associated with interindividual BP variation have been consistently identified[16,17], and our understanding of the genetic determinants of hypertension is in its early phase. Nonetheless, efforts in the dissection of BP genetics would create new targets for therapeutic approaches, and future use of individually tailored treatment will improve drug efficacy and reduce its toxicity.

TREATMENT RECOMMENDATIONS

If a specific secondary cause of hypertension is suspected or if the BP is persistently high despite six months of more intensive treatment, patient referral to a hypertension specialist is advised[2]. Recommendations on the treatment of secondary causes of hypertension are beyond the scope of this review.

Nonpharmacological therapy

This includes reversal of lifestyle factors contributing to treatment resistance, and discontinuation or minimisation of interfering substances. There is usually a beneficial result with lower salt diet (< 100 mEq of sodium/24 h), ingestion of low fat and high fibre diet, weight loss in obese or overweight patients, moderation of alcohol intake to no more than two drinks per day for most men and one drink for women or lighter-weight persons, advising smoking cessation, and regular physical activity. Do not offer calcium, magnesium or potassium supplements as a method for reducing BP[17].

Pharmacological therapy

Drug treatment of resistant hypertension involves, by definition, combinations of three or more drugs.

Diuretic therapy: Inappropriate volume expansion contributes to resistant hypertension, even among patients already on thiazide diuretics. In order to increase efficiency it may be necessary to add a diuretic, increase the usual dose or change to different diuretic class. In those without significant renal impairment, the long acting thiazide diuretic chlorthalidone is preferred over hydrochlorothiazide for the treatment of resistant hypertension[18]. It has a more potent antihypertensive effect, longer half-life and has been shown to achieve better BP control, in comparison with hydrochlorothiazide. Among patients with an estimated glomerular filtration < 30 mL/min per m^2, thiazide diuretics become less effective and loop diuretics should be used instead, such as furosemide, which needs at least twice daily dosing due to its short half-life. In the presence of refractory volume retention, medical intervention should focus on reducing dietary sodium intake and increasing the diuretic treatment intensity.

Aldosterone antagonists: The prevalence of primary aldosteronism in resistant hypertension patients seems to be much more common than previously acknowledged, and recent studies using aldosterone antagonists, such as spironolactone, eplerenone, and amiloride, reported significant antihypertensive benefits when added to a multiple drug regimen in patients with difficult-to-treat hypertension[19]. The antihypertensive effect of spironolactone was evaluated in randomized trials and has proven efficacy, although results were more modest than initial expectations. Compared to a placebo, spironolactone 25 mg significantly decreased mean daytime and nighttime ambulatory systolic BPs by 5.4 and 8.6 mmHg, respectively, with no significant change in diastolic pressure[20]. However, as suggested by the authors, it is possible that higher doses of spironolactone could have had a greater impact on BP levels. Regarding which add-on therapy to use in resistant hypertension, a recent study designed to compare spironolactone *vs* dual blockade of the renin-angiotensin-aldosterone system (RAS) has found that spironolactone has greater antihypertensive effect than dual blockade of the RAS in resistant hypertension[21].

Choice of regimen: Regimens should be simplified and long-acting combination agents should be preferred, to improve efficacy and adherence to treatment. Non-invasive hemodynamic studies assessing cardiac output, vascular resistance and intravascular volume may be considered to guide a pharmacological approach. While several studies have reported additive antihypertensive benefit by combining two different classes of drugs, few studies have systematically evaluated the combination of three or more medications in the treatment of resistant hypertension[2]. Considering no specific indication for a class of drugs, a reasonable approach would be to sequentially combine agents with different mechanisms of action to improve efficacy and drug tolerance. The use of same-class combinations such as dihydropyridine and non-dihydropyridine calcium channel blockers (CCB), combinations of diuretic, an angiotensin-converting enzyme inhibitor (ACEi) or an angiotensin receptor blocker (ARB) are not advocated over different classes of antihypertensive agents. The latter combination therapy, with a recognizably larger biologic effect, was evaluated in The Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial[22], and despite the reported small reduction in systolic BP in the combination-therapy group as compared with the ramipril group, no significant benefit was seen among patients receiving the two-drug therapy. However, combination therapy adverse effects were significantly increased (hypotension, syncope, renal dysfunction and hyperkalae-

