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EDITORIAL

# Annual reports of antibiotic use and resistance - for whom?

**O. Cars**

Professor of Infectious Diseases, Uppsala University, Uppsala, Sweden, Chairman of the Swedish Strategic Programme for Rational Use of Antimicrobial Agents and Surveillance of Resistance (STRAMA)

ABSTRACT

**Sweden, Denmark and the Netherlands, countries with low antibiotic use and low antimicrobial resistance, issue yearly reports on antimicrobial consumption and resistance. In these countries the reports have political priority and aim to disseminate information and promote antibiotic strategies within and between countries.**

The current worldwide increase of antibiotic-resistant bacteria and the simultaneous downward trend in the development of new antibiotics have serious implications. Although antibiotic resistance is increasingly affecting the management of infectious diseases, effective action to contain it has been largely lacking. In many parts of the world the problem is still not regarded as a challenge deserving priority action at the political level.

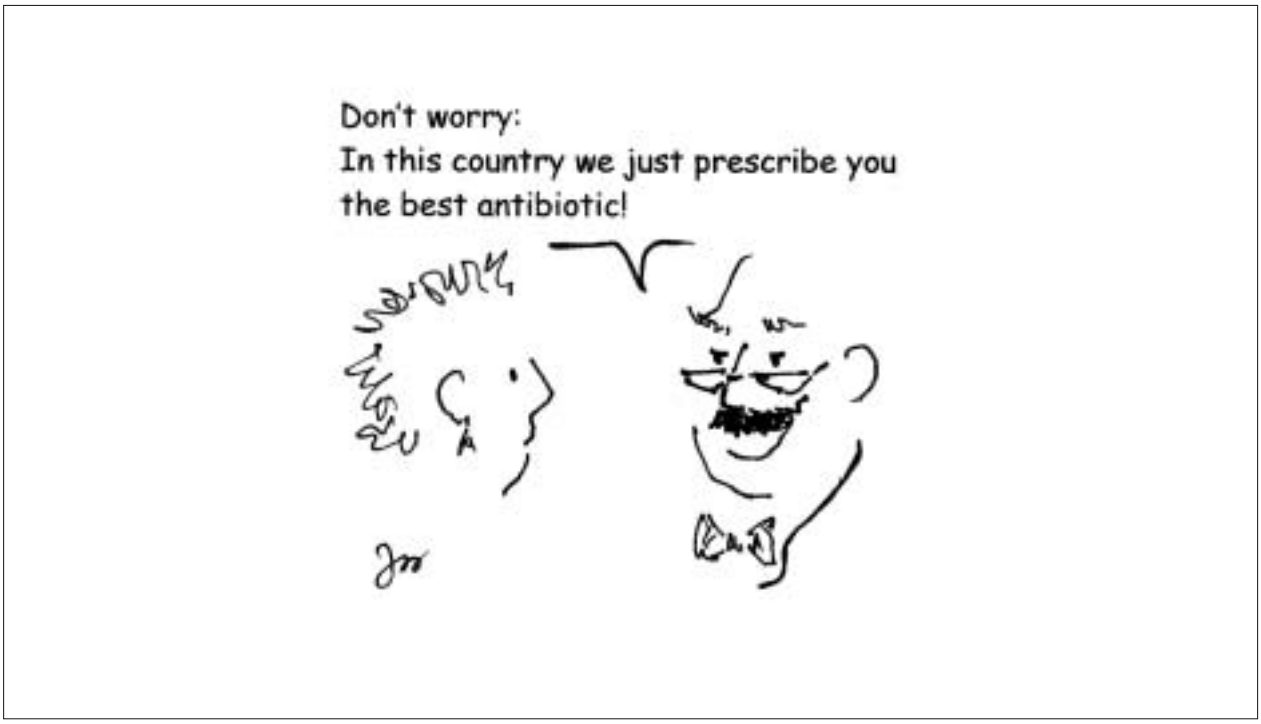
In the Netherlands and the Scandinavian countries, the awareness of antibiotic resistance as a potential threat to public health is high and funds have been allocated for surveillance programmes. A second edition of the report on consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands – NETHMAP 2004 – is now available. Similar annual reports have been published in Sweden (SWEDRES), Denmark (DANMAP) and Norway (NORM). What are the objectives of these reports and who should read them?

