Pharmacogenomics of Antihypertensive Treatment & Clinical Pharmacological Studies of Digoxin Treatment

PÄR HALLBERG
Dissertation presented at Uppsala University to be publicly examined in Enghoffsalen, Entrance 50, Uppsala University Hospital, Uppsala, Wednesday, May 25, 2005 at 13:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in English.

Abstract

In Part I we found that the CYP2C9 genotype appears to influence the diastolic blood pressure response to the angiotensin II-receptor antagonist irbesartan in patients with hypertension and left ventricular hypertrophy. Those with the *1/*2 genotype (slower metabolism) responded better than those with the *1/*1 genotype (normal metabolism), likely due to a slower elimination of the drug. We further found that a +9/-9 exon 1 polymorphism of the B2 bradykinin receptor gene – shown to affect mRNA expression - appears to influence the regression of left ventricular mass during therapy with irbesartan or the beta-blocker atenolol in the same patients. Subjects with the -9/-9 genotype (higher mRNA expression) had a greater regression than carriers of the +9 allele.

In Part II we found that women on digoxin therapeutic drug monitoring have higher serum digoxin concentrations (SDCs) as compared to men (1.54±0.04 [mmol/L±SE] vs 1.20±0.05 [mmol/L±SE], p<0.001), which could be of importance since an SDC >1.4 mmol/L has been associated with increased mortality. We further found that coadministration of P-glycoprotein inhibitors with digoxin was common (47%) among the same patients, and that the SDC increased in a stepwise fashion with the number of P-glycoprotein inhibitors (20-60%). Lastly, we found that patients admitted to Swedish coronary care units with atrial fibrillation without heart failure and who had been given digoxin had a higher 1-year mortality than those not given digoxin (RR 1.44 [95% CI 1.29-1.60], adjustment made for potential confounders).

In conclusion, Part I represents a further step in the pharmacogenomic prospect of tailoring antihypertensive therapy. Part II indicates that heightened attention to the digoxin-dose is warranted in women, that there is a need for awareness about P-glycoprotein interactions with digoxin, and that long-term therapy with digoxin is an independent risk factor for death among patients with atrial fibrillation without heart failure.

Keywords: pharmacogenomics, irbesartan, atenolol, hypertension, digoxin, RIKS-HIA, atrial fibrillation, heart failure

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To Yan and Alexandra

...och Omid. Jag glömmer inte.
List of Papers

This thesis is based on the following studies which will be referred to by their Roman numerals:


V The effect of digoxin on mortality – a cohort study of patients with atrial fibrillation, heart failure or both. Pär Hallberg, Johan Lindbäck, Bertil Lindahl, Ulf Stenestrand, Håkan Melhus. *Manuscript*.

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### Abbreviations

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<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ABC</td>
<td>ATP-binding cassette</td>
</tr>
<tr>
<td>ACC/AHA</td>
<td>American College of Cardiology/American Heart Association</td>
</tr>
<tr>
<td>ACE</td>
<td>angiotensin converting enzyme</td>
</tr>
<tr>
<td>AF</td>
<td>atrial fibrillation</td>
</tr>
<tr>
<td>ALAP</td>
<td>adipocyte-derived leucine aminopeptidase</td>
</tr>
<tr>
<td>Ang I / Ang II</td>
<td>angiotensin I / angiotensin II</td>
</tr>
<tr>
<td>ANP</td>
<td>atrial natriuretic peptide</td>
</tr>
<tr>
<td>ARB</td>
<td>Ang II type 1 receptor blocker</td>
</tr>
<tr>
<td>AT1,4</td>
<td>Ang II type 1-4</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>BP</td>
<td>blood pressure</td>
</tr>
<tr>
<td>CV</td>
<td>cardiovascular</td>
</tr>
<tr>
<td>CYP</td>
<td>cytochrome</td>
</tr>
<tr>
<td>DBP</td>
<td>diastolic blood pressure</td>
</tr>
<tr>
<td>EF</td>
<td>ejection fraction</td>
</tr>
<tr>
<td>EM</td>
<td>extensive metabolizer</td>
</tr>
<tr>
<td>ESC</td>
<td>European Society of Cardiology</td>
</tr>
<tr>
<td>ET-1</td>
<td>endothelin-1</td>
</tr>
<tr>
<td>HDL</td>
<td>high-density lipoprotein</td>
</tr>
<tr>
<td>HF</td>
<td>heart failure</td>
</tr>
<tr>
<td>HR</td>
<td>heart rate</td>
</tr>
<tr>
<td>HT</td>
<td>hypertension</td>
</tr>
<tr>
<td>I/D</td>
<td>insertion/deletion</td>
</tr>
<tr>
<td>IM</td>
<td>intermediate metabolizer</td>
</tr>
<tr>
<td>LBBB</td>
<td>left bundle branch block</td>
</tr>
<tr>
<td>LDL</td>
<td>low-density lipoprotein</td>
</tr>
<tr>
<td>LV</td>
<td>left ventricular</td>
</tr>
<tr>
<td>LVEF</td>
<td>left ventricular ejection fraction</td>
</tr>
<tr>
<td>LVMH</td>
<td>left ventricular hypertrophy</td>
</tr>
<tr>
<td>LVMi</td>
<td>left ventricular mass index</td>
</tr>
<tr>
<td>MI</td>
<td>myocardial infarction</td>
</tr>
<tr>
<td>NO</td>
<td>nitric oxide</td>
</tr>
<tr>
<td>NYHA</td>
<td>New York Heart Association</td>
</tr>
<tr>
<td>Pgp</td>
<td>P-glycoprotein</td>
</tr>
<tr>
<td>PM</td>
<td>poor metabolizer</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>RAAS</td>
<td>renin-angiotensin-aldosterone system</td>
</tr>
<tr>
<td>RIKS-HIA</td>
<td>Register of Information and Knowledge about Swedish Heart Intensive care Admissions</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>SBP</td>
<td>systolic blood pressure</td>
</tr>
<tr>
<td>SDC</td>
<td>serum digoxin concentration</td>
</tr>
<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
</tr>
<tr>
<td>TDM</td>
<td>therapeutic drug monitoring</td>
</tr>
<tr>
<td>TGF-β₁</td>
<td>transforming growth factor beta₁</td>
</tr>
</tbody>
</table>
Part I – Pharmacogenomics of antihypertensive treatment

Introduction

Diversity in response to antihypertensive therapy is well-documented\(^1\). Reasons include many variables in the biological system, such as body weight, age, sex, general condition of health, and genetic make-up.

Although individual human genomes are 99.9% identical, the 0.1% difference predicts as many as three million polymorphisms, the most common being the single nucleotide polymorphism (SNP). Many polymorphisms in the human genome will have no effect. Some, however, will affect protein expression or function, resulting in phenotypes affected for disease or with altered drug response.

Pharmacogenomics focuses on the link between polymorphism in genes and variable response to drugs. A gene is considered polymorphic when variants exist in a population with a frequency of at least 1\(^%\)^2. Today, most of the cases described are directly linked with metabolism modifications (mainly P450 cytochromes); however, studies have also observed the potential association between a genotype and the target of a given drug.

Blood pressure (BP) levels are maintained through complex interactions of many biochemical, physiological and anatomical traits organized into interrelated systems that exert redundant and counterbalancing pressor and depressor effects\(^3\). Although single factors may rarely cause BP to deviate into the hypertensive range, most hypertension (HT) has a multifactorial etiology that includes many genetic and environmental factors acting through the intermediate systems regulating BP level\(^4\). Antihypertensive drugs lower BP by acting on specific targets within these intermediate systems.

Since many components of the BP regulating systems are proteins that may vary in structure, configuration, or quantity because of genetic differences among individuals, it is reasonable to expect that interindividual variation in BP responses to these drugs would in part be genetically determined. Historically, the candidate gene approach to studies of genetic diseases has proven fruitful. Obvious candidate genes to influence BP responses are those that code for components of a system targeted by the
drug. Additional candidates are genes that code for components of the counter-regulatory systems opposing an initial drug-induced fall in BP.

The genetic approach to the study of the mechanisms underlying HT has led to the identification of some quantitative trait loci or genes that influence BP both in animal models and in patients, but relatively few examples of a pharmacogenomic approach to antihypertensive therapy are available. In particular, the association of different variants of angiotensin-converting enzyme (ACE) and angiotensinogen with the BP response to drugs interfering with the renin-angiotensin-aldosterone system (RAAS) has been studied.

The purpose of a clinical pharmacogenomic assay is to distinguish between those patients who are more and those who are less likely to respond to a drug, or conversely, those who are more and those who are less at risk for adverse events. With this information, better choices for drug therapies could be made to maximize the therapeutic response and to minimize the risk for adverse reactions.

Pharmacogenomics of antihypertensive treatment is still in its early stages, and a limited number of studies have been published so far. To better understand this field, I will first briefly review the pathophysiology of essential HT and the principles of its drug treatment. I will then continue with an overview of those pharmacogenomic studies of antihypertensive treatment which, to my knowledge, have been published so far and which deals with primarily two aspects: the BP lowering effect and the regression of left ventricular hypertrophy (LVH).
Hypertension

It is well established that HT increases the risk for cardiovascular (CV) and renal morbidity and mortality, and that control of elevated BP can significantly reduce these risks. Guidelines for HT management have accordingly become increasingly stringent and multifaceted. The recommended goal BP levels for patients with target organ disease, diabetes, or clinical CV disease have been revised downward, lower than the widely accepted threshold of 140/90 mm Hg. The need to use multiple antihypertensive agents to reach even the 140/90 mm Hg target BP has been demonstrated in several trials, and current recommendations call for antihypertensive therapy to provide protection against target organ damage, independent of BP reduction.

The prevalence of HT, defined as a BP >140/90 mmHg, was almost 29% in the United States in 1999-2000. During the same time, over 30% of all hypertensive individuals were unaware of their illness, 42% were not being treated, and 69% did not have their HT controlled. The increasingly rigorous guidelines for HT management and the apparent difficulties in achieving these goals in clinical practice, therefore, present considerable challenges.

CV sequelae imposed by HT occur at a two- to three-fold increased rate compared with normotensive persons of the same age. Coronary disease is the most common hazard of HT because of its greater incidence in the general population. Elevated BP has been found to be related to the development of CV disease in a continuous, graded fashion, with no indication of a critical value.

The 2003 report of the Seventh Joint National Committee on Detection, Evaluation, and Treatment of High BP (JNC VII) includes both systolic and diastolic levels in the classification of BP (table 1). The levels shown in table 1 should be based on at least two sets of readings over several weeks. HT is then categorized by either systolic or diastolic gradation into one of two stages. If the SBP and DBP correspond to different stages, the highest stage is used. Patients with preHT are at increased risk for progression to HT; those in the 130/80 to 139/89 mmHg range are at twice the risk to develop HT as those with lower values.

<table>
<thead>
<tr>
<th>CATEGORY</th>
<th>SYSTOLIC, MM HG</th>
<th>DIASTOLIC, MM HG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt;120</td>
<td>and &lt;80</td>
</tr>
<tr>
<td>PreHT</td>
<td>120-139</td>
<td>or 85-89</td>
</tr>
<tr>
<td>HT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 1</td>
<td>140-159</td>
<td>or 90-99</td>
</tr>
<tr>
<td>Stage 2</td>
<td>≥160</td>
<td>or ≥100</td>
</tr>
</tbody>
</table>

*Table 1. Classification of BP for adults ≥18 years according to JNC VII.*
The pathophysiology of HT

There is still much uncertainty about the pathophysiology of HT. A small number of patients (between 2% and 5%) have an identifiable cause for their raised BP (such as renal or adrenal disease)\(^9\). In the remainder, however, no clear single identifiable cause is found and their condition is labelled "essential HT".

A number of physiological mechanisms are involved in the maintenance of normal BP, and their derangement may play a part in the development of essential HT (fig 1). It is probable that a great many interrelated factors contribute to the raised BP in hypertensive patients, and their relative roles may differ between individuals.

![Diagram of factors involved in the pathogenesis of essential HT.](image)

**Figure 1.** An oversimplified scheme of some of the factors involved in the pathogenesis of essential HT.

1) RAAS and the kallikrein-kinin system

a) RAAS

The RAAS may be the most important of the endocrine systems that affect the control of BP\(^10\). Renin is secreted from the juxtaglomerular apparatus of the kidney in response to glomerular underperfusion or a reduced salt intake.
It is also released in response to stimulation from the sympathetic nervous system.

Renin is responsible for converting angiotensinogen to angiotensin I (Ang I), a physiologically inactive substance which is rapidly converted to angiotensin II (Ang II) by ACE in tissues such as the lungs. ACE is also responsible for the catabolism of various biologically important peptides (e.g., bradykinin) into inactive metabolites. Ang II is a potent vasoconstrictor and thus causes a rise in BP. In addition, it stimulates the release of aldosterone from the zona glomerulosa of the adrenal gland, which results in a further rise in BP related to Na\(^+\) and water retention.

The circulating RAAS is not thought to be directly responsible for the rise in BP in essential HT. In particular, many hypertensive patients have low levels of renin and Ang II (especially elderly and black people). There is, however, increasing evidence that there are important non-circulating "local" ACE-independent renin-angiotensin epicrine or paracrine systems, which also control BP. Angiotensinogen can be converted directly to Ang II by enzymes, such as tissue plasminogen activator, cathepsin G, and tonin, whereas chymostatin-sensitive Ang II-generating enzyme, chymase, and cathepsin G are able to catalyze the hydrolysis of Ang I to Ang II. Local renin systems have been reported in the kidney, the heart, and the arterial tree.

Regardless of the pathway by which it is formed, Ang II mediates its physiologic effects by a final common step: binding to highly specific receptors located on the cell membrane. In humans, at least four Ang II receptors have been described: Ang II type I (AT\(_1\)), AT\(_2\), AT\(_3\), and AT\(_4\). Only the first two of these have been well defined. The two latter have been proposed based on operational criteria, but their transduction mechanism are unknown and they have not yet been cloned. The AT\(_1\) receptor mediates most of the known physiological actions of Ang II. The AT\(_2\) receptor is found primarily during fetal development and appears to mediate programmed cell death or apoptosis. Despite belonging to the same receptor family, the AT\(_1\) and AT\(_2\) receptor subtypes differ markedly in their signalling cascades and biologic activities.

Virtually all of the known regulatory actions of Ang II on BP and osmoregulation have been attributed to the AT\(_1\) receptor. These include vasoconstriction, aldosterone and vasopressin release, renal tubular Na\(^+\) reabsorption, and decreased renal blood flow. AT\(_1\) receptor stimulation has been shown to mediate cell growth and proliferation of vascular smooth muscle cells, cardiomyocytes, and coronary endothelial cells. Accordingly, the AT\(_1\) receptor has been implicated in various CV, renal, and cerebral pathologies, such as LVH, vascular media hypertrophy, cardiac arrhythmias, atherosclerosis, glomerulosclerosis, stroke, and dementia.
b) The kallikrein-kinin system

Kinins are vasodepressor autacoids that play an important role in the regulation of CV and renal function. In mammals, the main kinins are bradykinin and lysyl-bradykinin (kallidin). They are released from substrates known as kininogens by serine protease enzymes known as kininogenases. The main kininogenases are plasma and tissue (glandular) kallikrein. These are separate enzymes that differ in function and are encoded by different genes. Kinins are destroyed by enzymes known as kininases, located mainly in the endothelial cells of the capillaries of the lungs and other tissues. Examples of kininases include ACE, neutral endopeptidases 24.11 and 24.15, and carboxypeptidases.

Kinins act mainly as local autocrine and paracrine hormones via two different types of receptor, B1 and B2. B1 receptors are expressed primarily during administration of lipopolysaccharides (such as endotoxin) and in inflammation. Most of the known effects of kinins are mediated by B2 receptors. This receptor belongs to a family of peptide hormone receptors with seven membrane-spanning regions linked to G proteins. Prostaglandins and nitric oxide (NO) mediate some of the effects of kinins.

Renal kallikrein is located in the connecting cells of the tubules; kinin receptors are present in the collecting ducts. Kinins play a role in regulation of the renal microcirculation and water and Na⁺ excretion. The natriuretic and diuretic effects of kinins are mediated in part by prostaglandin E₂.

Decreased activity of the kallikrein-kinin system may play a role in HT. Low urinary kallikrein excretion in children is one of the major genetic markers associated with a family history of essential HT, and children with high urinary kallikrein excretion have less probability of a genetic background of HT. Urinary kallikrein excretion is decreased in various models of genetic HT. Mice in which the bradykinin B2 receptor is deleted by homologous recombination (gene knockout) develop HT when fed a high-Na⁺ diet. Thus, low kinin activity may be involved in the development and maintenance of salt-sensitive high BP.

Components of the kallikrein-kinin system, especially tissue kallikrein, are present in the heart, arteries, and veins. Kinins are found in the venous effluent of isolated perfused hearts, and their release is rapidly increased during ischemia.