mia). The authors concluded that no additional advantage and possibly some harm could occur from this drug combination. Although the lack of an additional benefit from a substantial lowering of BP is puzzling, similar results were found in the VALIANT study[23], in which the combination of a full dose of captopril plus valsartan did not significantly reduce the occurrence of the primary outcome but there was an increase in treatment-related adverse effects. Nonetheless, these findings contrast with a previous clinical trial done in a heart failure population, CHARM[24], in which candesartan added to existing therapy with any ACEi was superior to placebo in reducing deaths or hospitalization for heart failure.

Evidence-based recommendations in primary hypertension consider an ACEi or ARB plus a CCB and a diuretic as the most rational triple drug treatment. Among patients who still have their BP uncontrolled with the triple drug regimen at maximum recommended and tolerable doses, spironolactone 25-50 mg/d should be added to the treatment. Further increases in spironolactone doses are not recommended in the absence of documented primary aldosteronism. Adverse effects of aldosterone antagonists are uncommonly seen with eplerenone use.

In primary hypertension, with no compelling indication for a beta-blocker (e.g., ischaemic heart disease or heart failure), patients should not receive a beta-blocker as the first-line of treatment. Several studies have reported that beta-blockers are not as effective as other antihypertensive drug classes in the prevention of cardiovascular events of uncomplicated hypertension, and the combination with thiazide diuretics increases the risk of developing diabetes mellitus[1]. Nonetheless, beta-blockers are a reliable therapeutic option for uncontrolled hypertension and complicated hypertension, especially those with vasodilating properties, such as carvedilol and nebivolol. Furthermore, the association between beta and alpha-blockers may potentiate their antihypertensive effect[25].

The work by Hermida *et al*[26] in resistant hypertension underlined the importance of ensuring at least one of the antihypertensive drugs is taken at bedtime, in comparison with subjects receiving all drugs on awakening. This chronotherapeutic approach resulted in a significant 60% increase in BP control, and a 32% improvement in nighttime BP and in the prevalence of a non-dipper pattern, and should be implemented in resistant-to-treatment patients.

Other available agents can be added sequentially in the presence of persistent uncontrolled BP, such as centrally acting agents (clonidine, rilmenidine), or direct vasodilators (minoxidil, hydralazine). The two former pharmacological agents are potent vasodilators which should be reserved for cases of hypertension refractory to the above-mentioned medications because of frequent adverse effects.

A work by Hanselin *et al*[27] retrospectively studied antihypertensive use in > 140 000 patients with resistant hypertension. With regard to antihypertensive class use, 96.2% of the resistant hypertension patients were on ACE inhibitors and/or ARBs, 93.2% were taking diuretics, 83.6% a CCB, and 80.0% were on beta-blockers. Only 3.0% of patients were taking chlorthalidone and 5.9% aldosterone antagonists. Moreover, a total of 15.6% of patients were instead treated with ACE inhibitors plus ARB, which is not a first-line drug association. The authors concluded that although guideline-recommended first-line agents were used frequently, effective medications such as chlorthalidone and aldosterone antagonists were still underused.