The overall aim of a national antibiotic strategy is to contain antibiotic resistance and thereby preserve the possibility of effective antibacterial treatment when it is needed. It is thus evident that *the problem* (resistance rates and trends) needs to be measured. For the same reasons, monitoring antibiotic use – *the major cause of the problem* – is important. Studies linking prescriptions to diagnosis should be encouraged to better evaluate the quality of antibiotic use. However, surveillance systems in themselves do not do any good unless the data are used for action. Antibiotic therapy is still mostly empirical and needs to be based on local guidelines. It is not likely that physicians will benefit from these national reports in their daily practice, but they may help to increase the general awareness of the problem and increase interest in local surveillance and consumption data. On the national level, the reports provide important feedback to 'those who need to know', for example members of drug and therapeutics committees, professional organisations and other bodies where the reports form a knowledge base for policy decisions, guidelines, interventions and research strategies.

Scandinavia and the Netherlands have managed to keep resistance rates and antibiotic use low and this fact is attracting a continuously increasing interest from many parts of the world. Since 2001, the European Surveillance of Antibiotic Consumption (ESAC) project has collected standardised and comparable data on antibiotic consumption. The Netherlands is a 'leading star' with the lowest antibiotic usage in all of Europe. In this context, the Dutch guidelines for restricted antibiotic use in otitis media and other respiratory tract infections are becoming well known and discussed at international meetings.

The annual report on antibiotic use and resistance clearly marks the problem as one of political priority and is a

valuable tool to spread information about the resistance problem to prescribers, healthcare administrators, politicians, patients and parents. Resistance that develops in one part of the world may easily spread to other areas through migration, trade and travel. No country can isolate itself from resistant bacteria. Another important objective of the annual report is therefore to disseminate information and promote successful strategies between countries. To contain the resistance problem a global concerted action is needed.




Cars. Annual reports of antibiotic use and resistance.

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EDITORIAL

# Highly active antiretroviral therapy for HIV infection: lessons for the future

**D.M. Burger, A.J.A.M. van der Ven, P.P. Koopmans**

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## ABSTRACT

**HIV-related morbidity and mortality has been greatly reduced since the introduction of highly active antiretroviral therapy. Issues regarding the patient, the virus, the drugs and the treatment team are discussed. HIV treatment remains complex with a rapidly changing field of possibilities and views, and should therefore be limited to specialised centres.**

In this issue of *the Netherlands Journal of Medicine*, Lowe, Prins and Lange from the International Antiviral Therapy Evaluation Treatment Centre (IATEC) review the studies on efficacy of HAART for the treatment of HIV infection. When all regimens are compared according to the outcome of a plasma viral load of HIV-1 RNA below the detection limit of 50 copies/ml after 48 weeks of therapy, an average value of 50% can be calculated with a wide range from 20 to 88%. The authors describe four factors that determine this wide range: the patient, the virus, the drug, and the treatment team. How can the lessons from this review be applied in daily clinical practice?

## THE PATIENT

Several factors related to the patient may determine treatment outcome. First of all, adherence to this therapy is the most important factor associated with success of therapy. A plethora of studies has been conducted to evaluate which factors determine adherence, and many factors have been found in at least one study to be predictive of adherence. However, only a few patient factors have consistently been associated with good adherence: the absence of depressive symptoms, the ability to implement HAART regimens in daily life, and a good patient-physician (or nurse) relationship.

Depression may occur in at least 20% of HIV-positive patients, which is at least double the incidence in non-HIV-infected individuals. Having a potentially fatal disease, social isolation, and having lost friends due to AIDS may all contribute. The presence of depressive symptoms may be associated with a negative view on treatment possibilities, and adverse effects are possibly less well accepted. Therefore, the diagnosis of depression is of utmost importance before starting HAART. If pharmacological treatment of depressive symptoms is indicated, one should of course be aware of the potential drug-drug interactions with HAART regimens. Unfortunately, many interactions exist between antidepressants and HAART, and consultation of a clinical pharmacologist may be wise.