2) The adrenergic system

The cathecolamines epinephrine and norepinephrine act in the body as neurotransmitters and hormones. Norepinephrine is the predominant
neurotransmitter, whereas epinephrine is the major hormone. Cathecolamines are released from the nervous system following depolarization. Sympathetic nervous system stimulation causes increased HR and heart contractility, arteriolar constriction and decreased blood supply to the splanchnic bed, the skin and the kidneys, and metabolic changes including increased hepatic glycogenolysis and lipolysis in fatty tissue. Thus the autonomic nervous system has an important role in maintaining a normal BP.

Cathecolamines exert their cellular action via binding to adrenoceptors through G-proteins. Studies have revealed many subtypes of adrenoceptors such as the $\alpha_{1a}$, $\alpha_{1b}$ of the $\alpha_1$ receptors, the $\alpha_{2a}$, $\alpha_{2b}$, $\alpha_{2c}$, $\alpha_{2d}$, $\alpha_{2e}$ and $\alpha_{2f}$ types of the $\alpha_2$ receptors, and $\beta_1$, $\beta_2$ and $\beta_3$ of the $\beta$ receptors.

Although there is growing evidence that essential HT is commonly neurogenic and is initiated and sustained by overactivity of the sympathetic nervous system, the precise causal mechanisms leading to sympathetic augmentation in hypertensive subjects are still not entirely clear. Among other, possible mechanisms include increased sympathetic nerve firing rates, altered neuronal norepinephrine reuptake, diminished arterial baroreflex buffering of sympathetic nerve traffic, and facilitation of norepinephrine release by neurohumoral factors such as Ang II. An increased sympathetic activity is thought to, at least in part, both initiate and sustain the BP elevation. High renal sympathetic tone contributes to HT development by stimulating renin secretion and through promoting renal tubular reabsorption of Na+. The effects of cathecolamines are important, not least because drugs that block the sympathetic nervous system do lower BP and have a well established therapeutic role. It is probable that HT is related to an interaction between the autonomic nervous system and the RAAS, together with other factors, including, e.g., Na$^+$ and circulating volume.

3) The vascular endothelium

Vascular endothelial cells play a key role in CV regulation by producing a number of potent local vasoactive agents, including the vasodilator NO and the vasoconstrictor peptide endothelin. Dysfunction of the endothelium has been implicated in human essential HT.

Stimulation of endothelial cells by acetylcholine, other agonists, and physical stimuli induces the release of NO in vascular smooth muscle cells, resulting in relaxation of vascular tone. Patients with essential HT have an impaired endothelium-dependent vascular relaxation in their arteries.

Studies have shown that acetylcholine-mediated vasodilation, linked to a defect in the NO pathway, is reduced in normotensive subjects with a familial history of essential HT, a finding that suggests that endothelium
dysfunction can precede the appearance of HT and that this abnormality might play a role in the pathogenesis of essential HT. Prostacyclin is a strong vasodilator that inhibits the growth of vascular smooth muscle cells and is also the most potent endogenous inhibitor of platelet aggregation. Therefore, it has been considered to play an important role in cardiovascular disease. Prostacyclin synthase is abundantly expressed in vascular endothelial and smooth muscle cells, and prostacyclin has been shown to inhibit collagen expression.

Modulation of endothelial function is an attractive therapeutic option in attempting to minimize some of the important complications of HT. Clinically effective antihypertensive therapy appears to restore impaired production of NO, but does not seem to restore the impaired endothelium dependent vascular relaxation or vascular response to endothelial agonists.

4) Renal Na\(^+\) handling

Many mechanisms affecting Na\(^+\) transport are involved in the maintenance of a normal BP. Human renal transplant studies show that there is a genetic component to renal factors that mediate essential HT. For example, previously normotensive renal transplant recipients without a family history of essential HT who receive a kidney from a donor with a family history of essential HT, compared with a donor without a family history of essential HT, develop HT more frequently and require more medication for BP control.

Data suggest that Na\(^+\) intake in excess of that needed to maintain normal extracellular fluid volume is necessary but not sufficient for HT to be manifest.

Because many subjects with essential HT do not reduce BP in response to dietary Na\(^+\) restriction, mechanisms other than Na\(^+\) intake must mediate their high BP. Some investigators postulate that essential HT is due to heterogenous nephron perfusion with narrowing of afferent arterioles of a minority of nephrons leading to local release of vasoconstrictors including Ang II. This phenomenon would cause vasoconstriction in adjacent normal glomeruli due to local release of Ang II and possibly other vasoconstrictors, and due to increased perfusion pressure to all afferent arterioles because of the increased systemic BP. Some studies suggest that an excess of other vasoconstricting substances or a deficit of vasodilating substances contribute to essential HT in some subjects. Some subjects with essential HT have increased plasma levels of arginine vasopressin, and selective inhibition of the V1 receptor reduces BP in these individuals, supporting a causal role for this mechanism in their HT. Still other studies show that HT itself reduces tonic release of NO, and that some subjects with essential HT have a primary
defect in agonist-induced NO\textsuperscript{25}. Also, atrial natriuretic peptide (ANP) is a hormone secreted from the atria of the heart in response to increased blood volume\textsuperscript{33}. Its effect is to increase Na\textsuperscript{+} and water excretion from the kidney as a sort of natural diuretic. A defect in this system may cause fluid retention and HT. In response to increases in blood volume or BP, ANP is released from the heart and act through natriuretic peptide receptor A (NPRA) in the kidneys, adrenals, and vasculature to increase natriuresis, diuresis, and vasorelaxation. Levels of ANP are elevated in both human patients and animal models of heart failure (HF) and cardiac hypertrophy.

5) Oxidative stress

Large amounts of reactive oxygen species (ROS), derived from oxygen, are produced in vascular cells, including superoxide (\cdot O\textsubscript{2}\textsuperscript{-}) and hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), and act as important intracellular signals. Oxidative stress describes the injury caused to cells by the oxidizing of macromolecules resulting from increased formation of ROS and/or decreased antioxidant reserve. A growing number of reports have provided a critical role for oxidative stress in the pathogenesis of CV diseases, including HT\textsuperscript{34}.

An enhanced production of ROS contributes to the dysregulation of physiological processes, which leads to structural and functional alterations in HT\textsuperscript{34}. For instance, an enhanced production of ROS causes a loss of NO bioavailability, which impairs endothelial function, causing (among others) a decreased endothelium-dependent vasodilation\textsuperscript{34}.

Studies also indicate that low-density lipoprotein (LDL) cholesterol and, in particular, its oxidative derivatives are injurious to the endothelium, whereas high-density lipoprotein (HDL) has been shown to prevent the oxidative modification of LDL\textsuperscript{35}.

Also, hyperhomocysteinemia is a known independent risk factor for the development of atherosclerotic vascular disease\textsuperscript{36}. In fact, increased plasma levels of homocysteine has been shown to damage endothelial cells through various mechanisms, including H\textsubscript{2}O\textsubscript{2} generation and the formation of oxidized lipids and proteins.

6) Metabolic abnormalities

There is a clustering of several risk factors, including obesity, HT, glucose intolerance, diabetes mellitus and hyperlipidemia, which is observed more frequently than by chance alone\textsuperscript{37}. This has led to the suggestion that these represent a single syndrome which is referred to as the metabolic syndrome.
Also, several reports have disclosed the relationship between essential HT and insulin resistance. Subjects with essential HT are more insulin resistant than normotensives.

Treatment of HT

HT is a major medical problem and, in spite of huge efforts, it still constitutes an important risk factor for CV morbidity and mortality. Antihypertensive treatment reduces morbidity and mortality for all stages of HT. Overall, the risk of all-cause mortality is reduced by 31%, and the risk of CV mortality by 60% among those on long-term therapy. More than half of the decline in coronary heart disease mortality in women and one-third to one-half of the decline in men could be attributed to improvements in risk factors, such as hypercholesterolemia, smoking, and HT.

Optimal CV protection in HT requires more than lowering the BP. Other potential risk factors to be taken into account are age, sex, smoking, diabetes, low level of exercise, obesity and high serum cholesterol levels. These factors often interact with, and even amplify each other. This explains the well-described high risk for CV morbidity that remains in treated hypertensives in spite of BP reduction, and underlines the importance of a multifactorial approach to the treatment of HT.

Treatment strategy

The algorithm suggested in JNC-VII for managing HT is shown in figure 2. Factors to be considered in selecting the appropriate drug for initial therapy include presence or absence of clinical CV disease, concomitant but unrelated symptoms or diseases, quality of life, and cost.

Most patients with HT will respond to one (approximately 50%) or two (approximately 30%) antihypertensive medications. Failure to respond to treatment suggests an identifiable cause of HT. Among patients who do not have a secondary cause of HT, inadequate drug treatment and noncompliance are among the most common causes of resistant HT.
Figure 2. Algorithm for the treatment of HT according to JNC VII.

Pharmacologic treatment of HT

Figure 3 in a simplified manner shows the principal mechanisms of action of the various antihypertensive agents most commonly used today.
Figure 3. Oversimplified scheme of the target sites of the main types of antihypertensive drugs. Only principal sites of action are given. Crossed arrows or “-“ denotes inhibition, arrows with dotted lines enzymatic reactions. ACE=angiotensin converting enzyme; ARBs=Ang II type 1 receptor blockers; AT1=Ang II type 2 receptor; AT2=Ang II type 2 receptor; TGFβ1=transforming growth factor β1.

Diuretics

Diuretics differ in structure and major site of action within the nephron. Thiazide diuretics, the first well-tolerated, orally effective antihypertensives, have enjoyed wide usage and popularity. They act by inhibiting Na⁺ and Cl⁻ cotransport across the luminal membrane of the early segment of the distal convoluted tubule, where 5 to 8% of filtered Na⁺ is normally reabsorbed. Plasma and extracellular fluid volume are thereby shrunken, and cardiac output (CO) falls.

Loop diuretics primarily block Cl⁻ reabsorption by inhibition of the Na⁺/K⁺/Cl⁻ cotransport system of the luminal membrane of the thick ascending limb of Henle’s loop, the site where 35 to 45% of filtered Na⁺ is
reabsorbed. Therefore, the loop diuretics are more potent and have a more rapid onset of action than do the thiazides\(^46\).

\(\text{K}^+\)-sparing agents act in the distal tubule to prevent \(\text{K}^+\) loss, spironolactone as an aldosterone antagonist, the others (i.e. amiloride) as direct inhibitors of \(\text{K}^+\) secretion\(^47\). By themselves, \(\text{K}^+\)-sparing agents are relatively weak antihypertensives. They are effective in reducing diuretic-induced \(\text{K}^+\) wastage, but progressive hypokalemia may still occur with their use\(^48\).

\(\beta\)-adrenoceptor blockers

Although there is no consensus as to the mechanisms by which \(\beta\)-blocking drugs lower BP, it is probable that some or all of the modes of action listed in table 2 are involved\(^49\).

**Proposed Mechanisms to Explain the Antihypertensive Actions of \(\beta\)-blockers**

| 1. | Reduction in CO |
| 2. | Central nervous system effect |
| 3. | Inhibition of renin |
| 4. | Reduction in venous return and plasma volume |
| 5. | Reduction in peripheral vascular resistance |
| 6. | Improvement in vascular compliance |
| 7. | Resetting of baroreceptor levels |
| 8. | Effects on prejunctional \(\beta\)-receptors: reduction in norepinephrine release |
| 9. | Attenuation of pressor response to catecholamines with exercise and stress |

Table 2.

\(\beta\)-blockers, alone and in combination with other antihypertensives, reduce BP in patients with combined systolic and diastolic HT and in most patients with isolated systolic HT in the elderly\(^30\). Some \(\beta\)-blockers are also found to reduce the risk of mortality in survivors of acute MI\(^51\).

Most antihypertensive drugs, including \(\beta\)-blockers, reduce left ventricular (LV) mass and wall thickness. It is not known, however, whether reversal of HT-induced cardiac hypertrophy improves the independent risk of CV morbidity and mortality associated with LVH.

**Calcium channel blockers**

Calcium channel blockers interact with the same calcium channel: the L-type voltage-gated plasma membrane channel\(^52\). They exhibit differences in their
structure and CV effects\textsuperscript{53}. The ability of calcium channel blockers to diminish cytosolic calcium concentrations within vascular smooth muscle cells probably explains their vasodilatory properties. Calcium channel blockers are more effective in constricted than in nonconstricted vascular beds, and greater vasodepressor responses occur in patients with higher levels of BP. Calcium channel blockers facilitate natriuresis, probably by improving renal blood flow, diminishing renal tubular Na\textsuperscript{+} reabsorption, and interfering with aldosterone secretion.

Diltiazem and verapamil are nonselective. At equivalent concentrations, they induce vasodilation, depress cardiac contractility, and inhibit atrioventricular conduction.

Dihydropyridines are predominantly vasodilators. The first generation, exemplified by nifedipine, had modest effects on cardiac contractility. The second generation, exemplified by amlodipine, is more vascular-selective vasodilators with no effect on cardiac contractility.

\textit{ACE inhibitors}

ACE inhibitors block the conversion of Ang I to Ang II.

The most obvious manner by which ACE inhibitors lower the BP is to markedly reduce the circulating levels of Ang II, thereby removing the direct vasoconstriction induced by this peptide. Some of the effects of ACE inhibitors may be mediated via their inhibition of the breakdown of bradykinin\textsuperscript{54}. In addition, multiple other effects likely contribute to the antihypertensive effect:

\begin{itemize}
  \item a decrease in aldosterone secretion\textsuperscript{55}, which may cause natriuresis or at least a lack of reactive renal Na\textsuperscript{+} retention as the BP falls;
  \item blunting of the expected increase in sympathetic nervous system activity typically seen after vasodilation\textsuperscript{56};
  \item suppression of endogenous endothelin secretion\textsuperscript{57};
  \item improvement in endothelial dysfunction\textsuperscript{58}
\end{itemize}

\textit{Ang II type 1 receptor blockers}

Ang II type 1 receptor blockers (ARBs) lower BP by blocking the action of Ang II, the main peptide effector of the RAAS\textsuperscript{59}. Ang II contributes in two major ways to the clinical picture of HT: it raises BP through its direct and indirect vasoconstrictor actions, and it has trophic actions on the heart and blood vessels that might contribute directly to vascular structural change and to CV and renal events.
ARBs selectively block the binding of Ang II to the AT₁ receptor, thereby reducing vascular resistance and lowering BP⁶⁰. Ang II may continue to bind with the AT₂ receptor, which may further counteract the harmful effects of AT₁ receptor stimulation⁶¹.
LV hypertrophy

HT is an established independent and modifiable promoter of CV disease. It damages and compromises the vascular supply to the heart, brain, kidneys and limbs. It also directly alters the structure and function of the myocardium by promoting LVH. LVH is a major and independent risk factor for CV morbidity and mortality. The risk of adverse events such as HF, MI and stroke increases when HT induces LVH. A population-based investigation of individuals with essential HT suggests that for each 39 g increase in LV mass per m², there is a 40% increase in CV events.

Morphological studies indicate detrimental structural remodelling of the hypertensive heart. These include a disproportionate accumulation of fibrillar collagen in arteriolar adventitia and interstitial necrotic and microscopic myocardial scars replacing necrotic myocytes. There is also some evidence suggesting that LVH may be associated with the development of HT indicating that there may be a common factor that is promoting both. Alterations in the RAAS and catecholamines have been implicated in both the etiology of HT and the development of LVH. Hemodynamic stress, both pressure and volume overload, is fundamental to the development of LVH; however, a host of nonhemodynamic factors contribute, with the RAAS implicated strongly in the hypertrophic response. Also, data concerning the involvement of the sympathetic nervous system are growing. Although studies have found that LVH is induced by administration of catecholamines in vivo, hypertrophy of cultured adult myocardial cells was not induced by catecholamines in vitro. A recent study of hypertensive patients, however, demonstrated that hypertensive LVH is correlated with increased sympathetic activity largely confined to the heart, suggesting that trophic effects of increased cardiac sympathetic nerve firing and norepinephrine release are related to the development of hypertensive LVH.

The RAAS was initially viewed as a hormonal system predominantly involved in BP and volume regulation. More recently, RAAS activation has been understood to be an important inducer of tissue hypertrophy and interstitial fibrosis, with Ang II acting as a potent growth factor in vascular smooth muscle cells and cardiac myocytes. Animal studies have provided evidence that supports the importance of Ang II in the fibrous tissue response. Blocking of the RAAS not only has important quantitative effects on myocyte hypertrophy, but also has cardioprotective and cardio reparative properties that affect the development and reversal of pathologic fibrosis.

Interestingly, the AT2-receptor appears to down-modulate actions mediated by the AT1-receptor and results in decreased cellular proliferation, decreased levels of serum arginine vasopressin, or decreased vasoconstrictor
Although the function of the AT₂-receptor is less well understood than that of the AT₁-receptor, evidence of up-regulation of AT₂-receptors in pathologic CV conditions\(^7\) suggest a function of the AT₂-receptor to counteract the growth-promoting effects of the AT₁-receptor.