A recent study evaluated the patterns of hypertension treatment, medication adherence, and treatment intensification (either increase in medication class or dose) in a resistant hypertension population[9]. The authors found that only 55% of the patients achieved BP control one year after resistant hypertension identification. In the first year of follow up the majority of patients were adherent to their antihypertensive medications (median medication adherence rates were nearly 85%); however, the use of many antihypertensive medication classes declined over 1 year, and one of the largest drops was seen in diuretic use. The latter underlines the difficulty to maintain adherence in patients with multiple antihypertensive regimens. One of the most important findings of this study was the evaluation of the role of therapy intensification in resistant hypertension patients. Over 1 year of follow-up, the intensification of the treatment regimen occurred in only 21.6% of visits with an elevated BP documented (10% had a class addition and 32% had a dose increase), which is much less than the expected intensification of the antihypertensive treatment for this high-risk group. However, the treatment intensification, but not treatment adherence, associated with improved BP control supports the importance of therapy optimization in resistant hypertension management.

Currently, BP remains uncontrolled in many cases despite the availability of multiple antihypertensive drugs and the patient's adherence to treatment. The absence of novel antihypertensive drugs targeting new pathways has led to new treatment strategies using different combinations of available drugs, particularly those targeting sodium balance and RAS, which are the major recognizable factors affecting BP control. Some of these new strategies combine different diuretic drugs that act on different nephron segments[28] and others combine different RAS blockers[29], both approaches theoretically minimise counter-regulatory mechanisms that often limit the antihypertensive effect of the medical regimens. A recent work by Bobrie *et al*[30] compared the efficacy and safety of two stepped-care strategies of sequential nephron blockage (SNB) *vs* sequential RAS blockage (SRASB) added to a triple standardized therapy regimen (including an ARB, CCB and thiazide) in patients with resistant hypertension. The SNB strategy consisted of the sequential addition of low doses of one to three other diuretics acting at different nephron segments (spironolactone, furosemide and amiloride), rather than the standard ap-

proach of increasing the dose of a single diuretic or changing the class of the diuretic. The rationale of this novel approach is to minimise the effects of intrarenal counterregulatory mechanisms that are triggered by the use of diuretics acting at a single site. The SRASB strategy consisted of reinforcing RAS blockage by adding an ACEi and beta-blocker, in order to neutralise RAS counterregulatory mechanisms. In this study, the SNB was more effective than SRASB. The incidence of adverse events was similar in both groups and both treatment strategies were well tolerated. The authors concluded that progressive sodium depletion by the SNB strategy is effective to reduce BP in resistant hypertension patients. Moreover, antinatriuretic counterregulatory mechanisms seem to contribute more to uncontrolled hypertension pathophysiology than counterregulation inside the RAS. The choice of other diuretic classes instead of thiazide-type drugs, such as chlorthalidone, or bumetanide in place of furosemide, their doses and optimal sequence of administration needs further study and definition.

Device-based therapies

Renal denervation: The role of afferent and efferent sympathetic nerves in essential hypertension has been long recognised and recently reappraised. Efferent sympathetic outflow produces renal vasoconstriction, decreases renal blood flow, and increases renin release as well as sodium uptake, and afferent signals increase central sympathetic activity and contribute to neurogenic hypertension. A catheter-based radiofrequency ablation has been developed to selectively disrupt the renal nerves, and its efficacy and safety are supported by the Symplicity HTN-2 trial[31] comprising resistant hypertension subjects who had a baseline systolic pressure ≥ 160 mmHg (≥ 150 mmHg in diabetes type 2 patients). After 6 mo, office BP in the renal denervation group reduced by 32/12 mmHg from baseline, and home BP had a less pronounced reduction of 11/7 mmHg. The comparatively large discrepancy between the effects on clinic and ambulatory BP is noteworthy, and has been reported similarly by other study groups[32,33]. The antihypertensive effect seems to be sustained over 2 years. Moreover, the renal denervation registered a very favourable safety profile and was of minor complexity. Although procedure effects on cardiovascular outcomes are not well established, subsequent reports suggested important benefits in coexisting metabolic syndrome and sleep apnoea. Witkowski *et al*[32] concluded that renal denervation improves indices of insulin action and glucose metabolism (plasma glucose concentration 2 h after glucose administration: 7.0 mmol/L *vs* 6.4 mmol/L; hemoglobin A_{1C}: 6.1% *vs* 5.6%) as well as sleep apnea (apnea-hypopnea index: 16.3 events/h *vs* 4.5 events/h) at 6-mo follow up. A study by Brandt *et al*[34] has investigated the effect of catheter-based renal sympathetic denervation on left ventricular hypertrophy and systolic and diastolic function, parameters related to chronic activation of the sympathetic nervous system and to increased mortality, in patients with resistant hypertension. This study found a significant reduction in left ventricular mass, left ventricular filling pressures and left atrium dimensions, and a significant increase in ejection fraction. The prognostic importance of these results is not yet clear, and it is not possible to distinguish what proportion of these changes were caused by BP reduction or were related to the sympathetic denervation *per se*. Nevertheless, the long-term improvement in left ventricular dimension and function may have significant morbidity and mortality impact, in addition to BP-lowering effect. Furthermore, the Symplicity HF trial, designed to address the efficacy of renal denervation in heart failure patients, will further define the usefulness of this technique in other clinical settings besides resistant hypertension.