When HAART was introduced around 1996, many drug combinations existed of three times daily intake, food instructions and a high tablet count (>10 tablets/day was not unusual). In recent years, much has improved, and nowadays regimens are always twice daily or once daily, food restrictions have become less important, and the number of tablets per day is usually less than six to eight. In the near future, HAART regimens consisting of one to two tablets taken once daily will become available.

Although obvious, a good patient-physician (or nurse) relationship may not always be present. Within a medical team, several types of professionals need to be present to deal with the heterogeneous patient population (IV drug addicts, immigrants, etc). The role of dedicated HIV nurses

should not be underestimated. They usually have more time to discuss social aspects of therapy, barriers for 100% adherence, etc. For that reason, the Dutch government has required the presence of at least one HIV nurse at every HIV treatment centre in the Netherlands.

## THE VIRUS

Two aspects of the virus may be relevant here: (1) the magnitude of the plasma viral load, and (2) the presence of primary mutations. As Lowe *et al*. describe, there are conflicting data on the effect of a high baseline viral load (i.e. >100,000 or >1,000,000 copies/ml) on treatment outcome. It is beyond doubt, that it takes longer to achieve an undetectable viral load when the baseline value is high. This may increase development of resistance, although the evidence for this is not strong. Four drug regimens have been tried to achieve a faster and more durable virological response in patients with very high baseline viral load. However, the results were disappointing; in particular the side effects did not balance the effect. At the moment, three drugs should therefore remain the mainstay of HAART until more data are available.
So far, the presence of primary resistance has been documented to be less than 10% in most of the cohort studies. It can be expected, however, that this figure will rise, especially against agents with a low barrier against resistance, such as lamivudine, efavirenz and nevirapine. Unfortunately, the mutations selected by these agents may disappear from the plasma after some time, but remain present in other compartments. Therefore, a negative result of a resistance test on a plasma sample before starting treatment may give false information. Nevertheless, response to HAART may be suboptimal in the case of primary resistance. Recommendations for testing for primary resistance vary within the literature, but Dutch guidelines recommend testing in selected groups of patients.

## THE DRUG(S)

Lowe *et al*. pay relatively little attention to the selection of drugs for the initial HAART regimen while this may be the most important influence a physician may have in assuring treatment response. With the availability of more than 15 different drugs for initial treatment of HIV infection, there is an urgent need for standardisation of first-line treatment both within centres and within the country. Unlike regionalised treatment guidelines for treatment of malignancies, a patient may receive three different first-line regimens in three different HIV treatment centres in the same city. National guidelines for the treatment of HIV infection have therefore been developed recently. Of even more concern is the use of unapproved treatment combinations in the absence of evidence-based criteria. For instance, a number of physicians have started to use triple nucleoside therapy as initial or maintenance treatment while there were no data available about the efficacy and safety of these approaches. Later, data from clinical and cohort studies indicated inferior efficacy of these triple nucleoside therapies, and they are now no longer in use. Some patients, however, have been treated with these inferior regimens while there was no urgent reason for not selecting the recommended regimens. We advocate the use of regional or national treatment guidelines, and that choices for individual patients are made in multidisciplinary teams so that convenience of the regimen, drug-drug interactions, toxicity, comorbidity and potential salvage ability can be taken into account.