Although the prognostic implications of the regression of LVH are far from established\(^7\), the risk for CV events in individuals with persistent LVH has been shown to be greater than in subjects in whom LVH has resolved\(^7\). Therefore, it would be of interest to treat hypertensive patients with LVH with drugs that decrease LVH more compared to other drugs. There is no doubt that antihypertensive therapy is able to cause regression of LVH but, even within a relatively homogenous group, individual responses vary greatly. The understanding of the molecular basis of LVH may provide us with new and more specific pharmacological agents, and perhaps the ability to individualize treatment and maximize the reduction in risk of morbidity and mortality from CV disease.

Pharmacogenomic studies of HT – an overview

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<th>RAAS + kallikrein-kinin</th>
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<th>Metabolic abnormalities</th>
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</table>

Table 3. An overview of the number of pharmacogenomic studies of antihypertensive therapy as of January 2004.

1) RAAS and the kallikrein-kinin system

ACE

Studies have reported discordant influence of polymorphisms in the RAAS. The by far most studied is the insertion/deletion (I/D) polymorphism of the ACE gene\(^7\). While the association between the D allele and an increased
serum and tissue level of ACE is rather well established, the association with HT as well as its accompanied target organ damages is less clear. The D allele has been shown to be associated with an increased risk of coronary artery disease, myocardial infarction (MI), hypertrophic cardiomyopathy and LVH, but other studies have produced different results. Also, studies of the association with HT have produced contradictory results.

Differences may be the result of the heterogenous genetic makeup and allele frequencies of different populations, environmental factors and other confounders, as well as the usually low number of individuals included in the studies. This can also be said about the pharmacogenomic studies which have investigated the influence of the ACE I/D polymorphism on antihypertensive response. Some studies have found a positive correlation to the D allele and response to antihypertensive therapy, others to the I allele, and still others have found no influence of the polymorphism, again addressing the difficulties in reproducing results. One study has reported a sex-dependent association between the polymorphism and response to hydrochlorothiazide, hypertensive women with the II genotype and hypertensive men with the DD genotype having the largest fall in BP.

Recently, by far the most comprehensive study was published by Harrap et al., who reported data from the PROGRESS trial. This large-scale randomized trial was designed to determine the effects of an ACE inhibitor, perindopril, on the risks of major vascular events among individuals with a history of stroke or transient ischemic attack. No association between ACE genotype and any CV endpoint was found. This study may have produced the final word in the discussion about the association between the ACE I/D polymorphism alone on therapeutic response to ACE-inhibitors. However, one cannot yet rule out an impact of the polymorphism in conjunction with others. Also, one can only draw conclusions from the specific category of patients studied.

In addition, most studies have focused on the therapeutic response to ACE-inhibitors, whereas only a few have used beta-blockers, calcium channel blockers, and diuretics. Another aspect of pharmacogenomics is studies of drug related side effects. So far, few studies have had this approach. One study in Chinese subjects have found an association between the ACE II genotype and a higher incidence of ACE inhibitor-related cough. However, the association has been disputed by others.

The AT1-receptor

The second most studied polymorphism in the RAAS is the AT1-receptor gene polymorphism A1166C. Presence of the C allele has been associated with e.g., essential HT and increased LV mass. Again, inconclusive and
contradictory results from pharmacogenomic studies have appeared, showing a positive association with antihypertensive response with the C allele, the A allele, or no association with either in response to infused Ang II.

Angiotensinogen

Studies of the third most investigated polymorphism, the angiotensinogen M235T polymorphism, have also produced contradictory results. The T variant was found to be more frequent in patients with HT, and the TT genotype to be associated with a significantly higher angiotensinogen level. Pharmacogenomic studies have again produced divergent results, in some demonstrating a positive association between the T allele and response to antihypertensive therapy, and in others a negative association. Another polymorphism in the angiotensinogen gene, the T174M, has also been associated to antihypertensive response in one study.

Aldosterone synthase

A fourth polymorphism that has appeared in studies is the T-344C aldosterone synthase (CYP11B2) gene polymorphism. The T-allele has been associated with HT, whereas hypertensive subjects with the CC genotype were characterized by a pattern of early eccentric LV hypertrophy in another study. One pharmacogenomic study has found a positive association between the C-allele and a more profound response to antihypertensive treatment, whereas another found the opposite.

Transforming growth factor beta1

Transforming growth factor beta1 (TGF-β1) regulates extracellular matrix production, and overproduction has been associated with cardiac hypertrophy and histopathological changes such as accumulation of extracellular matrix protein in the heart. In addition, a recent study showed that patients with essential HT have higher plasma-levels of TGF-β1 than normotensive controls. In the same study, hypertensives with target organ damage such as LVH had higher levels than hypertensives with no target organ damage. There is growing evidence that cardiac fibrosis induced by Ang II may be mediated by TGF-β1. Both ACE inhibitors and ARBs can reduce the TGF-β1 mRNA levels in hypertensive rats. In a recent study, Yu et al. demonstrated that treatment with an ARB was associated with a reduced TGF-β1 expression in the heart after acute MI. Production of TGF-β1 is in part under genetic control. The G+915C SNP, which changes codon 25 in the signal sequence and substitutes an
arginin for a proline in the protein, is functionally associated with TGF-β₁ production. Awad et al. showed that lymphocytes from individuals homozygous for the G allele have a higher production of TGF-β₁ in vitro than heterozygotes.

In a study comparing the efficacy of the ARB irbesartan vs the beta-blocker atenolol in patients with essential HT and LVH, LV mass index (LVMI) change depended on genotype in the irbesartan group. Regression of LVMI in this group was about two-fold in patients carrying the C allele compared with patients with the G/G genotype at 48 weeks of treatment. In the atenolol group, on the other hand, LVMI change did not differ between the genotypes.

**Aminopeptidases**

Aminopeptidases play a role in the metabolism of several peptides that may be involved in BP regulation and the pathogenesis of HT. Adipocyte-derived leucine aminopeptidase (ALAP) has recently been identified as a member of the M1 family of zinc-metallopeptidases. The peptidase hydrolyzes a variety of bioactive peptides in vitro, including Ang II and kallidin, and is widely expressed in human tissues. The enzyme is thought to play a role in the regulation of BP through inactivation of Ang II and/or generation of bradykinin.

Recently, the Arg528 variant of a Lys528Arg (A1583G) polymorphism in the ALAP gene was shown to be associated with essential HT. Since ALAP seems to be particularly abundant in the heart, it could be involved in the modulation of LVH. In one pharmacogenomic study of patients with essential HT and LVH given the ARB irbesartan or the beta-blocker atenolol, regression of LVH was markedly greater among irbesartan treated patients, suggesting a role for this polymorphism in the determination of ARB efficacy.

**2) The adrenergic system**

Two frequent polymorphisms in the gene encoding the β₁-adrenoeceptor have been found, Ser49Gly and Arg389Gly.

Concerning the Ser49Gly polymorphism, in vitro studies have shown that the 49Gly variant of the receptor had a more profound adenylyl cyclase activity on agonist stimulation, was more sensitive to the inhibitory effect of the antagonist metoprolol and showed a greater down-regulation on long-term agonist stimulation, suggesting that the Gly49 variant of the β₁-AR may be associated with an inherent cardioprotective effect. However, the
polymorphism did not influence the BP or HR reduction in patients with essential HT and LVH in one pharmacogenomic study.\textsuperscript{136}

Concerning the Arg389Gly polymorphism, in vitro studies have demonstrated that this polymorphism alters the function of the receptor so that the Gly389-variant exhibits lower basal levels of adenylyl cyclase activity and reduced responsiveness upon agonist stimulation.\textsuperscript{137} This finding suggests that the polymorphism could influence BP level and HR, as well as an individual’s response to β\textsubscript{1}-blockade. A large case-control association study by Bengtsson et al in Scandinavians identified individuals homozygous for the Arg389 allele as having increased risk to develop HT.\textsuperscript{138} One pharmacogenomic study found a 3-fold greater reduction in DBP among hypertensives homozygous for the Arg389 variant treated for 4 weeks with metoprolol,\textsuperscript{139} and another found a greater improvement of LV EF among Arg389-homozygote patients with HF as compared to Gly389-homozygotes during treatment with the combined α- and β-adrenergic receptor blocker carvedilol.\textsuperscript{140} However, the polymorphism did not affect BP response to treatment with atenolol\textsuperscript{136, 141} or bisoprolol\textsuperscript{141} among hypertensives in other studies. The latter is disputed in one other study, showing a greater response in Arg389 homozygotes.\textsuperscript{142}

\textit{G proteins}

The G\textsubscript{s} protein system is essential to the activation of adenylyl cyclase in cardiac and vascular smooth muscle and therefore plays an important role in neuroendocrine regulation of CO and peripheral resistance.\textsuperscript{143} Abnormalities of G\textsubscript{s} proteins have been an obvious target for investigation in essential HT. In the CV system, the α-subunit of G\textsubscript{s} couples β\textsubscript{1} and β\textsubscript{2} adrenoceptors to the stimulation of cAMP production. A common silent polymorphism (ATT → ATC, Ile131) that has been identified in exon 5 of the α-subunit of the G\textsubscript{s} protein, was found to be related to the level of untreated SBP in white hypertensives, and also to BP response to β-blockade.\textsuperscript{144} Since the polymorphism is silent, the finding may suggest that a functional trait locus may be present in or near the α-subunit of the G\textsubscript{s} gene.

A C825T polymorphism has been described in exon 10 of the gene encoding the β\textsubscript{3}-subunit of G proteins (GNB3),\textsuperscript{145} resulting in a shortened splice variant that gives rise to enhanced signal transduction via pertussis toxin-sensitive G proteins. The T allele has been associated with low plasma renin,\textsuperscript{146} and impaired LV diastolic filling, an early marker of hypertensive heart disease, in hypertensive subjects.\textsuperscript{147} One pharmacogenomic study found a greater BP lowering response to hydrochlorothiazide in hypertensives who were TT homozygotes, as compared to CC homozygotes.\textsuperscript{148} Another found a greater reduction in SBP,
total peripheral resistance and pulse wave velocity in response to clonidine in carriers of the T allele among a group of young, healthy male subjects.  

3) The endothelial system

Endothelin

The endothelins are a family of peptides that are extremely potent vasoconstrictors. Endothelin-1 (ET-1), the major isoform in the vascular endothelium, is generated in two steps from the precursor preproET-1. The precursor is converted into the polypeptide bigET-1, from which ET-1 is cleaved by endothelin-converting enzymes. ET-1 exerts both arterial and venous vasoconstriction, direct positive inotropic and chronotropic effects on the heart, and hypertrophic effects on vascular smooth muscle cells, fibroblasts and isolated cardiomyocytes. The effects of ET-1 appear to be mediated through two receptor subtypes, ETA and ETB. Both ETA- and ETB-receptors are present in the human myocardium.

HT has been associated with increased ET-activity. Plasma ET-1 levels are higher in patients with essential HT than in normotensive subjects, and parallels the degree of cardiac hypertrophy.

The G5665T polymorphism of the preproET-1 gene causes a Lys/Asn change at codon 198 of the protein. Studies have shown that overweight patients carrying the T-allele have higher BP than those with the G/G genotype. Another study demonstrated that SBP during pregnancy was higher among carriers of the T allele in a group consisting of normal and pre-eclamptic women, and that plasma ET-1 levels were significantly higher among T/T homozygotes.

There was a significant difference between genotypes in the reduction of SBP among men at 12 weeks of antihypertensive treatment in one pharmacogenomic study of patients with essential HT and LVH given either irbesartan or atenolol. Carriers of the T-allele responded in average with a more than two-fold greater reduction than those with the G/G genotype, irrespective of treatment.
4) Renal Na\(^+\) handling

The epithelial Na\(^+\) channel

The amiloride-sensitive epithelial Na\(^+\) channel is composed of three subunits, α, β and γ, with similar structures\(^{157}\). The α subunit supports Na\(^+\) conductance when expressed alone, whereas the β and γ subunits, which by themselves do not support Na\(^+\) conductance, greatly enhance channel activity when expressed in conjunction with the α subunit. Liddle's syndrome is caused by mutations of subunits of the epithelial Na\(^+\) channel that result in increased Na\(^+\)-channel activity in the distal renal tubule with excess Na\(^+\) reabsorption\(^{158}\). This Na\(^+\) retention causes the high BP and the characteristic suppression of the RAAS seen in Liddle's syndrome. High BP in these patients responds well to reduction of salt intake or to amiloride, which acts specifically to reduce the activity of the abnormal channels.

The clinical features of Liddle's syndrome overlap with those of some patients with essential HT. In particular, black patients with HT are known to be sensitive to changes in salt intake and have low plasma renin activity. Na\(^+\)-channel activity is increased in lymphocytes from patients with the T594M mutation of the Na\(^+\) channel β subunit. Therefore, it is possible that this Na\(^+\)-channel mutation in patients with essential HT could contribute to the rise in BP by increasing renal tubular Na\(^+\) reabsorption. Among black London people the T594M mutation occurs more frequently in people with HT than those without\(^{158}\). Also, black patients carrying the mutated allele had a pronounced antihypertensive effect of monotherapy of amiloride, a drug usually considered a weak antihypertensive agent\(^{159}\).

\(\alpha\)-adducin

The cytoskeletal protein adducin is a heterodimer or heterotetramer of α and β subunits that is critical for the assembly of the actin-spectrin network and has been implicated in cell-signal transduction\(^{160}\). The 460Trp allele was associated with a larger BP increase after saline infusion\(^{161}\), faster proximal tubular reabsorption\(^{162}\), lower plasma renin activity, and larger BP fall after diuretics\(^{163}\). The 460Trp allele has also been associated with greater response to hydrochlorothiazide in two pharmacogenomic studies of patients with essential HT\(^{89, 164}\). In another study, hypertensive patients carrying the 460Trp variant and who were treated with diuretics had a lower risk of the combined outcome of MI and stroke than patients treated with ACE-inhibitors, beta-blockers or Ca-antagonists\(^{160}\).
Drug metabolism

Many factors, such as dietary intake, age, and concurrent drug therapies, affect a person’s response to medications. Importantly, genetic makeup determines inherent pharmacokinetics, and gives rise to interpersonal differences in drug absorption, distribution, metabolism, and excretion. Some of these differences can be explained by polymorphisms of genes encoding proteins which affect drug absorption (e.g., P-glycoprotein and organic anion transporting polypeptide), and cytochrome (CYP) P450 or phase II drug-metabolising enzymes (acetyltransferase 2 or thiopurine S-methyltransferase), which affect drug metabolism.

Genotype and Phenotype

Genotype is the term denoting the genetic constitution of an individual, either overall or at a specific locus. Phenotype is the observed characteristic (as influenced by dietary intake and environmental exposure) of a patient’s enzyme activity, and includes such designations as “poor metabolizer” (PM), “intermediate metabolizer” (IM), “extensive metabolizer” (EM), and “ultrarapid extensive metabolizer.” Patients who express dysfunctional or inactive enzymes are considered PMs. EMs are patients who express enzymes that have normal (extensive) activity, in whom the anticipated medication response would be seen with standard doses of drugs. Ultrarapid EMs are patients who have higher quantities of expressed enzymes because of gene duplication. Normal doses of drugs in these patients may result in reduced or no efficacy (or toxicity with prodrugs) because of rapid metabolism.

Determining cytochrome P450 activity before prescribing medications is not routine. However, genotyping or phenotyping for some of the P450 enzymes may become commonplace for drugs with a narrow therapeutic range (e.g., phenytoin or warfarin). Standard drug doses are based on pharmacokinetics in healthy volunteers, who are most likely to be EMs.

CYP2D6

The lack of pharmacogenomic studies of CYP enzymes is somewhat surprising given that many antihypertensive drugs are metabolized through the CYP450 system. For example, beta-blockers such as metoprolol, carvedilol and timolol are metabolized via the highly polymorphic CYP2D6. Currently, more than 70 different alleles have been described. Non-functional or null alleles are caused by altered splicing sites, frameshift mutations, deletion of the gene, premature stop codons or missense
mutations. Other alleles with alterations of the amino acid sequence are associated with a reduction of in-vivo enzymatic activity. A PM phenotype results if all inherited alleles are null alleles. In Caucasians, about 7% of the population are PMs. The lack of metabolizing capacity impairs elimination of drugs which are dependent on this oxidative pathway of metabolism. One study showed that individuals characterized by genotyping as PMs and IMs had 6.2-3.9-fold higher metoprolol plasma concentrations, respectively, as compared to EMs\textsuperscript{168}. A clinical significance of these differences is supported by a recent study showing a 5-fold greater likelihood for CYP2D6 PMs to develop adverse effects when given metoprolol as compared to other genotypes\textsuperscript{169}.
Aims of Part I

The hypothesis of studies I-II was that the response to antihypertensive treatment is in part genetically determined. The aim was to determine whether specific gene polymorphisms were related to:

– the antihypertensive response to the ARB irbesartan and the beta$_1$ adrenoceptor blocker atenolol (study I)

– the change in LV mass in response to antihypertensive treatment with the ARB irbesartan and the beta$_1$ adrenoceptor blocker atenolol (study II)
Theoretical background to study I and II

Study I: CYP2C9 is the principal CYP2C in human liver\(^{170}\). It metabolizes many clinically important drugs including the diabetic agent tolbutamide, the anticonvulsant phenytoin, the s-enantiomer of the anticoagulant warfarin\(^{170}\), the ARB and losartan\(^{171}\), and several other drugs including the antidiabetic drug glipizide\(^{171}\). A rare polymorphism was reported in the metabolism of tolbutamide and phenytoin as early as the 1970s\(^{172, 173}\). Subsequently, the impaired metabolism was shown to be due to a rare allele, CYP2C9*3, which carries an Ile359Leu mutation\(^{174}\). Clinical problems with toxicity and dosage adjustment of both warfarin and phenytoin have been found in CYP2C9 PMs\(^{175, 176}\). This allele has a frequency of approximately 7% in Swedish subjects (the frequency of homozygous CYP2C9*3 PMs is about 0.7%)\(^{177}\).