Although catheter-based renal denervation opens new interesting therapeutic perspectives, there are several limitations in the studies supporting this novel procedure that were recently reviewed by Steichen *et al*[35]. In the Symplicity HTN-1 trial[36] there was no control group with which to make comparisons regarding BP and glomerular filtration rate responses over time. The number of patients included in the pivotal studies[31,36] was small (205 patients), and only a few have reached the 24-mo follow-up time point. This limited follow-up appears not to be appropriate to exclude delayed adverse effects that may derive from the percutaneous technique. The study design was weak and may have exposed the investigators to several biases. It is of note that secondary hypertension and white-coat hypertension were not properly excluded. The BP reductions should be carefully interpreted, as no intervention was proposed in the control group. It would be preferable to optimize the pharmacological approach in a standardized fashion in the two groups before randomisation and to carry out a factitious intervention (sham) in the control group, to clearly establish the real added value of the denervation procedure compared to the optimal medical therapy. In addition, office-based measurements of BP were the main method used to evaluate procedure efficacy, which is not the preferable approach, particularly in an open study (placebo effect). The benefit for the BP control was much more modest in the ambulatory BP group than in the clinic reading group. Only a relatively small number of the randomized patients had 24-h BP monitoring before and 6 mo after denervation, and the average reduction in systolic BP was only 11 mmHg, much less impressive than the 32 mmHg reported in the office-based BP measurements. The difference compared to the control group was only 8 mmHg, similar to the effect expected with the addition of a new antihypertensive drug class. The experience from surgical renal denervation showed that the reduction in BP could be maintained over time[37]; however, long-term BP reduction after percutaneous renal denervation is uncertain. In the latter, denervation is less complete and sympathetic reinnervation may possibly be seen, a phenomenon well documented after renal transplantation. Moreover, the maintenance of the BP

Table 1 Summary of therapeutic approaches in resistant hypertension

	Usefulness	Limitations
Medical treatment		
Aldosterone antagonists	High prevalence of primary aldosteronism Higher efficacy than dual RAS blockade	70% of resistant-to-treatment hypertension cases are not controlled with ⩾ 4 antihypertensive drugs Aldosterone antagonists cause frequent adverse effects, particularly in kidney disease
Sequential nephron/RAS blockade[1]	Act on sodium balance and RAS (major blood pressure determinants) Sequential nephron blockade superior to RAS blockade	Sequential blockade benefits need further validation Sequential nephron blockade regimen complexity Potential higher incidence of adverse effects/poor tolerance Needs regular medical evaluation
Device-based therapy		
Catheter-based renal denervation	Novel therapeutic target for resistant to drug treatment patients Promising effects on glycemic control, sleep apnea and heart structure/function Potential benefits in heart failure patients	Needs further validation of study results Needs complete evaluation of delayed procedure complications Not available to all uncontrolled blood pressure patients Procedure success variability related to operator/patient factors
Carotid baroreflex activation	Novel therapeutic target for resistant to drug treatment patients	Needs further validation of study results Implantation's complexity and invasiveness

[1]Added to triple antihypertensive treatment (ARB, thiazide, CCB). ARB: Angiotensin receptor blocker; CCB: Calcium channel blockers; RAS: Renin-angiotensin-aldosterone system.

reduction was only reported in a small group of patients followed over 24 mo (18 patients)[36]. A prolonged follow-up of these patients with noninvasive imaging would be essential to evaluate long-term possible lesions induced by the denervation ablation. Finally, there are no available results on morbidity or mortality. Despite the above considerations, the European Society of Cardiology Council for Cardiology Practice[38] considers catheter-based renal denervation useful, particularly in the treatment of patients with hypertension coexisting with sleep apnea and metabolic syndrome.

Carotid baroreflex activation: Another device-based therapy for resistant hypertension is carotid baroreflex activation, consisting of an implanted pulse generator and electrodes around carotid sinuses. The Rheos Baroreflex Hypertension Therapy System enhances afferent nerve outflow from the baroreceptors to the cardiovascular control centres of the brain, reducing sympathetic outflow and, subsequently, BP. The Device-Based Therapy in Hypertension Trial was a non-randomised study which documented carotid baroreflex activation effect on office and ambulatory BP of patients with resistant hypertension[39]. The office BP reduced by an average of 21/12 mmHg and heart rate decreased by 8 beats/min from baseline, and the antihypertensive effect was sustained through the 24-mo follow up. However, in home BP monitoring the systolic BP was reduced nonsignificantly by 6 mmHg ($P = 0.10$), but with a significant decrease in diastolic BP (4 mmHg, $P = 0.04$), and in heart rate (5 beats/min, $P < 0.001$). Conversely, the subsequent randomised controlled trial reported less convincing results, due to failure to meet endpoints for acute responders and procedural safety[40]. Moreover, the implantation's complexity and invasiveness are a noteworthy limitation. Nonetheless, the development and investigation of baroreflex activation therapy continues, and future clinical trials will further define its therapeutic benefit.

Table 1 summarizes the most relevant therapeutic approaches in resistant hypertension discussed in the manuscript.

CONCLUSION

Hypertension is one of the most important preventable causes of premature morbidity and mortality, and despite the availability of effective antihypertensive drugs, it remains uncontrolled in too many patients. The causes of uncontrolled hypertension and the distinction between pseudoresistance and true resistant hypertension should be carefully pursued using a systematic approach to the patient. Understanding the pathophysiology of uncontrolled BP will help direct future efforts to improve the outcome in this high-risk population. The management of resistant hypertension should be standardised and follow the current treatment guidelines, including device-based therapy in specific cases, which has shown encouraging results in BP, metabolic control, and heart dimensions and function. In the absence of novel antihypertensive drugs targeting new pathways, an active pursuit of treatment intensification and the use of different combinations of antihypertensive drugs, particularly those targeting renal antinatriuretic counter-regulatory mechanisms, may be an effective approach to manage truly resistant hypertension patients.

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Acknowledgments to reviewers of *World Journal of Cardiology*

We acknowledge our sincere thanks to our reviewers. Many reviewers have contributed their expertise and time to the peer review, a critical process to ensure the quality of our World Series Journals. Both the editors of the journals and authors of the manuscripts submitted to the journals are grateful to the following reviewers for reviewing the articles (either published or rejected) over the past period of time.