A second mutant allele of CYP2C9 (CYP2C9*2 containing an Arg144Cys substitution) has been reported to have decreased catalytic activity\(^{178}\). CYP2C9*2 has a frequency of approximately 11% in Swedish subjects\(^{177}\) but has a lower frequency in African-Americans and appears to be virtually absent in Asians\(^{174}\).

In vitro studies have provided evidence that CYP2C9 plays a major role in irbesartan metabolism\(^{179, 180}\), as is the case for losartan, for which the CYP2C9 genotype determines the metabolic rate\(^{181}\). In the light of these studies, it might be suspected that the CYP2C9 genotype would influence the clinical response to treatment with ARBs in the same manner as for warfarin and phenytoin. This has, however, not been investigated. In study I, the aim was to evaluate whether the CYP2C9 genotype influences the BP response to irbesartan.

Study II: A number of studies demonstrate that bradykinin mediates important CV effects that may protect against LVH\(^{182}\). Brull et al\(^{183}\) showed that a +9/-9 exon 1 polymorphism of the B2 bradykinin receptor was associated with LV growth response among normotensive white males undergoing a 10 week physical training programme\(^{183}\). The –9 allele of the B2 bradykinin receptor has been shown to result in more B2 bradykinin receptor mRNA than has the +9 allele\(^{184}\). Individuals with the greatest increase in LV mass carried the +9/+9 genotype\(^{183}\). A further influence of the ACE D/I polymorphism was also shown; individuals with both the D/D and the +9/+9 genotype had markedly greater growth than individuals with the I/I and -9/-9 genotype. Study II aimed to investigate whether the B2 bradykinin receptor genotype would influence regression of LV mass in hypertensive patients with LVH during antihypertensive treatment.
Study population and study course

The subjects participated in the SILVHIA trial\textsuperscript{185}, in which Caucasian men and women with mild-to-moderate essential HT, and echocardiographically verified LVH were enrolled, with the primary goal of evaluating the efficacy of irbesartan compared to atenolol on BP reduction and regression of LVH. LVH was considered present if LVMI was $>131$ g/m$^2$ for men and $>100$ g/m$^2$ for women. The Penn convention was used for calculation of LV mass, which was corrected for body mass index (BMI)\textsuperscript{186}. The inclusion criteria constituted a DBP of 90-115 mmHg. Altogether 166 patients eligible for inclusion were enrolled. Secondary HT was excluded by means of a physical examination and routine laboratory tests. All antihypertensive agents were withdrawn before the start of a 4-6 week, single-blind, placebo lead-in period. At the end of the placebo period, patients were determined eligible for the double-blind part of the study if diastolic BP (DBP) was 90-115 mmHg. A total of 115 qualifying patients, of which 89\% had been treated with antihypertensive medication previously, were randomized in a double-blind fashion to receive either irbesartan 150 mg or atenolol 50 mg once daily as monotherapy. The doses were doubled after six weeks if DBP was $\geq$ 90 mmHg. If DBP remained $\geq$ 90 mmHg at week 12, hydrochlorothiazide (12.5-25 mg once daily) was added. At week 24, felodipine (5-10 mg once daily) was added if required. In all, 102 patients completed the first 12 weeks of monotherapy, and 101 patients completed the whole 48 week study. Complete echocardiographic and blood pressure data were available in 90 at that time. Of the 14 who discontinued, nine (six irbesartan, three atenolol) discontinued due to adverse events. Three patients (one irbesartan, two atenolol) requested to be withdrawn, and two patients (both irbesartan) discontinued because of DBP $> 115$ mmHg, i.e. above the upper limit according to the protocol. Echocardiography was performed at baseline and at weeks 12, 24 and 48, while blood pressure was measured more frequently. An overview of the study course is given in figure 4. The local ethics committees approved the study, the participating patients gave their informed consent, and the study was completed in accordance with institutional guidelines. The additional pharmacogenomic studies performed were approved by the local ethics committees, and blood samples for DNA extraction were collected after ethical approval of the study.
Figure 4. An overview of the study course and drop-out rates.
Methods

Study I: BP was measured by trained study-nurses using a mercury sphygmomanometer, after resting for at least 10 minutes in a seated position, and was determined as the average of three measurements taken one minute apart. The inclusion criteria constituted a DBP of 90-115 mmHg at two examinations within a week, with values differing no more than eight mmHg. During treatment BP was measured at trough (24 ± 3 hours after the last dose). A total of 102 patients completed the first 12 weeks of monotherapy in the SILVHIA trial, and it is data from those first weeks that are considered in this study. Of the 102 patients, 49 patients had been treated with irbesartan and 53 with atenolol. Baseline characteristics of all patients are shown in table 4.

Genomic DNA was extracted from leukocytes using QIAamp DNA Mini Kit (QIAGEN, Germany) using the blood spin protocol. Genotyping for CYP2C9 was performed using two sets of three oligonucleotide primers, two for the PCR-amplification and one extension (detection) primer for the minisequencing reaction, required for the detection for each of the mutated variants. The primers required for the PCR-amplifications were taken from a publication from Steward et al\textsuperscript{175} with the removal of TCC at the 5´-end and addition of TT at the 3´-end of the reverse primer for amplifying the segment containing the *2-polymorphism, and the biothinylation of the 5´-end of the forward primers. Extension primers for detecting the substituted nucleotides were 5´ GCCGGGCTTCTTCTTGAACAC 3´ for Arg144 and 5´ GCTGGTGGGGAGAAGGTCAA 3´ for Ile359. All DNA-primers were synthesized by Thermo Hybaid (Germany). PCR-amplification was conducted using AmpliTaq Gold PCR-kit and the PCR-engine GeneAmp\textsuperscript{®} PCR System 9700 (Applied Biosystems, USA). The reaction mixtures consisted of 0,5 mM dNTP, 0,2 μM of each primer, 1 x PCR Buffer II, 100 ng of purified DNA, 1 U AmpliTaq Gold polymerase, and either 1,75 mM (amino acid 144) or 2,5 mM (amino acid 359) MgCl\textsubscript{2}, in a final volume of 50 μl. PCR amplification conditions comprised an initial 10 min denaturation at 95°C, 35 cycles of 95°C for 30 s, 64°C (amino acid 144) or 61°C (amino acid 359) for 30 s and 72°C for 60 s, after which a 10 min final extension at 72°C concluded the reactions. The PCR products were analyzed and the patients thus genotyped by solid-phase minisequencing after capture of biothynylated PCR products in the streptavidin-coated microtiter wells Combiplate 8 (Thermo Labsystems, Finland) and single-nucleotide extension of the specific detection primers by a radioactively labelled nucleotide\textsuperscript{187, 188}.

Changes in BP were calculated as percent changes. Differences between genotypes were tested with two-tailed Student’s \textit{t}-test, with p<0.05 taken as statistically significant.
Table 4. Baseline characteristics of the study population. n, number of subjects. BMI, body mass index. SBP, systolic BP. DBP, diastolic BP. Data are mean values ± SD.

Table 5. Baseline patient characteristics stratified by B2BKR genotype. BMI=body mass index; SBP=systolic BP; DBP=diastolic BP; HR=HR; LVMI=LV mass index. Mean values±[SE].
Results

**Study I:** The mean dose of irbesartan was 251 mg and 70 mg for atenolol at 12 weeks. Approximately 67% of the patients treated with irbesartan and 42% of the patients treated with atenolol received the higher doses (300 versus 100 mg). The CYP2C9 genotypes of the 49 patients treated with irbesartan and the 53 patients treated with atenolol are shown in table 6. Of the patients with the CYP2C9 *1/*1 genotype, 70% had been given full dose irbesartan treatment (300 mg) at 12 weeks, whereas the corresponding percentage among patients with the *1/*2 genotype was 50%. Of the patients with the *1/*1 genotype 42% had reached DBP <90 mmHg, and 50% of the patients with the *1/*2 genotype. These differences between genotypes did not, however, reach statistical significance.

The changes in DBP, with respect to genotype at 12 weeks of treatment with irbesartan, are presented in figure 5 and table 6. A significant difference (p=0.036) between *1/*1 and *1/*2 was found (figure 5). A similar tendency (p=0.23) was seen for SBP (figure 5). The number of patients with genotypes *1/*3, *2/*2 and *2/*3 were not sufficient for statistical analyses in the group of patients treated with irbesartan, and no patients with genotype *3/*3 were present. In contrast to the findings with irbesartan, no relation between the CYP2C9 genotype and change in BP was seen in patients treated with atenolol (table 6).

![Figure 5. Reduction of diastolic and systolic blood pressure in patients treated with irbesartan, divided by CYP2C9 genotype (*1/*1 vs *1/*2); n=33 for *1/*1 and n=12 for *1/*2.](image)
Table 6. Patient characteristics and blood pressure response with respect to treatment and genotype. n, number of subjects. BMI, body mass index. SBP, systolic blood pressure. DBP, diastolic blood pressure. Data are mean values ± SD.

Study II: The genotype distribution was consistent with Hardy-Weinberg equilibrium. Baseline patient characteristics and change in BP and HR did not differ significantly between B2 bradykinin receptor genotypes as shown in table 1, nor did they differ significantly between the two treatment groups, as shown in the SILVHIA-trial. Among patients given atenolol, 48% received 50 mg, 39% 100 mg and the remaining either 75 or 25 mg. Among patients given irbesartan, 66% received 300 mg, 29% 150 mg, and the remaining either 75 or 225 mg. A total of 57% of the patients required additional treatment with hydrochlorothiazide (56%) and felodipine (28%). The +9/-9 and -9/-9 genotypes were considered as one group in the analyses, since their mean adjusted LVMI changes were almost identical (-21.2 [3.7] for -9/-9 and -21.9 [2.7] for -9/+9 at 48 weeks). Although LVMI was reduced more in the irbesartan (16%) than in the atenolol group (9%) in the SILVHIA study, there was no significant interaction between B2BKR genotype and type of treatment.

The regression in LV mass (figure 6) was about half in patients with the +9/+9 genotype compared to carriers of one or two -9 alleles at 48 weeks in the multivariate model (-10.0 g/m² [4.6] vs -21.6 g/m² [2.2], p=0.03). This was almost identical to the univariate mean change in LVMI (-9.7 g/m² [4.1] vs -21.7 g/m² [3.0], p=0.07), indicating a lack of confounding of the variables included in the multivariate model. A similar tendency was evident already at 12 weeks (adjusted mean change in LVMI 1.3 g/m² [4.2] vs -5.6 g/m² [2.0], p=0.15, and univariate mean change 3.5 g/m² [6.0] vs -6.1 g/m² [2.1], p=0.07).
Figure 6. Response of LV mass index (LVMI) to antihypertensive treatment in relation to B2 bradykinin receptor genotype. Mean values ± SEM. Adjustment is made for age, gender, dose, baseline systolic and diastolic BP (SBP and DBP) and LVMI, changes in SBP and DBP, and at 48 weeks also additional antihypertensive medication.
Limitations

There are several limitations to the reported studies herein:

a) The majority of the patients had been treated previously, which probably affected baseline values.

b) Due to their post-hoc nature, the studies should be regarded as hypothesis-generating and need to be reproduced in prospective studies specifically designed from a pharmacogenomic view and with larger samples.

c) The sample size of the SILVHIA-trial was too small to allow testing of multiple genotypes in the same analyses.

d) The results do not necessarily mean that all patients will exhibit the same genotype-based response to drug therapy; the SILVHIA-trial had selected, as most clinical trials do, a homogenous cohort of patients.
Discussion and future aspects

**Study I:** The CYP2C9 genotype thus seems to predict the DBP response to irbesartan in mild-to-moderate HT. As expected, no influence of CYP2C9 genotype on the reduction of BP was seen in patients treated with atenolol, which is not eliminated by this enzyme. Thus, this treatment group rather served as an active control group and further support the findings of a CYP2C9 genotype dependent response to irbesartan.

It is reasonable to believe that patients with the *1/*2 genotype respond better to irbesartan treatment than patients with the *1/*1 genotype due to a slower elimination of the drug and thus higher blood concentrations.

Taken together with the findings by Yasar et al\(^{181}\), who showed that the rate of losartan metabolism is determined by the CYP2C9 genotype, our findings suggest that response to treatment with two common AT\(_1\)-receptor antagonists is under genetic influence much in the same way as warfarin and phenytoin. This also stresses the possibility of pharmacokinetic interaction between these AT\(_1\)-receptor antagonists and other drugs metabolized by CYP2C9, such as warfarin and phenytoin, which might have clinical importance.

**Study II:** It seems that the B2 bradykinin receptor genotype influences not only exercise-induced LV growth in normotensive people\(^{183}\), but also LV mass regression during antihypertensive treatment with two common antihypertensive drugs. Thus, subjects with high concentrations of the B2 bradykinin receptor (-9/-9 genotype) seems to respond better than those with low concentrations (carriers of the +9 allele).

An ultimate goal of pharmacogenomic knowledge is to advance beyond the current approach to antihypertensive drug therapy to more individualized approaches. Since proteins are the targets of antihypertensive drugs, the complete mapping and sequencing of all genes in the human genome implies that potential targets of virtually every antihypertensive drug in due time will be discovered. The challenge will be to ascertain the functions of many newly discovered genes, assess the extent and impact of their polymorphisms, and identify those gene products which are valid drug targets. The latter task will be aided by identification of genes that influence disease activity, inasmuch as disease genes may be candidates to influence drug response. Conversely, drug response genes may become candidates to influence disease process. The ability to obtain complete “molecular profiles” of disease and drug response genes in individual patients should provide efficient and accurate methods to identify individuals prior to drug
administration who are likely to have favorable or unfavorable responses to treatment with a particular drug or class of drug. Reliable methods to ensure that a drug is only administered to those in whom it will be effective and non-toxic may also serve to maintain the usefulness of drugs that are found to be toxic in a few but are efficacious in most.

Knowledge of genes that contribute to the disease process and genes that influence drug responses should facilitate the development of new drugs and therapeutic approaches that are based on a deeper understanding of the molecular determinants of the disease and the response to therapy. Drugs that are more specific for the molecular characteristics of individual patients should contribute to greater efficacy and reduced toxicity.

Several factors should be taken into account at the design stage of further pharmacogenomic studies. First, they should have a sufficient sample size to detect the efficacy of the drugs across different genotypes. Second, more attention should be paid to the standardization of the phenotype, and researchers should not be surprised should the results obtained seem contradictory to those found by others, who may have studied a different set of patients with a different phenotype. Third, there should be a focus on the use of haplotypes to define variation at a gene locus more accurately. Fourth, studies should focus not only on specific, highly selected and well-known points in the various biological systems involved in HT, but instead widen the repertoire of studied genes to whole pathways, including both pharmacodynamics and pharmacokinetics. Whereas small influences of polymorphisms in single candidate genes may remain undetected, a combined effect of several and at various levels in a given pathway may prove dramatically greater and thus detectable. Fifth, researchers should also focus on the interaction between genetic and environmental factors. Sixth, one should not forget to study possible genetic predisposition of drug-related side-effects. Seventh, virtually all pharmacogenomic studies of antihypertensive treatment today are based on post-hoc analyses of trials not primarily designed for pharmacogenomic studies; we also need studies specifically designed for pharmacogenomics.