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World Journal of Cardiology

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Events Calendar 2012

January 18-21, 2012
Ninth Gulf Heart Association Conference
Muscat, Oman

January 27, 2012
ESC Global Scientific Activities at the 23rd Annual Conference of the Saudi Heart Association
Riyadh, Saudi Arabia

January 29–31, 2012
Integrated management of acute and chronic coronary artery disease
Innsbruck, Austria

January 30, 2012
Webinar on "Best of Euroecho 2011"
Sophia Antipolis, France

February 1-3, 2012
American Heart Association and American Stroke Association International Stroke Conference 2012
New Orleans, Louisiana, United States

February 3-5, 2012
6th Asian-Pacific Congress Of Heart Failure 2012
Chiang Mai, Thailand

February 9, 2012
4th British Society for Heart Failure Medical Training Meeting
London, United Kingdom

February 23–25, 2012
Advanced Invasive Cardiac Electrophysiology
Sophia Antipolis, France

February 24–26, 2012
International Congress of Cardiology
Hong Kong, China

February 28, 2012
Echocardiography evaluation of patient with multivalvular disease
Sophia Antipolis, France

February 29-March 3, 2012
Winter ISHNE 2012
Zakopane, Poland

March 8–10, 2012
Cardiac Pacing, ICD and Cardiac Resynchronisation
Vienna, Austria

March 8–10, 2012
24th Colombian Congress of Cardiology and Cardiovascular Surgery
Cali, Colombia

March 10-11, 2012
23rd International Meeting "Cardiology Today"
Limassol, Cyprus

March 14-18, 2012
Ninth Mediterranean Meeting on Hypertension and Atherosclerosis
Antalya, Turkey

March 15-17, 2012
e-Cardiology 2012
Osijek, Croatia

March 15-18, 2012
China Interventional Therapeutics 2012-CIT
Beijing, China

March 16-17, 2012
12th Annual Spring Meeting on Cardiovascular Nursing
Copenhagen, Denmark

March 16-17, 2012
3rd European Meeting: Adult Congenital Heart Disease
Munich, Germany

March 16-18, 2012
JCS2012 - The 76th Annual Scientific Meeting
Fukuoka, Japan

March 20–23, 2012
32nd International Symposium on Intensive Care and Emergency Medicine
Brussels, Belgium

March 25-29, 2012
16th International Symposium On Atherosclerosis 2012
Sydney, Australia

March 28–31, 2012
Rome Cardiology Forum 2012
Rome, Italy

March 28–31, 2012
Annual Spring Meeting of the Finnish Cardiac Society 2012
Helsinki, Finland

March 30-April 1, 2012
Frontiers In CardioVascular Biology 2012
London, United Kingdom

April 5-7, 2012
EAE Teaching Course on New echocardiographic techniques for myocardial function imaging
Sofia, Bulgaria

April 12-14, 2012
Cardiovascular Risk Reduction: Leading The Way In Prevention 2012
National Harbor, MD, USA

April 12-15, 2012
NHAM Annual Scientific Meeting 2012
Kuala Lumpur, Malaysia

April 18-21, 2012
World Congress of Cardiology Scientific Sessions 2012
Dubai, United Arab Emirates

April 19-21, 2012
Delivering Patient Care in Heart Failure
Sophia Antipolis, France

April 20-22, 2012
7th Clinical Update on Cardiac MRI and CT
Cannes, France

April 25-27, 2012
Angioplasty Summit 2012
Seoul, South Korea

April 25-28, 2012
The 61st International Congress of the European Society of Cardiovascular and Endovascular Surgery
Dubrovnik, Croatia

April 28-29, 2012
24th Annual Scientific Meeting of the SCS
Singapore, Singapore

May 3–5, 2012
EuroPRevent 2012
Dublin, Ireland

May 15–18, 2012
EuroPCR Congress 2012
Paris, France

May 17-20, 2012
2nd International Meeting On Cardiac Problems In Pregnancy 2012
Berlin, Germany

May 19-22, 2012
Heart Failure 2012
Belgrade, Serbia

May 23-26, 2012
46th Annual meeting of the Association for European Pediatric and Congenital Cardiology
Istanbul, Turkey

May 26-27, 2012
Cardiovascular Spring Meeting 2012
Vienna, Austria

June 7–9, 2012
6th Congress of Asian Society of Cardiovascular Imaging
Bangkok, Thailand

June 7-9, 2012
6th Congress of Asian Society of Cardiovascular Imaging 2012
Bangkok, Thailand

June 15–17, 2012
13th Annual Cardiology Update
Bhurban, Pakistan

June 21-24, 2012
10th International Pulmonary Hypertension Conference and Scientific Sessions 2012
Orlando, Florida, United States

July 19-22, 2012
13th Annual South African Heart Congress
Sun City, South Africa

August 16-19, 2012
60th annual scientific meeting of CSANZ
Brisbane, Australia

August 25-29, 2012
ESC Congress 2012
Munich, Germany

September 29-October 4, 2012
International Society of Hypertension 24th Annual Scientific Meeting 2012
Sydney, Australia

October 4-6, 2012
Magnetic Resonance in Cardiology
Riva Del Garda, Italy

October 20-23, 2012
Acute Cardiac Care 2012
Istanbul, Turkey

World Journal of Cardiology

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GENERAL INFORMATION

World Journal of Cardiology (*World J Cardiol*, *WJC*, online ISSN 1949-8462, DOI: 10.4330) is a monthly peer-reviewed, online, open-access (OA), journal supported by an editorial board consisting of 362 experts in cardiology from 43 countries.

The biggest advantage of the OA model is that it provides free, full-text articles in PDF and other formats for experts and the public without registration, which eliminates the obstacle that traditional journals possess and usually delays the speed of the propagation and communication of scientific research results. The open access model has been proven to be a true approach that may achieve the ultimate goal of the journals, i.e. the maximization of the value to the readers, authors and society.

Maximization of personal benefits

The role of academic journals is to exhibit the scientific levels of a country, a university, a center, a department, and even a scientist, and build an important bridge for communication between scientists and the public. As we all know, the significance of the publication of scientific articles lies not only in disseminating and communicating innovative scientific achievements and academic views, as well as promoting the application of scientific achievements, but also in formally recognizing the "priority" and "copyright" of innovative achievements published, as well as evaluating research performance and academic levels. So, to realize these desired attributes of *WJC* and create a well-recognized journal, the following four types of personal benefits should be maximized. The maximization of personal benefits refers to the pursuit of the maximum personal benefits in a well-considered optimal manner without violation of the laws, ethical rules and the benefits of others. (1) Maximization of the benefits of editorial board members: The primary task of editorial board members is to give a peer review of an unpublished scientific article *via* online office system to evaluate its innovativeness, scientific and practical values and determine whether it should be published or not. During peer review, editorial board members can also obtain cutting-edge information in that field at first hand. As leaders in their field, they have priority to be invited to write articles and publish commentary articles. We will put peer reviewers' names and affiliations along with the article they reviewed in the journal to acknowledge their contribution; (2) Maximization of the benefits of authors: Since *WJC* is an open-access journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJC* official website, thereby realizing the goals and significance of the communication between authors and peers as well as public reading; (3) Maximization of the benefits of readers: Readers can read or use, free of charge, high-quality peer-reviewed articles without any limits, and cite the arguments, viewpoints, concepts, theories, methods, results, conclusion or facts and data of pertinent literature so as to validate the innovativeness, scientific and practical values of their own research achievements, thus ensuring that their articles have novel arguments or viewpoints, solid evidence and correct conclusion; and (4) Maximization of the benefits of employees: It is an iron law that a first-class journal is unable to exist without first-class editors, and only first-class editors can create a first-class academic journal. We insist on strengthening our team cultivation and construction so that every employee, in an open, fair and transparent environment, could contribute their wisdom to edit and publish high-quality articles, thereby realizing the maximization of the personal benefits of editorial board members, authors and readers, and yielding the greatest social and economic benefits.