Until now, only few pharmacogenomic studies of antihypertensive treatment have been conducted. So far, studies have focused almost exclusively on genes encoding proteins in the RAAS, the adrenergic system and its downstream receptor signaling pathway and α-adducin. The possible impact of polymorphisms in genes involved in renal Na⁺ handling, metabolic abnormalities, endothelial dysfunction and oxidative stress have been somewhat overlooked. Also, pharmacogenomic trials of antihypertensive treatments other than ACE-inhibitors and ARBs are scarce. Widening the repertoire of candidate genes and candidate drugs may prove very fruitful. To aid the selection of candidate genes, data from association studies in HT, as well as studies of the functionality of polymorphisms in genes encoding proteins involved in the regulation of BP etc, and in proteins both directly
and indirectly targeted by antihypertensive drugs may prove useful. To date, a vast number of such studies have been performed, and the number of candidate genes is growing. Given the polygenetic nature of essential HT, future pharmacogenomic studies need to focus also on these if the prospect of a “tailored” pharmacologic therapy is to be realized. This way, doors may be opened to the development of new antihypertensive drugs, targeted at other proteins. However, association studies have the disadvantage that functionality of polymorphisms that only become evident when provoked by drug treatment may elude detection. Equally important may be studies of antihypertensive drug related side-effects and their possible relation to genetic polymorphisms. To date, only a handful of such studies have been performed.

A last but not irrelevant question is the additional cost which will be introduced should tailored antihypertensive treatment be realized in the future. Genotyping will inevitably be needed, but to date, such technique is not readily available to all. However, it has already been proven by Liljedahl et al.\(^{189}\) that “antihypertensive chips”, capable of genotyping numerous individuals for hundreds of SNPs at the same time to a limited cost is possible. The matter would rather be a question of proving the value of such genotyping in large trials of antihypertensive treatment. In the near future, one could imagine selected centra that can provide this kind of service, which in itself is not overwhelmingly difficult but requires experience and resources. If genotyping will prove to be an accurate way of knowing in beforehand what type of antihypertensive treatment the individual patient is most likely to respond to, the cost of such genotyping would likely be small as compared to the reduced health-care costs. However, there is still a long way to go until such a goal can be realized.
Part II – Clinical pharmacological studies of digoxin treatment

Introduction

Heart failure

Cardiovascular disease has been the most common cause of morbidity and mortality in the Western world for the past century. The prevalence of HF increases with age and ranges from <1% for patients <50 years of age to 5% for patients 50 to 70 years of age and 10% for all patients >70 years of age. The mean age at diagnosis has increased from approximately 60 years in the 1950s to 80 years in the last decade.190

The clinical syndrome of HF arises from the heart’s inability to fill/relax and/or eject blood from the ventricle. The inability of the ventricle to fill with blood, due to a problem with ventricular relaxation, is referred to as diastolic dysfunction. The inability of the ventricle to empty/eject blood is referred to as systolic dysfunction, typically defined as an ejection fraction (EF) ≤45%. LV dysfunction classically presents as pulmonary congestion (rales), an S3 gallop, dyspnea on exertion, shortness of breath, paroxysmal nocturnal dyspnea, and/or orthopnea. While two-thirds of patients have HF from ischemic heart disease, others have identifiable causes such as HT, valvular disease, thyroid disease, alcohol use, myocardiitis, sepsis, anemia, and nonischemic cardiomyopathy. A small percentage of people will have no identifiable cause for ventricular dysfunction (idiopathic dilated cardiomyopathy).191

Functional classification of HF

Symptomatic HF is described by functional limitation, using a New York Heart Association (NYHA) classification system. “NYHA class I” refers to patients who have no symptoms at any level of exertion. Normal physical activity does not cause them undue fatigue or dyspnea, but such patients (as
well as the patients in the other functional classes) have ventricular dysfunction. NYHA functional class II patients have symptoms with ordinary exertion, while class III patients have symptoms with less-than-ordinary exertion, and class IV patients have symptoms at rest. The NYHA classification is one of the best predictors of survival. Only 50% of patients remain alive 5 years after the diagnosis of HF, and those with severe symptoms (NYHA functional class III or IV) have a 1-year survival rate of only 40%.

Today, patients with HF have many different characteristics compared with what was seen 30-40 years ago. Coronary artery disease has replaced HT and valvular heart disease as the most common etiology for HF, accounting for nearly 70% of cases. The prognosis in patients with severe LV dysfunction and coronary artery disease is worse than the prognosis of HF in patients with normal coronary arteries.

There is an increased prevalence of patients with HF and preserved systolic function. Of patients admitted to hospitals due to chronic HF, 30-40% of cases occur in the setting of preserved systolic function. Although diastolic dysfunction is, per se, a rare cause of HF, when combined with HT, diabetes, coronary artery disease, and/or atrial fibrillation (AF), it results in a clinical syndrome of HF with preserved systolic function. Studies thus far show that these patients are more likely to be women, generally older, and have AF more often than those with LV systolic dysfunction.

Systemic and pulmonary congestion is seen less frequently, even in patients with severe LV dysfunction. In the Studies of LV Dysfunction (SOLVD), which enrolled patients with an EF ≤0.45, <35% of the patients had signs of systemic congestion. This is probably the combined result of early recognition of LV dysfunction and the efficacy of available treatments that reduce or eliminate the signs and symptoms of congestion.

Despite advances in the recognition and treatment of HF, mortality remains very high. Most patients with HF die suddenly, presumably of ventricular arrhythmias, before they develop serious symptoms or after their symptoms have been controlled. This is very different from what was seen decades ago, when most patients were dying with progressive circulatory failure.

The pathophysiology of HF

Our understanding of the pathophysiology of chronic HF with reduced systolic function is constantly growing. Once considered a simple pump dysfunction, HF has come to be understood as a highly complex clinical
syndrome in which coronary artery disease and its progression, neurohormonal activation, ventricular remodeling, as well as genetic factors play important roles\textsuperscript{199}.

HF is initiated after an event damages the heart muscle or disrupts the ability of the myocardium to contract and/or relax. This event may have an abrupt onset (eg, an acute MI) or an insidious onset (eg, hemodynamic pressure or volume overload from long-standing HT or valvular disease), or it may be hereditary (eg, dilated cardiomyopathy)\textsuperscript{199}. These events lead to subtle or overt ventricular dysfunction, resulting in decreased pumping capacity.

\textit{Coronary artery disease}

In the United States, coronary artery disease is the underlying etiology for HF in \textgreater70\% of the cases\textsuperscript{193}. A sustained reduction in the coronary blood flow leads to a tissue perfusion that is sufficient to maintain viability but insufficient to maintain a normal contractility (hibernation)\textsuperscript{200}. However, hibernation cannot be maintained indefinitely, and eventually, myocardial necrosis ensues if coronary blood flow is not restored\textsuperscript{200}. Ischemia and hibernation may lead to myocyte apoptosis\textsuperscript{200}, which may result in progression of LV dysfunction\textsuperscript{200}. Finally, episodes of reversible myocardial ischemia caused by coronary artery disease, when superimposed on a left ventricle with already depressed systolic function, may cause an exacerbation of HF\textsuperscript{193}.

\textit{Neurohormonal and cytokine activation}

Decreased cardiac performance characterized by a reduction in CO and/or an increase in wall stress results in activation of neurohormonal systems, such as the adrenergic system, the RAAS, and the hypothalamic–neurohypophyseal system\textsuperscript{201}. Continued activation of the adrenergic system increases ventricular afterload and, therefore, the hemodynamic burden placed on the failing ventricle. It may also cause hypertrophy, ischemia, tachyarrhythmias, and myocyte damage\textsuperscript{201}.

Activation of the RAAS results in an increased level of Ang II, which increases ventricular afterload and causes myocyte hypertrophy, apoptosis, interstitial fibrosis, cardiac and vascular remodeling, and the secretion of aldosterone\textsuperscript{201}.

There is increasing evidence of interplay between the adrenergic system and the RAAS. Thus, in patients with HF, ACE-inhibition has been found to reduce enhanced peripheral sympathetic nerve impulse traffic and cardiac adrenergic drive\textsuperscript{201}. Conversely, \(\beta\)-blockade reduces the secretion of renin, therefore reducing the levels of angiotensin and aldosterone\textsuperscript{202}. 

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Arginine vasopressin is synthesized in the hypothalamus, and its release from the neurohypophysis is enhanced by osmolar stimuli as well as elevated concentrations of norepinephrine and Ang II. Increased release of arginine vasopressin in HF causes vasoconstriction (through binding to vasopressin$_1$ receptors), water retention, and dilutional hyponatremia (through binding to vasopressin$_2$ receptors$^{201}$).

The vasodilator peptides, such as ANP and brain natriuretic peptide, are also overexpressed in chronic HF and exert a counterregulatory or beneficial effect$^{33}$. However, renal responsiveness to their action is impaired. The decreased responsiveness leads to enhanced local actions of Ang II and the sympathetic system in the kidney, resulting in salt retention and further deterioration of renal function and increased vasoconstriction$^{33}$.

Remodeling

The development of myocardial hypertrophy initially represents an important adaptive mechanism to hemodynamic stress and is characterized by structural changes at the myocyte level that are translated into alterations in chamber size and geometry$^{201}$. The systemic vasoconstriction translates into increased afterload, which further reduces cardiac performance. In patients with HF, a marked increase in the LV diastolic pressure may be responsible for changes in the shape of the left ventricle from an ellipsoid to a more spherical configuration$^{203}$. This change in ventricular geometry may result in papillary muscle rearrangement and secondary mitral insufficiency$^{203}$. In addition, the elevated LV end-diastolic pressure (wall stress) can cause subendocardial ischemia$^{204}$ that is perpetuated by tachycardia, which shortens the diastole and decreases the coronary filling time. All these changes may lower the threshold for malignant ventricular arrhythmias.

Treatment of HF

The management of HF is comprehensive and includes the following$^{205}$: (1) accurate diagnosis, assisted by the use of echocardiography; (2) identification and treatment of etiologic factors, such as ischemia or HT; (3) syndrome definition, for instance, HF with systolic dysfunction versus HF with preserved systolic function, right-sided versus left-sided HF, HF with congestion versus without congestion, or high-output versus low-output state; (4) correction of precipitating causes, such as noncompliance with drugs, use of nonsteroidal anti-inflammatory drugs, anemia, infections, dietary indiscretion, inactivity, etc; and (5) therapy, which has 4 major components: (a) life-style interventions such as exercise, diet; (b)
pharmacologic therapy; (c) electrical therapy (implantable cardioverter defibrillators, biventricular pacing); and (d) surgical therapy (coronary artery bypass grafting, mitral valve repair). Only pharmacological therapy will be discussed here.

*ACE inhibitors*

*Background*

ACE inhibitors act by blocking the conversion of Ang I into Ang II, breaking down the RAAS that is activated in HF. This leads to a decrease in preload and afterload, with an improvement in the hemodynamic profile. Aldosterone production also diminishes, and thus the Na⁺ and water retention decreases. Inhibition of Ang II leads to a decrease in cardiac remodeling, hypertrophy, apoptosis (either directly or by decreasing aldosterone production), sympathetic activity, and vasopressin levels. ACE inhibitors also increase plasma concentrations of bradykinin, NO, and vasodilating prostaglandins.

*Benefits of ACE inhibitors*

*Clinical effects*

ACE inhibitors improve symptoms, NYHA functional class, and exercise capacity in patients with HF, as shown in randomized, double-blind, placebo-controlled trials. For example, the Captopril Multicenter Research Group showed that captopril treatment improved the NYHA class in 61% of patients compared with only 24% of patients taking placebo over a 12-week period.

*Effects on mortality and hospitalization*

The most important benefit of therapy with ACE inhibitors is the increase in survival seen in patients with NYHA class II to IV and in all patients with LV systolic dysfunction after an acute MI. The first trial to show a survival benefit, the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS) was conducted in patients with NYHA class IV who were randomized to receive enalapril or placebo. At the end of the study (20 months), patients treated with enalapril had a significant 27% reduction in total mortality, the primary end point. It appeared that enalapril had no effect on sudden death but decreased mortality from progressive HF by 50%.
Recommendations on the use of ACE inhibitors

Based on data from published trials, the American College of Cardiology/American Heart Association (ACC/AHA) guidelines recommend ACE inhibitors as the first-line therapy for symptomatic HF with reduced systolic function and for asymptomatic LV dysfunction. They should be used in conjunction with a diuretic to maintain the Na⁺ balance and prevent the development of fluid overload. A β-blocker should be added in all patients without contraindications. ACE inhibitors should be started at low doses and gradually increased to the doses that have been shown to decrease mortality in clinical trials.

β-adrenergic receptor blockers

Background

Long-term activation of the sympathetic system is associated with an increase in ventricular volumes and pressures by causing peripheral vasoconstriction and by impairing Na⁺ excretion by the kidney. Norepinephrine causes myocyte hypertrophy, and induces myocardial ischemia. Sympathetic activation is also correlated with increased arrhythmogenesis and sudden death. β-blockers act by inhibiting the adverse effects of the sympathetic nervous system activation in patients with HF.

Benefits of β-blockers

Clinical effects

Several randomized, double-blind, placebo-controlled trials have shown that long-term use of β-blockers is associated with improved clinical status in patients with HF. For instance, in the Metoprolol in Dilated Cardiomyopathy (MDC) trial, metoprolol increased exercise tolerance, enhanced quality of life, and improved NYHA class at 12 months, but not at 6 months. It appears that clinical improvement with β-blocker therapy becomes evident after a longer period compared with ACE inhibitor treatment.

Effects on mortality and hospitalization

The effects of β-blockers on mortality have been evaluated in several trials. For instance, the MERIT-HF trial, evaluating metoprolol controlled-release/extended-release, was conducted in almost 4,000 patients, mostly (96.4%) with NYHA class II to III. These patients had an EF <0.40 and were receiving ACE inhibitors, diuretics, and digoxin at the time of
randomization. Overall, a 34% risk reduction in all-cause mortality was reported in the metoprolol group, with a 49% risk reduction in death caused by HF and 41% decrease in sudden death. The effects appeared to be more pronounced in the 3.6% of patients who were in NYHA class IV210.

**Recommendations on the use of β-blockers**

Based on the results from available studies, the ACC/AHA guidelines recommend that β-blockers should be routinely prescribed to all patients with clinically stable HF caused by LV systolic dysfunction, unless they have a contraindication or have been shown to be intolerant to treatment with these drugs205.

**Diuretics**

**Benefits of diuretics**

**Clinical effects**

Trials have demonstrated the ability of diuretics to decrease signs of fluid retention in patients with HF211. In these short-term studies, diuretic use has led to a reduction in jugular venous pressures, pulmonary congestion, peripheral edema, and body weight, all observed within days. Diuretics have been shown to improve cardiac function, symptoms, and exercise tolerance in patients with HF212.

**Effects on mortality and hospitalization**

There have been no long-term studies of diuretic therapy in HF, and, thus, their effects on morbidity and mortality are not known. A retrospective analysis of the SOLVD trials showed that non–K⁺-sparing diuretic use is associated with an increased risk of arrhythmic death (relative risk, 1.33; 95% confidence interval, 1.05 to 1.69; P = 0.02)213. Use of a K⁺-sparing diuretic alone or in combination with other diuretics was not associated with increased risk of arrhythmic death (relative risk, 0.9; 95% confidence interval, 0.61 to 1.31; P = 0.6)213, suggesting that diuretic-induced electrolyte disturbances may result in fatal arrhythmias in patients with systolic LV dysfunction. The Torasemide in Congestive HF (TORIC) study compared the efficacy of torsemide and furosemide in HF214. Although not designed as a mortality study, TORIC showed fewer deaths in the torsemide-treated patients (2.2% vs 4.5% in the torsemide and furosemide group, respectively)214. Another published trial suggested that the use of torsemide is associated with fewer HF rehospitalizations and fewer hospital days for HF admission than with the use of furosemide215. Although the above trials enrolled a relatively small number of patients, had an open-label design, and
the percentage of patients receiving ACE inhibitors and β-blockers was small, these observations deserve further investigation.

Recommendations on the use of diuretics
Diuretics improve symptoms within hours or days, whereas the clinical effects of ACE inhibitors or β-blockers may take weeks or months to become apparent. ACC/AHA guidelines recommend that diuretics be prescribed to all patients who have evidence of fluid retention and that they should be combined with an ACE inhibitor and a β-blocker. The treatment goal is to eliminate physical signs of fluid retention. Once fluid retention has resolved, treatment with the diuretic should be maintained to prevent the recurrence of volume overload.

Aldosterone antagonists

Background
Despite treatment with an ACE inhibitor, patients with HF may demonstrate elevated aldosterone levels. Mechanisms of "aldosterone escape" include alternative stimuli for aldosterone synthesis (such as adrenocorticotrophic hormone and endothelin), K⁺-dependent aldosterone secretion, and reduced aldosterone clearance. Potentially harmful effects of this aldosterone escape include magnesium loss, sympathetic activation, parasympathetic inhibition myocardial ischemia and fibrosis and production of ventricular arrhythmias.

Benefits of aldosterone antagonists

Clinical benefits and effects on mortality and hospitalization
The effect of aldosterone-receptor blockade with spironolactone in patients with NYHA class III to IV who are treated with ACE inhibitors, diuretics, and digoxin was evaluated in the Randomized Aldactone Evaluation Study (RALES). This trial showed a 30% reduction in all-cause mortality, a 31% reduction in cardiac deaths, and a 35% reduction for hospitalization for worsening HF. Patients also had a net improvement in symptoms and in NYHA class.