Aims and scope

The major task of *WJC* is to rapidly report the most recent developments in the research by the cardiologists. *WJC* accepts papers on the following aspects related to cardiology: arrhythmias, heart failure, vascular disease, stroke, hypertension, prevention and epidemiology, dyslipidemia and metabolic disorders, cardiac imaging, paediatrics, nursing, and health promotion. We also encourage papers that cover all other areas of cardiology as well as basic research.

Columns

The columns in the issues of *WJC* will include: (1) Editorial: To introduce and comment on major advances and developments in the field; (2) Frontier: To review representative achievements, comment on the state of current research, and propose directions for future research; (3) Topic Highlight: This column consists of three formats, including (A) 10 invited review articles on a hot topic, (B) a commentary on common issues of this hot topic, and (C) a commentary on the 10 individual articles; (4) Observation: To update the development of old and new questions, highlight unsolved problems, and provide strategies on how to solve the questions; (5) Guidelines for Basic Research: To provide guidelines for basic research; (6) Guidelines for Clinical Practice: To provide guidelines for clinical diagnosis and treatment; (7) Review: To review systemically progress and unresolved problems in the field, comment on the state of current research, and make suggestions for future work; (8) Original Articles: To report innovative and original findings in cardiology; (9) Brief Articles: To briefly report the novel and innovative findings in cardiology; (10) Case Report: To report a rare or typical case; (11) Letters to the Editor: To discuss and make reply to the contributions published in *WJC*, or to introduce and comment on a controversial issue of general interest; (12) Book Reviews: To introduce and comment on quality monographs of cardiology; and (13) Guidelines: To introduce consensuses and guidelines reached by international and national academic authorities worldwide on the research in cardiology.

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SPECIAL STATEMENT

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Biostatistical editing
Statistical review is performed after peer review. We invite an expert in Biomedical Statistics to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, Ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc*. The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

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Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

Statement of informed consent
Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

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Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

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Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Baishideng Publishing Group Co., Limited, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the ICMJE to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www.clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

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Title: Title should be less than 12 words.

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Authorship: Authorship credit should be in accordance with the standard proposed by ICMJE, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

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There are unstructured abstracts (no less than 256 words) and structured abstracts (no less than 480). The specific requirements for structured abstracts are as follows:

An informative, structured abstracts of no less than 480 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections. AIM (no more than 20 words): Only the purpose should be included. Please write the aim as the form of "To investigate/study/…; MATERIALS AND METHODS (no less than 140 words); RESULTS (no less than 294 words): You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$; CONCLUSION (no more than 26 words).

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Text

For articles of these sections, original articles and brief articles, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both. The main text format of these sections, editorial, topic highlight, case report, letters to the editors, can be found at: http://www.wjgnet.com/1949-8462/g_info_20100312194155.htm.

Illustrations

Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Figures should be either Photoshop or Illustrator files (in tiff, eps, jpeg formats) at high-resolution. Examples can be found at: http://www.wjgnet.com/1007-9327/13/4520.pdf; http://www.wjgnet.com/1007-9327/13/4554.pdf; http://www.wjgnet.com/1007-9327/13/4891.pdf; http://www.wjgnet.com/1007-9327/13/4986.pdf; http://www.wjgnet.com/1007-9327/13/4498.pdf. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: …*etc.* It is our principle to publish high resolution-figures for the printed and E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. $^{a}P < 0.05$, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of *P* values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of *P* values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine con-

tributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

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The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that..."

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

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Pleased provide PubMed citation numbers to the reference list, e.g. PMID and DOI, which can be found at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in E-version of this journal.

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Authors: the name of the first author should be typed in bold-faced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

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Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h,

blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/1949-8462/g_info_20100312200347.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

Examples for paper writing

Editorial: http://www.wjgnet.com/1949-8462/g_info_20100312192220.htm

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