Recommendations on the use of aldosterone antagonists
Currently, the ACC/AHA guidelines recommend that spironolactone should be used in patients with recent or current NYHA class IV symptoms, irrespective use of ACE inhibitors, β-blockers, digoxin, and diuretics.
**ARBs**

**Background**

These agents block the action of Ang II at the receptor level and conceivably could block the effects of Ang II produced not only through the classical ACE pathway but also by, e.g., the chymase pathway. Available ARBs block only the Ang II type 1 receptors (associated with hypertrophy and remodeling) and enhance the activation of Ang II type 2 receptors, e.g., causing vasodilation[^14].

**Benefits of ARBs**

**Effects on mortality and hospitalization**

The Evaluation of Losartan in the Elderly (ELITE) trial was conducted to determine whether losartan offers safety and efficacy advantages over ACE inhibition with captopril in the treatment of elderly patients with HF[^218]. Although not the primary end point of the study, death and/or hospital admissions for HF were reduced by a nonsignificant 32% in the losartan group. This was attributed to a 46% reduction in all-cause mortality in patients receiving losartan, considered to be because of a reduction in sudden death. The HF hospitalization rate was identical in the 2 groups (5.7%)[^218]. The hypothesis that losartan might reduce mortality when compared with captopril was tested in a larger follow-up study. ELITE II showed no significant differences in mortality between the two treatment groups[^219].

Recently, results from the VALIANT study was published[^220]. This study compared death from any cause (primary endpoint) in patients with myocardial infarction complicated by LV systolic dysfunction, HF, or both and who received, in a double-blind fashion, the ARB valsartan (n=4909), the ACE-inhibitor captopril (n=4909), or the combination of the two (n=4885). During a median follow-up of 24.7 months, 979 patients in the valsartan group died, as did 941 patients in the valsartan-and-captopril group and 958 patients in the captopril group (no statistically significant differences). The valsartan-and-captopril group had the most drug-related adverse events. With monotherapy, hypotension and renal dysfunction were more common in the valsartan group, and cough, rash, and taste disturbance were more common in the captopril group.

**Recommendations on the use of ARBs**

The ACC/AHA guidelines recommend that ARBs should not be considered equivalent or superior to ACE inhibitors in the treatment of HF with reduced systolic function[^205]. They should only be used if a patient has intolerance to ACE inhibitors secondary to intractable cough or angioedema.
**Digoxin**

**Background**

The inotropic effect of digoxin is generally accepted to be due to high affinity binding of cardiac glycosides to the Na⁺, K⁺-ATPase. This leads to inhibition of the enzyme and an increase in intracellular Na⁺. Hence, the Na⁺/Ca²⁺-exchanger activity is affected so that less Ca²⁺ is extruded from the cell. Consequently, more Ca²⁺ is accumulated in the sarcoplasmic reticulum and is available during subsequent contractions leading to increased force of contraction.

Besides their direct positive inotropic effect, cardiac glycosides improve hemodynamics also by inhibition of extra-cardiac Na⁺, K⁺-ATPase. Hence, cardiac glycosides may restore baroreceptor activity in congestive HF. The increased vagal activity may also be generally beneficial mimicking β-adrenoceptor antagonist treatment. By inhibiting Na⁺, K⁺-ATPase in the kidney, digoxin reduces the renal tubular reabsorption of Na⁺, resulting in the suppression of renin secretion from the kidneys. These observations have led to the hypothesis that digoxin acts in HF primarily by attenuating the activation of neurohormonal systems and not as a positive inotropic drug.

A close correlation has been observed between decreased LV ejection fraction (LVEF) and decreased Na⁺, K⁺-ATPase concentration. An interesting question is, whether the reduction of Na⁺, K⁺-ATPase is part of the reason for cardiac dysfunction and/or hypertrophy, whether it serves compensatory purposes with effects comparable to digitalis treatment, or whether it just coincides with the disease progress.

**Benefits of digoxin**

**Clinical effects**

The beneficial effects of digoxin in HF include reduced symptoms, improvement in NYHA class, increased exercise time, modestly increased LVEF, enhanced CO, and decreased HF hospitalizations. When digoxin is withdrawn, these benefits are lost, as shown in the Randomized Assessment of Digoxin and Inhibitors of Angiotensin-Converting Enzyme (RADIANCE) and the Prospective Randomized Study of Ventricular Function and the Efficacy of Digoxin (PROVED) trials. Their design was similar, involving patients in normal sinus rhythm, except that the RADIANCE trial included ACE inhibitors and diuretics as background therapy, whereas in the PROVED study, patients received diuretics only.
Effects on mortality and hospitalization

The Digitalis Investigation Group (DIG) trial tested the effects of digoxin on survival in patients with HF in normal sinus rhythm230. The trial enrolled 7,788 patients, of whom 87% had systolic dysfunction. They were randomized to a mean dose of 0.25 mg of digoxin or placebo, with a background therapy of ACE inhibitors and diuretic agents (average follow-up 37 months). Before enrollment, >50% of the patients in this trial were not receiving digoxin. For both groups, the all-cause mortality was 35% and the cardiovascular mortality was 30%230. There was a trend toward a decrease in mortality caused by HF in the digoxin group, but this was offset by an increase in death for other causes that included deaths presumed to result from arrhythmias. On the other hand, the DIG trial showed that digoxin reduced the risk of hospitalizations, including the risk for hospitalizations because of worsening HF230, both regarding the number of patients hospitalized and the frequency of such hospitalizations per patient, confirming the results of the PROVED and RADIANCE trials228, 229. The benefits were more marked in patients with NYHA class III-IV230. However, digoxin therapy did not appear to influence the quality of life, as measured in a sub-group of patients in the DIG-trial231.

Recommendations on the use of digoxin

The ACC/AHA guidelines recommend that use of digoxin should be considered to improve the symptoms and clinical status of patients with HF, in conjunction with diuretics, an ACE-inhibitor, and a beta-blocker. Digoxin may be used early to reduce symptoms in patients who have been started on, but have not yet responded symptomatically to, treatment with an ACE-inhibitor or a beta-blocker. Alternatively, treatment with digoxin may be delayed until the patient’s response to ACE-inhibitors and beta-blockers have been defined and used only in patients who remain symptomatic despite therapy with the neurohormonal antagonists. If a patient is taking digoxin but not an ACE-inhibitor or a beta-blocker, treatment with digoxin should not be withdrawn, but appropriate therapy with the neurohormonal antagonists should be instituted. The ACC/AHA guidelines further state that although some physicians have advocated using serum levels to guide the selection of the dose of digoxin, there is litte evidence to support such an approach. Although physicians have traditionally been taught that digitalis produces frequent side-effects, the drug is well tolerated by most patients with HF.

However, the latter statements have been discussed, as follows.
The traditional therapeutic range for serum digoxin concentration (SDC) (1.2-2.5 nmol/L) was developed not to assess efficacy but toxicity. In the mid-1960s, initial investigation was done with tritium-labeled drug in determining SDC. Smith et al. developed a radioimmunoassay to determine SDC and to assess therapeutic versus toxic SDC.

A recent retrospective cohort analysis of the combined PROVED and RADIANCE databases indicates that patients with a low SDC (0.6 to 1.2 nmol/L) were no more likely to have worsening symptoms of HF on maintenance digoxin than those with moderate (1.2 to 1.5 nmol/L) or high (>1.5 nmol/L) SDCs. Other studies and analyses in the 1990s have provided evidence supportive of a reduced upper therapeutic limit for SDC. These reports have evaluated hemodynamic and neurohormonal effects of digoxin in relationship to SDC. Digoxin ameliorates autonomic dysfunction as reflected by increased parasympathetic and baroreceptor sensitivity during therapy. Although higher concentrations of digoxin are associated with increased inotropic effect, its neurohormonal effects, such as decrease in HR and plasma norepinephrine, are reached at lower SDC.

The median maintenance dose of digoxin in the DIG Study was 0.25 mg per day — 70% of patients were maintained on this dose. The steady-state SDC in patients receiving this dose (available in a subset of patients) averaged between 1.0 nmol/L and 1.2 nmol/L.

Recently, a post-hoc subgroup analysis of the patients enrolled in the DIG trial showed that women who were randomly assigned to digoxin had a higher rate of death than women who were randomly assigned to placebo (33.1% vs 28.9%). In the multivariable analysis, digoxin was associated with a significantly higher risk of death among women, but it had no significant effect among men. However, because SDCs were measured in <33% of patients at 1 month, the trial had insufficient statistical power to test whether the interaction between sex and digoxin therapy was independent of sex-based differences in SDC. A further subgroup analysis of the DIG trial recently showed a relation between SDC and mortality in men. The risk of death increased significantly if the digoxin level exceeded 1.3 nmol/L, whereas a SDC of 0.6 to 1.0 nmol/L was associated with a decrease in all-cause mortality. This relation was maintained after multivariable adjustment: hazard ratio, 0.8 (95% CI, 0.68 to 0.94). A similar analysis for women could not be done because the SDC was not available in most women.

Although further studies with larger numbers of patients is desirable to support the aiming for a lower SDC, current evidence suggest that maintaining a lower SDC will minimize risk of digoxin toxicity without sacrificing efficacy in managing HF.
Atrial fibrillation

Chronic HF and atrial fibrillation (AF) each affect 1–2% of the population, and the prevalence of both rises steeply with age. They share common risk factors and consequently frequently coexist. Chronic HF may affect more than 50% of patients with AF while the prevalence of AF increases in proportion to the severity of chronic HF. Advances in the treatment of HF have decreased mortality and have perhaps led to a reduction in the incidence of associated AF, while effective management of AF will improve symptoms and may retard the progression of chronic HF.

Therapy of AF – with special reference to digoxin

Acute therapy

Electrical cardioversion is initially successful in 70% or more of patients selected for this procedure. Pharmacological cardioversion does not require general anesthesia but the rate of cardioversion is much lower. In patients in whom both rapid rate control and cardioversion are considered appropriate amiodarone would appear to be the drug of choice. Even if amiodarone is found to be only modestly effective in cardioverting acute AF, pre-loading with amiodarone has been shown to improve subsequent electrical cardioversion rates and maintenance of sinus rhythm thereafter. Cardioversion occurs with amiodarone alone in 15–20% of patients with chronic AF over a period of one month.

A study by Stambler et al suggests that ibutilide may be an effective pharmacological agent for cardioversion of acute AF, but further evidence of its safety in HF is necessary. In contrast, class I antiarrhythmic drugs should generally not be used in chronic HF because of the risk of proarrhythmia and the danger of exacerbating chronic HF.

Currently there is no evidence to suggest that digoxin has any effect on cardioversion in AF in the presence or absence of HF. A small, but well-designed randomized double-blind placebo-controlled trial in 36 patients with recent onset AF, and without HF, who were given either 1.4 mg digoxin orally over 14 hours or placebo capsules, reported reversion to sinus rhythm in eight out of 18 patients taking placebo and nine out of 18 patients taking digoxin. This, of course, was not statistically significant. The mean time to conversion in those patients who returned to sinus rhythm during the observation period was 5.1 hours in the digoxin group compared with 3.3 hours in the placebo group. A number of similar studies have produced very similar results, including a much larger (239 patients), multicentre Swedish study. These are referenced in the latest
guidelines for the management of AF, published jointly by the AHA/ACC and the European Society of Cardiology (ESC). These guidelines state that "digitalis glycosides are generally no more effective than placebo for conversion of recent onset AF to sinus rhythm. Digoxin may prolong the duration of episodes of paroxysmal AF in some patients".

Although intravenous digoxin may effectively slow the HR at rest, there is a delay of at least 60 minutes before onset of a therapeutic effect in most patients, and a peak effect does not develop before up to 6 hours. If rate control alone is the purpose of treatment then intravenous diltiazem appears to be a potentially more effective alternative to digoxin, although the experience is comparatively small. Some data also support the use of the beta-blocker esmolol, but there are no data on the safety and efficacy of beta-blockers in acute AF and HF and concerns remain about their safety in severe, unstable HF. Only a few studies have investigated the role of beta-blockers in the management of chronic AF in the setting of chronic HF, presumably due to fears about their negative inotropic effects.

**Control of HR in persistent AF**

When restoration of sinus rhythm is not possible or is not attempted in patients with AF, control of the ventricular rate is essential. The risk of long-term side-effects of amiodarone renders alternatives preferable for the long-term control of ventricular rate. Amiodarone should be reserved for those in whom a policy of cardioversion is being actively pursued or to increase the chance of maintaining sinus rhythm after cardioversion. For long-term use, beta-blockers are relatively safe to control heart rate (HR) in AF patients. Verapamil and diltiazem reduce HR both at rest and during exercise better than placebo. However, the negative inotropic effect of oral calcium antagonists requires that they be used cautiously in patients with HF. Calcium antagonists are preferred over beta-blockers for long-term use in patients with chronic obstructive pulmonary disease. Digoxin is generally effective for rate control in persistent AF, at least at rest, particularly when congestive HF is present.

**Maintenance of sinus rhythm after cardioversion**

Studies of electrical cardioversion in patients with predominantly mild chronic HF suggest that about 70% will relapse into AF by 1 year without prophylactic antiarrhythmics. Amiodarone increases the chance of remaining in sinus rhythm to 42–85% at 1 year and appears to have a neutral effect on survival in chronic HF. However, doubts remain about the long-term toxicity and tolerance of amiodarone and its safety in those with previous drug-induced torsade-de-pointes. However, currently it appears the safest
and most effective prophylactic drug for the maintenance of sinus rhythm in patients with chronic HF. Novel class III antiarrhythmics could replace amiodarone for conversion and maintenance of sinus rhythm if they are shown to be safe and effective in patients with chronic HF, but they may not control the ventricular rate. So far only dofetilide has been shown not to increase mortality in chronic HF whilst being modestly effective in cardioverting and prolonging the arrhythmia-free interval in the subgroup of chronic HF patients with AF.

What of the widespread practice of using digitalis as prophylactic therapy in patients with paroxysmal AF? There are no comparable randomized placebo-controlled studies of this strategy, but a study of 139 episodes of AF during ambulatory monitoring in 72 patients did not support it. Thirty-one of the patients were taking digoxin, and there was no difference between those taking and those not taking digoxin, either in the frequency of attacks or in the ventricular rate during attacks (140/minute vs 134/minute). Furthermore, digoxin therapy was associated with a significantly greater number of prolonged attacks of AF (defined as those lasting more than 30 minutes). In keeping with this and other observations, the AHA/ACC/ESC guidelines state that "the evidence available does not support a role for digitalis in suppressing recurrent AF in most patients."

Rate control vs rhythm control

Recently, the results of the AFFIRM trial, involving 4060 patients with AF randomly allocated to a "rhythm control" versus a "rate control" strategy, were published. This trial showed no difference in mortality and other important secondary endpoints, including quality of life, between the two strategies. A sub-study of 1968 patients from the rate-control arm of AFFIRM found that both β-blockers and calcium-channel blocking agents were effective as first-line agents in about 50–70% of patients, and that digoxin (which was allowed to be added as a second-line agent) appeared to increase the rate control efficacy of these agents modestly. A further analysis found digoxin to be the sole drug that was significantly related to survival; it increased death rate.
P-glycoprotein

In 1976, a 170 kDa glycosylated membrane protein was isolated from colchicine-resistant Chinese hamster ovary cells. Since this glycoprotein appeared unique to sublines displaying altered drug permeability, it was named P-glycoprotein (Pgp). A significantly decreased uptake and retention of daunorubicin and adriamycin was also found in sublines of mouse P388 leukemia resistant to these anthracyclines. About ten years later, the human MDR1 gene was isolated from multidrug-resistant KB carcinoma cells, and was demonstrated to encode human Pgp.

Pgp belongs to a large group of functional proteins that share common structural and functional properties. This superfamily is known as the ATP-binding cassette (ABC) superfamily. To date, more than 40 human ABC transporter genes have been identified and sequenced. Hitherto reported human ABC transporters are classified into 7 subfamilies, ABCA to ABCG. In this new system of nomenclature, Pgp is defined as ABCB1.

Over the last decade, it has been elucidated that human Pgp is expressed in normal tissues including the liver, kidneys, small and large intestines, brain, testis, muscle tissue and placenta. It is well-accepted that Pgp confers an intrinsic resistance to normal tissues by exporting unnecessary or toxic exogeneous substances or metabolites out of the body. Intestinal Pgp locates in the brush border membrane of enterocytes and limits absorption of the Pgp substrates. Pgp in the capillary endothelial cells of brain and testis also limits the transport of substrates into the extravascular space. Pgp in the luminal membrane of renal proximal tubules accelerates their secretion into the urine. Thus, Pgp expressed throughout the body regulates the pharmacokinetics of drugs which are Pgp substrates. Generally speaking, drug transporters, e.g. Pgp, and drug metabolizing enzymes, e.g. cytochrome P450s (CYPs), are the two major biological factors determining the pharmacokinetics of drugs.

More than 200 years ago digitalis glycosides were first used by William Withering in heart disease studies. Digoxin is still one of the most commonly prescribed drugs in the treatment of congestive HF. It was early recognized that safe administration of digitalis glycosides was a difficult task, and their use were very often complicated by cardiac side-effects. Digoxin has a very narrow therapeutic window; drug/digoxin interactions which modulate SDC may result in hazardous clinical consequences, e.g., verapamil can increase the plasma concentration of digoxin in a dose-dependent way up to 60-90% at a daily verapamil dose of 240 mg and thus can cause digoxin intoxication, especially in patients with a high digoxin level before starting verapamil therapy. In a study done by Klein et al. in 1982 seven of 49 patients showed signs of digoxin intoxication after combination therapy of digoxin with verapamil. Also, quinidine and amiodarone coadministered with...
digoxin have been shown to cause clinical toxicity. Both drugs are Pgp inhibitors like verapamil. In vitro studies and an in vivo study showed that the digoxin/verapamil interaction occurs at the apical membranes in renal tubular cells by blocking Pgp, the specific pump which extrudes digoxin out of the cells. Verapamil substantially decreases the renal clearance of digoxin. In most cases it does not lead to digoxin toxicity. However, caution has been recommended regarding the use of this drug combination.

Digoxin is excreted exponentially, with an elimination half-life of 36 to 48 hours in patients with normal renal function. Renal excretion of digoxin is proportional to the glomerular filtration rate (and hence to creatinine clearance). With daily maintenance therapy, a steady state is reached when daily losses are matched by daily intake. For patients not previously given digoxin, institution of daily maintenance therapy without a loading dose results in development of steady-state plateau concentrations after 4 to 5 half-lives, or 7 to 10 days, in subjects with normal renal function. If the elimination rate of the drug is prolonged, the length of time before a steady state is reached on a daily maintenance dose is also prolonged proportionately. A patient's estimated lean body mass should be used in the calculation for maintenance dosing. Also, recent evidence suggests that the steady-state volume of distribution of digoxin (Vdss) is decreased in chronic renal failure, and therefore loading doses of digoxin as well as maintenance doses should be decreased in these patients.

Disturbances of cardiac impulse formation, conduction, or both are hallmarks of digoxin toxicity. Among the most common ECG manifestations are ectopic beats of atrioventricular, junctional or ventricular origin, first-degree atrioventricular block, an excessively slow ventricular rate response to AF, or an accelerated atrioventricular junctional pacemaker. These manifestations require only a dosage adjustment and monitoring as clinically appropriate. More severe manifestations include severe bradycardia and heart block; in cases where concomitant electrolyte abnormalities are present, malignant ventricular arrhythmias may occur.

**Adverse drug reactions**

To estimate the contribution of drug-related events to admission rates, several studies have investigated consecutive hospital admissions during defined time periods. The proportions of patients admitted to hospital secondary to an ADR vary between 2.4-16.8% . While some adverse drug events are unpredictable (such as anaphylaxis from an unrecognized allergy), many others can be anticipated and prevented.
Drug-drug interactions

Drug-drug interactions are a particularly important type of adverse drug event because they are often predictable based on previous reports, clinical studies, and an understanding of pharmacologic principles. For example, digoxin toxicity can develop in patients simultaneously treated with clarithromycin because the latter inhibits Pgp\textsuperscript{276}. Patients admitted with digoxin toxicity (n=1051) were about 12 times more likely to have been treated with clarithromycin (adjusted odds ratio, 11.7; 95% confidence interval, 7.5-18.2) in the previous week\textsuperscript{277}. Many hospital admissions of elderly patients for drug toxicity occur after administration of a drug known to cause drug-drug interactions. Many of these interactions could be avoided.

Pharmacodynamic effects are usually monitored by direct measurement of physiological indices of therapeutic responses, such as lipid concentrations, blood pressure or blood glucose. However, for many drugs a readily available effective measure is lacking or is insufficiently sensitive. Furthermore, a large interindividual variability with regard to dose or concentration and response can make individualizing drug dosage difficult. This is especially the case for drugs with a narrow therapeutic index, large interindividual variability in pharmacokinetics, or a concentration-dependent pharmacokinetics. Drug level monitoring (therapeutic drug monitoring, TDM) combines the measurement of drug concentrations in body fluids (especially in plasma, serum, whole blood) with pharmacokinetics and pharmacodynamics. Hence, TDM can be a valuable and useful tool for some specific drugs to optimize and individualize pharmacotherapy. Furthermore, TDM may contribute to minimize the risk of concentration-dependent adverse drug reactions and therefore, may be an essential part of clinical management. This also applies to digoxin which is still a widely used drug for congestive HF and for AF, even though its effectiveness and safety are debated.
Aims of Part II

The aims of Part II of this thesis were;

a) to determine whether there is a sex-based difference in SDCs and adverse drug reactions to digoxin

b) to characterize the prevalence and clinical significance of P-gp interactions among patients on digoxin therapy

c) to explore the effect of digoxin on mortality in patients with AF, HF, or both
Theoretical background to studies III-V

**Study III:** In a post-hoc analysis of the DIG-trial, Rathore et al.\(^{238}\) found that digoxin therapy is associated with an increased risk of death among women but not among men. The trial had insufficient statistical power to test whether the interaction between sex and digoxin therapy was dependent on sex-based differences in SDCs. Was the digoxin dose too high for women?

**Study IV:** Concerns have been raised about the high rate and the quality of drug use in older people\(^ {278}\). The high rate has been attributed in part to the accumulation of diseases with aging. Use of several drugs is justified in the treatment of multiple chronic diseases but it increases the risk of adverse drug events\(^ {279}\). Drugs most often associated with adverse events involve those most commonly used by older people, such as cardiovascular drugs.

Adverse drug events occur in 14.6% to 35% of older people, depending on the population setting and measures employed for their identification\(^ {280-282}\), and have been shown to increase exponentially as the number of drugs used increases\(^ {283}\). One important risk factor for adverse drug events in older people is pharmacokinetic drug-drug interactions. In fact, it has been shown that 43% to 53% of the occurrence of inappropriate drug use among the elderly population concerned drug interactions, drug duplication or use of drugs inappropriate for long-term use\(^ {278, 280, 284, 285}\). Thus, many adverse drug reactions today can be attributed to pharmacokinetic problems. Alterations of P450-catalyzed reactions in the liver are the most studied. However, the field has expanded to encompass the full spectrum of drug disposition and excretion, and drug-drug interactions at the absorptive and excretional level may turn out to be equally important as those at the metabolic level. At these former levels, energy-dependent efflux proteins, i.e. the ABC-transporters, have emerged as possible sites of interactions. Pgp is the most extensively studied ABC-transporter and is located to epithelial barriers in the kidneys, liver, intestine and brain, where it functions as an efflux pump and affects bioavailability, distribution and elimination of certain drugs\(^ {268}\). So far, studies on the clinical significance of drug-drug interactions with Pgp has been limited.

Digoxin is a high-affinity Pgp-substrate\(^ {286}\). Recent publications have shown a sex-based difference in mortality\(^ {238}\), and subsequently an increased mortality for men with SDC > 1.5 nmol/L\(^ {239}\). In this context, heightened attention to a patient’s SDC is warranted.

Digoxin is commonly used in the treatment of congestive HF and AF. Among individuals aged 75 and older, almost 20% were on medication on digoxin in 1996\(^ {287}\). Despite declining use in the last few years, digoxin is still one of the most frequently prescribed drugs; it was listed twice among the top 200 prescriptions in 2000\(^ {288}\). It is associated with intoxication and
adverse drug effects such as cardiac arrhythmia, which sometimes can be serious, especially as the dosing of the drug can be difficult. In 1995, it was the drug most often monitored therapeutically because of its narrow therapeutic window and potentially serious side effects. Risk factors for intoxication such as high age, impaired renal function and low body weight are well-known to clinicians. In spite of this, statistics show that unintended digoxin intoxication remains a common problem.

Several drugs demonstrate an inhibitory effect on Pgp in vitro, and raise the SDC in vivo, and hence Pgp is recognized as a site for drug-drug interactions. In study IV, we hypothesized that drug-drug interactions with Pgp is a major determinant of digoxin serum concentration.

Study V: Digoxin is widely used in clinical practice for treatment of HF and AF. While the efficacy of digoxin in AF has been disputed, the beneficial effects of digoxin in HF include reduced symptoms, improvement in NYHA class, increased exercise time, modestly increased LVEF, enhanced cardiac output, and decreased HF hospitalizations. Furthermore, digoxin withdrawal has been associated with an increased hospitalization rate and decreased LVEF, as shown in the PROVED and RADIANCE trials.

Although the DIG-trial found no effect of digoxin on mortality overall, several recent studies have suggested digoxin to be an independent risk factor for mortality, i.e., in patients with HF in sinus rhythm, survivors of ventricular fibrillation, and following an acute myocardial infarction, but not in patients with severe HF. Most recently, a post-hoc study of the AFFIRM trial found digoxin to increase death rate (R=1.41), but it was suggested that this may have represented the prescription of digoxin for patients at greater risk of death, such as those with HF.

Further studies are needed to test the effect of digitalis therapy on mortality and in particular to test the notion that digoxin may increase mortality in some patient categories, e.g. AF. In this study, we studied the 1-year mortality rate among patients admitted to coronary care units in Sweden with AF, HF or AF+HF and prescribed or not prescribed digoxin, with the aim of testing the effect of long-term therapy with digoxin on mortality in these patient groups, primarily in those with AF.
Study III & IV: All patients on digoxin TDM at our hospital over the last three years were considered for these studies. Patients were included if they were on oral digoxin treatment, steady-state concentration was reached, the serum sample was measured at trough and information about concomitant treatment and serum creatinine was available (n=618). The SDCs had been determined using fluorescence polarization immunoassay. Patient characteristics are shown in table 7.

In study III, we compared the SDCs between men and women, adjusting for the potential covariates age, dose and serum creatinine, to determine if there is a sex-based difference in SDCs in a normal, clinical setting.

In study IV, we classified the concomitantly administered drugs according to Pgp inhibiting capacity. Medline was systematically searched for the INN substance name and English spelling combined with the terms “P-gp”, “Pgp” and “MDR1”. Substances were classified as Pgp inhibitors when demonstrating a clear inhibitory effect on Pgp in cellular transport assays, ATPase activity assays, cellular uptake assays or in animal models using mdr1a(-/-)mice. Any effect of each drug on digoxin pharmacokinetics in vivo was also documented.

To evaluate whether only Pgp inhibitors with well-recognized digoxin interactions in vivo contribute to a change in SDC, the Pgp inhibitors were further divided into two groups: ClassI Pgp inhibitors, with well-documented effects on digoxin pharmacokinetics in vivo and ClassII Pgp inhibitors with established Pgp inhibitory effect in vitro, and putative effects on SDC in vivo. ClassI and II Pgp inhibitors were compared to drugs that had no or unknown effect on Pgp. Only substances administered orally were included in the classification. Verapamil, cyclosporine A, amiodarone, quinidine, quinine, dipyrimadole, carvedilol, atorvastatin and spironolactone comprised ClassI, and bromocriptin, flupentixol, glibencamide, isradipine, lansoprazole, loperamide, medroxyprogesterone, omeprazole, pantoprazole, paroxetine, sertraline, simvastatin and terfenadine ClassII.

The estimated adjusted mean SDCs were calculated with the GLM (general linear models) procedure of the SAS software (SAS Institute, NC, USA) for each Pgp-class. Two different models were used: one univariate and one multivariate including the potential covariates age, dose of digoxin, total number of prescribed drugs for each individual (all continuous), and sex.
Study V: This was a retrospective cohort study. Study patients were obtained from the Register of Information and Knowledge about Swedish Heart Intensive care Admissions (RIKS-HIA) between 1995 and 2001. RIKS-HIA contains detailed information on all patients admitted to most coronary care units at Swedish hospitals, as previously described. We obtained 1-year mortality data by combining information from the RIKS-HIA database with that from the National Cause of Death Register, which includes the vital status of all Swedish citizens.

An overview of the inclusion of patients is given in figure 8. The study subjects consisted of three categories; a) patients with AF, b) patients with HF, and c) patients with AF+HF. The AF group consisted of patients with an ECG-finding of AF at admission or at discharge, or patients with a discharge diagnosis of AF (ICD-10 I48), without a concomitant diagnosis of HF or pulmonary edema. The HF group consisted of patients with a medical history of HF, a diagnosis of HF at discharge (ICD-10 I55.1, I55.9), or with pulmonary edema at admission, without concomitant AF. The AF+HF group consisted of patients with an ECG-finding of AF at admission or at discharge, or patients with a discharge diagnosis of AF, with a concomitant medical history of HF, a diagnosis of HF at discharge or pulmonary edema at admission. Patients who died during hospital stay were excluded. Information on digoxin therapy was lacking in 20% of the cases, but varied considerably between reporting hospitals. Only patients with known digoxin therapy status derived from hospitals with <25% missing data regarding digoxin therapy were included, giving a total number of 57,203 patients. These three groups were divided into two subgroups, according to digoxin therapy. Mortality rates during the one year after discharge were obtained for each category, and then compared between those who were prescribed digoxin versus those not prescribed the drug. A further division according to sex was also done, since a sex-based difference in mortality among patients taking digoxin has been described.

The average relative risk (RR) of death for patients discharged with digoxin as compared to those without digoxin was estimated using Cox-regression analysis. Separate estimates of the RR were also calculated for men and women. Since this was a retrospective study we did not know the probability of receiving digoxin therapy for each patient as we would have in a randomised controlled clinical trial. In order to estimate these probabilities we applied a propensity score method using logistic regression analysis. Propensity scoring is a well-established method used in observational studies such as the present, and have been used extensively in previous studies. Similar propensity score methods have also been used in other studies of data from the RIKS-HIA. The number of patients available for complete case analyses in the adjusted model were a total of 46,341 (n FF=16,465; n HF=17,036; n AF+HF=12,840). The variables included in the logistic regression analysis were: age, sex, smoking status, diabetes mellitus,
hypertension, medications at admission (ACE inhibitors, antiarrhythmics, diuretics, nitroglycerin, beta-blockers, ASA/platelet inhibitor, lipid lowering drugs, anticoagulant drugs), history of heart failure, admission ECG (ST-elevation, LBBB), pacemaker, and CPAP (continuous positive airway pressure). Several two-way and one three-way interactions were included after selection by a stepwise procedure using the Akaike information criteria for inclusion/exclusion. The propensity score was then entered together with digoxin and discharge treatments (ACE inhibitors, antiarrhythmics, diuretics, nitroglycerin, beta-blockers, ASA/platelet inhibitor, lipid lowering drugs, anticoagulant drugs) in the Cox-regression analysis in order to adjust for differences between the digoxin and non-digoxin patients regarding the variables listed above. We thus compared patients with similar probability of receiving digoxin in order to distinguish between the risk of digoxin treatment and the risk of the indication for which digoxin was given. All statistical analyses were done using R, version 1.8 (R foundation for Statistical Computing, Vienna, Austria).
Results

Study III: The mean SDC was significantly higher in women than in men. Adjustment for age, dose, and serum creatinine had only a minor effect on the estimates.

We also searched the Swedish national register of adverse drug reactions to see whether adverse reactions to digoxin are more common among women than among men. The results are shown in table 7.
**Study IV:** Only results from the adjusted model are presented as it differed little from the univariate model. In addition, sub-class analyses including the variables p-creatinine and weight did not alter the results significantly. Overall, patients with concomitant Pgp inhibitors had higher SDCs than patients without (1.55 ± 0.04 compared to 1.26 ± 0.04 nmol/L, p < 0.001, mean values ± SE) (Fig. 7A). Also, patients with SDCs above the recommended therapeutic range (> 2.5 nmol/L at that time) were more likely to have Pgp inhibitors co-administered compared to patients with SDC ≤ 2.5 nmol/L (68% vs. 47%, p = 0.01). There were significant differences in mean SDCs for patients taking 0 Pgp inhibitors compared to patients taking 1 or 2 ClassI Pgp inhibitors (Fig. 7B). The effect on the mean SDCs among patients with ClassI Pgp inhibitors was not solely attributed to the most frequently co-administered Pgp inhibitors spironolactone and verapamil, since exclusion of patients with these drugs gave similar, however smaller, differences. Patients taking 1 or 2 ClassII Pgp inhibitors also tended to have elevated SDCs compared to patients with no Pgp inhibitors. However, these differences were not significant in the adjusted model.

**Figure 7.** The association between SDC and the number of prescribed P-gp inhibitors. (A) Adjusted* SDC means for the patients without ('0') (n = 328, SDC mean ± SE, 1.26 ± 0.04 nmol/L) or with ('1'), P-gp inhibitors (n = 290, SDC mean ± SE, 1.55 ± 0.04 nmol/L). (B) Adjusted* SDC means for patients taking zero, one, two or three P-gp inhibitors. The number of patients were 328, 204, 78 and eight, respectively. The SDC means ± SE (nmol/L) were 1.26 ± 0.04, 1.51 ± 0.05, 1.59 ± 0.08 and 2.00 ± 0.25.

*Adjusted for age, sex, digoxin dose and total number of prescribed drugs.
Study V: Unadjusted patient characteristics according to digoxin therapy status are shown in Table 8. Table 9 shows the same characteristics within propensity score quintiles. As can be seen from Table 8, there are differences in characteristics between patients discharged with or without digoxin. Within propensity score quintiles the characteristics are more balanced between the groups (Table 9). The comparison groups generated using propensity score were well-balanced with respect to the collected baseline risk factors. The likelihood of being discharged with digoxin, regardless of digoxin therapy at the time of admission, decreased significantly for each year; from about 30-35% in 1995 to 20-23% in 2001. Patients with AF had an overall 1-year mortality of 9.5%, which was lower than for those with HF; 25.4%. The highest 1-year mortality was seen in those with AF+HF; 26.7%.
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<th>HF</th>
<th>AF+HF</th>
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Table 8. Unadjusted patient characteristics according to digoxin therapy status. Values are percentages unless otherwise stated. BMI=body mass index; ACE=angiotension converting enzyme; CPAP=continuous pulmonary airway pressure; PTCA=percutaneous transluminal coronary angioplasty; CABG=coronary artery by-pass grafting; ASA=acetylsalicylic acid; ECG=electrocardiography.
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Table 8 (cont.) Unadjusted patient characteristics according to digoxin therapy status. Values are percentages unless otherwise stated. BMI=body mass index; ACE=angiotension converting enzyme; CPAP=continuous pulmonary airway pressure; PTCA=percutaneous transluminal coronary angioplasty; CABG=coronary artery by-pass grafting; ASA=acetylsalicylic acid; ECG=electrocardiography.
Table 9. Patient characteristics of patients with or without digoxin therapy within propensity score quintiles 1-5 (Q1-5). Values are percentages unless otherwise stated. BMI=body mass index; ACE=angiotension converting enzyme; CPAP=continuous pulmonary airway pressure; PTCA=percutaneous transluminal coronary angioplasty; CABG=coronary artery by-pass grafting; ASA=acetylsalicylic acid; ECG=electrocardiography.

* 25th, 50th and 75th percentile

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Table 8. Patient characteristics of patients with or without digoxin therapy within propensity score quintiles 1-5 (Q1-5). Values are percentages unless otherwise stated. BMI=body mass index; ACE=angiotension converting enzyme; CPAP=continuous pulmonary airway pressure; PTCA=percutaneous transluminal coronary angioplasty; CABG=coronary artery by-pass grafting; ASA=acetylsalicylic acid; ECG=electrocardiography.

* 25th, 50th and 75th percentile
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Table 9 (cont.) Patient characteristics of patients with or without digoxin therapy within propensity score quintiles 1-5 (Q1-5). Values are percentages unless otherwise stated. BMI=body mass index; ACE=angiotension converting enzyme; CPAP=continuous pulmonary airway pressure; PTCA=percutaneous transluminal coronary angioplasty; CABG=coronary artery by-pass grafting; ASA=acetylsalicylic acid; ECG=electrocardiography.
**Patients with AF**

The estimated one-year survival probability for patients with AF is shown in Figure 9, according to digoxin therapy status. Patients who were discharged with digoxin did worse than those who did not receive the drug. According to the adjusted data, RR for death was 1.44 [95% CI 1.29-1.60]. There was no statistically significant sex-based difference (RR for death among women 1.39 [95% CI 1.19-1.62] and among men 1.49 [95% CI 1.30-1.72]).

**Patients with HF**

The estimated one-year survival probability for patients with HF is shown in Figure 10, according to digoxin therapy status. Patients who received digoxin at the time of discharge did somewhat worse than those who did not receive the drug. According to the adjusted data, RR for death was 1.12 [95% CI 1.04-1.21]. There was no statistically significant sex-based difference (RR for death among women 1.11 [95% CI 1.00-1.24] and among men 1.13 [95% CI 1.02-1.24]).

**Patients with AF+HF**

The estimated one-year survival probability for patients with AF+HF is shown in Figure 11, according to digoxin therapy status. There was no difference between patients who were discharged with digoxin as compared to those who did not receive the drug; according to the adjusted data, RR for death was 1.04 [95% CI 0.97-1.12]. No significant sex-based difference was observed (RR for death among women 1.12 [95% CI 1.01-1.25] and among men 0.99 [95% CI 0.91-1.09]).
AF
Inclusion criteria (any of the following):
- ECG-finding of AF at admission
- Discharge diagnosis of AF (ICD-10 I48)

Exclusion criteria (any of the following):
- Medical history of HF (ICD-10 H50.0, H50.1 or H50.9)
- Diagnosis of HF at discharge (ICD-10 H50.0, H50.1 or H50.9)
- Pulmonary edema at admission

HF
Inclusion criteria (any of the following):
- Medical history of HF (ICD-10 H50.0, H50.1 or H50.9)
- Diagnosis of HF at discharge (ICD-10 H50.0, H50.1 or H50.9)
- Pulmonary edema at admission

Exclusion criteria (any of the following):
- ECG-finding of AF at admission
- ECG finding of AF at discharge
- Discharge diagnosis of AF (ICD-10 I48)

AF+HF
Inclusion criteria (must fulfil at least one criteria in both section A and B):
A
- ECG-finding of AF at admission
- Discharge diagnosis of AF (ICD-10 I48)

B
- Medical history of HF (ICD-10 H50.0, H50.1 or H50.9)
- Diagnosis of HF at discharge (ICD-10 H50.0, H50.1 or H50.9)
- Pulmonary edema at admission

n total=78,155

Exclusion of patients who died during hospital stay
n total=70,936

Exclusion of patients derived from hospitals with >25% drop-out regarding information on digoxin therapy
n total=63,689

Exclusion of patients with unknown digoxin therapy status
n total=57,203

Patients with AF
n women=8,521
n men=12,191

Patients with HF
n women=9,921
n men=11,711

Patients with AF +HF
n women=6,681
n men=9,178

Figure 8. Inclusion of study-patients registered in RIKS-HIA.
Figure 9. 1-year estimated survival probability – atrial fibrillation. Estimated survival probability for patients discharged with (dashed line) and without (solid line) digoxin. The left figure shows the crude survival probability, whereas the right shows survival probability adjusted for propensity score and other discharge treatments. RR = relative risk (95% confidence interval).

Figure 10. 1-year estimated survival probability – heart failure. Estimated survival probability for patients discharged with (dashed line) and without (solid line) digoxin. The left figure shows the crude survival probability, whereas the right shows survival probability adjusted for propensity score and other discharge treatments. RR = relative risk (95% confidence interval).
Figure 11. 1-year estimated survival probability – atrial fibrillation + heart failure. Estimated survival probability for patients discharged with (dashed line) and without (solid line) digoxin. The left figure shows the crude survival probability, whereas the right shows survival probability adjusted for propensity score and other discharge treatments. RR=relative risk (95% confidence interval).
Limitations

**Study III and IV:** There are several limitations to study III and IV. First, the studies were not prospective, randomized trials, meaning that they may have been affected by confounders. For instance, treatment indication for digoxin was unknown, as was the medical history of the patients. Second, patients on digoxin therapeutic drug monitoring do not necessarily represent the usual patient on digoxin therapy. Third, the digoxin doses may have been adjusted according to s-digoxin measurements and according to concomitant therapy, although this would rather underestimate the differences between groups in study IV. Fourth, weight and lean body mass was known only for a minority of patients.

**Study V:** Several limitations to this study need to be considered. First, the RIKS-HIA database does not include serum digoxin concentrations or digoxin dose. It may be argued that the higher observed mortality for patients with AF discharged with digoxin as compared to those with HF could be due to a comparably higher dose of digoxin in the former group. Subgroup analysis from the DIG trial recently showed a relation between serum digoxin level and mortality.\(^{239}\) The risk of death increased significantly if the digoxin level exceeded 1 ng/mL, whereas a digoxin level of 0.5 to 0.8 ng/mL was associated with a decrease in all-cause mortality in men. This relation was maintained after multivariable adjustment: hazard ratio, 0.8 (95% CI, 0.68 to 0.94). A similar analysis for women could not be done because the digoxin level was not available in most women. Although we did not have the doses of digoxin for the patients in our study, the findings in patients with concomitant AF and HF, who was likely given a dose similar to that for patients with AF alone, argues against the possibility of a dose-related difference in mortality. Second, although our results are based on a large cohort and have been adjusted for many confounding factors, a registry study cannot compensate for all confounders and, hence, cannot replace a randomized controlled trial. Propensity analyses are inherently limited by the number and accuracy of the variables evaluated. Nevertheless, propensity scoring is a well-established tool that enables excellent matching of baseline characteristics\(^ {405}\), and there are limitations to randomized controlled trials, e.g., strict inclusion and exclusion criteria may limit the applicability of the study results to other, perhaps more typical, patient populations. Third, the RIKS-HIA database did not include data on renal function, an important determinant of mortality.\(^ {306}\) Fourth, this study did not investigate the effect of digoxin on mortality in the emergency setting, only during long-term use.
Discussion

**Study III**: Evidently, these sex differences cannot be explained by an interaction between digoxin and progestin in combined hormone-replacement therapy, as suggested by Rathore et al., nor can they be explained by differences in digoxin prescriptions. Thus, the findings of a sex-based difference in mortality in the DIG-trial could well have been due to sex-based differences in SDC. We conclude that digoxin therapy in women should be administered with heightened attention to the appropriate dose.

**Study IV**: In this report we show that co-administration of digoxin and Pgp inhibitors as a group is associated with significant elevations in SDCs. Juurlink et al showed that patients hospitalized for digoxin intoxication were profoundly more likely to be on medication with the Pgp inhibiting drug clarithromycin. However, many clinically used drugs are Pgp inhibitors, clarithromycin being only one of them, and the clinical implication of pharmacokinetic interactions between these drugs as a group and digoxin has not previously been addressed.

Pharmacokinetic interactions with digoxin can arise from mechanisms other than Pgp, e.g., changes in gut motility or pH, disturbance of digoxin metabolizing intestinal bacteria or possibly from interactions with other transport proteins. Pgp seems, however, to be a major determinant for digoxin pharmacokinetics. Spironolactone is known to interfere with digoxin analysis, but at doses higher than those taken by the patients in this study. Possible explanations for the more pronounced effect of ClassI Pgp inhibitors on SDCs compared to ClassII Pgp inhibitors may be that a majority of the ClassI inhibitors are given at higher doses compared to ClassII inhibitors (the median and range of the lowest recommended dose is 40 (10-200) mg for ClassI vs 10 (0.25-60) mg for ClassII), and that ClassI inhibitors often demonstrate greater inhibitory effect on Pgp.

As the mortality rate, at least among men with HF, was recently shown to increase at SDCs above 1.5 nmol/L, pharmacokinetic Pgp interactions may in fact influence mortality rate, as pointed out by de Denus et al. Particular notice should be taken when prescribing Pgp inhibitors to digoxin patients with increased risk to develop toxicity, such as the elderly, patients with impaired renal function, and females. As a great proportion of the digoxin TDM patients in this study, 47%, had one or more Pgp inhibitor prescribed, and a clinically important elevation of the SDC (1.25 ± 0.04 vs 1.65 ± 0.07 nmol/L) was observed already with one ClassI Pgp inhibitor co-administered, we conclude that an increased awareness about Pgp interactions is warranted among prescribing clinicians. That inappropriate prescribing as a cause of adverse drug events is preventable is especially
important in the treatment of an already frail older population. This means that knowledge of the basic principles for appropriate prescribing is of fundamental importance. Our data show that awareness about Pgp interactions is important. It is our belief that an increased awareness among prescribing clinicians about this pharmacokinetic principle can help avoid many cases of Pgp substrate intoxication, e.g. digoxin intoxication.

**Study V:** The results of this study suggest an increased 1-year mortality rate after discharge from coronary care units in Sweden for patients with AF without HF given digoxin. This increase persisted after adjustment for potential confounders. On the other hand, digoxin therapy did not alter mortality in patients with HF+AF, whereas patients with HF without AF took an intermediate position. The relative increase in mortality for patients with AF found in this study is in accordance with previous experiences from the AFFIRM trial. This study found digoxin to be the sole drug that was significantly related to survival; it increased death rate. It has been suggested that rather than reflecting a deleterious effect of digoxin on survival, the result may have represented the prescription of digoxin for patients at greater risk of death, such as those with HF. The results of the present study indicate that the latter theory is unlikely and rather suggest that digoxin is an independent risk factor for death among patients with AF without HF on long-term therapy with this drug.

The use of digoxin in patients with AF has been controversial. Although still widely used in clinical practice, perhaps due to the theoretical advantage of a positive inotropic effect and a modifying effect on the ventricular rate, there is no evidence to suggest that digoxin has any effect neither on cardioversion in AF in the presence or absence of HF, nor in suppressing recurrent AF. Although intravenous digoxin slows the ventricular rate at rest, there is a delay of about 1 h and a maximum response occurs after about 6 hours. During exercise and in patients with increased sympathetic activity, digoxin alone does not control ventricular response unless large doses that are likely to produce intoxication are used. Digoxin is generally not recommended as first-line therapy for management of AF, except in patients with concomitant HF or left ventricular dysfunction. In persistent AF there are more effective alternatives such as calcium channel- and beta-blockers, but digoxin is usually considered a potentially useful adjunct therapy. The present study did not evaluate the effect of digoxin on mortality in the emergency setting, but the results cast doubts on the use of digoxin for long-term treatment of AF in the absence of HF.

Chronic HF and AF each affect 1–2% of the population, and the prevalence of both rises steeply with age. They share common risk factors and consequently frequently coexist. No study has so far examined the effect on mortality in patients with both HF and AF. Patients enrolled in the DIG
trial were in normal sinus rhythm. Our study suggests that digoxin therapy does not influence mortality in this patient category. A possible explanation may be that these patients represent a group with overall more severe HF; it has been shown that chronic HF may affect more than 50% of patients with AF\textsuperscript{240} and that the prevalence of AF increases in proportion to the severity of chronic HF\textsuperscript{241}. Although the RIKS-HIA database does no include NYHA class, the stepwise higher 1-year mortality between those with AF vs HF vs AF+HF in the studied material indicates an increasing severity of heart disease in the mentioned order. In the DIG-trial, a positive effect on hospitalization rate appeared to be greater among patients with severe HF, i.e., NYHA III-IV\textsuperscript{230}. This finding goes well in hand with that of the present study, i.e. of a stepwise decrease in the difference in mortality between those on digoxin vs those not on digoxin among patients with AF vs HF vs AF+HF. It may be that the combined positive inotropic and negative chronotropic effect of digoxin is beneficial, or at least not harmful, in patients with severe HF, while it may be more of harm than benefit in those with a lesser degree of HF, and in particular those without. Previous research suggest that the worse the heart failure, the lower the ejection fraction; the greater the impairment in myocardial relaxation, the greater the benefit from digoxin\textsuperscript{227}. Thus, long-term therapy with digoxin appears to negatively affect survival in patients with AF without HF, while exerting a neutral effect in patients with AF+HF, the group with the worst overall 1-year mortality. Patients with HF without AF take an intermediate position. A possible mechanistic explanation involves the observed close correlation between decreased LVEF and decreased Na\textsuperscript{+}, K\textsuperscript{-}-ATPase concentration\textsuperscript{226}. An interesting question is, whether the reduction of Na\textsuperscript{+}, K\textsuperscript{-}-ATPase serves compensatory purposes with effects comparable to digitalis treatment.

A cohort study cannot be compared to a prospective, randomized trial, but it deserves to be pointed out that the strength of the present study is that it reflects the normal patient clientele. Patients included in the DIG-trial were highly selected and the results do not necessarily apply to real life. The patient cohort in this study included unselected, consecutive patients. There were no exclusions due to presence or absence of specific risk factors or comorbidities. The representativeness of the cohort was also strengthened by the inclusion of patients from the general population at centers with different levels of care, including 61/71 of the coronary care units within the entire country. The findings of this study of patients with HF suggest that long-term therapy with digoxin should be undertaken cautiously in patients with HF in the absence of AF.

In summary, our study indicates that Swedish citizens admitted to coronary care units due to AF without HF and are discharged with digoxin, and to a lesser extent those with HF without AF discharged with the drug, has an increased mortality as compared to those not given the drug while
digoxin does not seem to affect the mortality among patients with both HF+AF.

Ideally, these findings should be confirmed in prospective, randomized, controlled trials, but since such trials have no commercial interest, they are unlikely to ever be performed. The results of the present study are in accordance with that of the AFFIRM trial and indicates that digoxin is an independent risk factor for death among patients on long-term therapy with AF rather than reflecting the prescription of digoxin for patients at greater risk of death, such as those with HF. We conclude that digoxin may increase mortality when used for long-term treatment of AF without HF, and that caution is advised for long-term therapy with digoxin in HF without AF. The current practice of digoxin use in patients with AF may have to be revised.
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factor-beta1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. 


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