## THE TREATMENT TEAM

Very limited data are available on what knowledge is needed to treat HIV-infected patients. Daily practice teaches, however, that extensive knowledge on (new) drugs, changes in toxicity profiles, and (new) drug combinations are a prerequisite for treating HIV infection. Patients frequently interrupt or change their initial regimen because of drug toxicity, while therapy changes as well as interruptions are associated with a less favourable treatment outcome. As Lowe *et al*. indicate, one study has related greater HIV-treatment experience with better survival. In most European countries, HIV care is restricted to specialised centres. In the Netherlands, the Dutch government has selected 22 treatment centres for approximately 8000 to 10,000 HIV-infected adults and children. These treatment centres are selected based on historical arguments and have to fulfil a number of quality criteria. We are awaiting peer review of these treatment centres. In addition, it is well known that there are patients still being treated in hospitals not listed as HIV-treatment centres. Apparently, there is no mechanism to protect patients against this practice. We plead for adherence to the guidelines for treating these patients in specialised centres only and for self-control among the group of medical specialists.

## CONCLUSIONS

Great improvements have been made in the treatment of HIV infections. Mortality has been reduced to less than 5%, and according to current knowledge a newly infected patient may have the same life expectancy as a non-HIV-infected subject, assuming good adherence and tolerability to the drugs. Nevertheless, HIV treatment is remains complex, is a rapid changing field of possibilities and views, and should therefore be limited to specialised centres.

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REVIEW

# Ischaemic preconditioning: from molecular characterisation to clinical application - part II

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**This article is the second part of two papers on ischaemic preconditioning. The first part of this review was published in the November issue of this journal.[1]**

## ABSTRACT

**Ischaemic preconditioning was originally described in animal hearts as histological infarct-size limitation by a previous brief episode of ischaemia. In humans, ischaemic preconditioning has been demonstrated in several *in vitro* and *in vivo* models, including coronary artery bypass grafting and percutaneous transluminal coronary angiograplasty, using surrogate markers of ischaemia and reperfusion injury. Increasing knowledge of the molecular signalling pathways mediating protection by ischaemic preconditioning has provided rational targets for pharmacological intervention. Several widely used drugs are able to mimic ischaemic preconditioning (e.g. adenosine, adenosine-uptake inhibitors, ACE inhibitors, angiotensin II antagonists, statins, opioids, volatile anaesthetics and ethanol), whereas others inhibit ischaemic preconditioning-induced protection (e.g. sulphonylureas and adenosine antagonists). The present review focuses on these different classes of drugs. Prudent use or avoidance of these drugs in patients who are at risk for myocardial infarction could theoretically limit ischaemia and reperfusion injury.**

## INTRODUCTION

In the first part of this review on ischaemic preconditioning, we described the infarct size limiting effects of the naturally occurring phenomenon of ischaemic preconditioning and the time windows in which this effect occurs.[1] Moreover, the interesting observation that a short period of ischaemia also renders distant organs resistant to a subsequent prolonged period of ischaemia was discussed. Finally, the most important triggers, mediators and end-effectors of ischaemic preconditioning that have been identified so far were summarised. However, most data described in this part were derived from animal experiments. Because these studies have convincingly shown that ischaemic preconditioning is the strongest form of *in vivo* protection against myocardial ischaemic injury other than early reperfusion, the possibility of using this phenomenon in clinical practice would be very desirable. Despite state-of-the-art reperfusion strategies, 30-day mortality of myocardial infarction is still around 7%.[2] In addition, the prevalence of cardiac failure is rapidly increasing and is often caused by (ischaemic) death of cardiomyocytes. Thus, there is a need for additional therapeutic strategies that increase tolerance to ischaemia and reperfusion. Exploitation of ischaemic preconditioning may offer such a strategy.

To adequately exploit this mechanism in the everyday clinical setting, three more issues need to be addressed. First, the evidence that preconditioning also occurs in the human heart needs to be discussed. Secondly, if indeed protection can be seen in humans, could it be exploited to develop therapeutic strategies to protect the human heart against ischaemic injury? In clinical practice, it is often not desirable or feasible to precondition myocardium with ischaemia. Fortunately, the accumulating knowledge about the molecular mechanisms mediating preconditioning has provided us with the possibility to modulate ischaemia and

** P. Smits was not involved in the handling and review process of this paper.

reperfusion injury pharmacologically, thus limiting infarct size in the same way as ischaemic preconditioning. Finally, it is essential to identify those patients who may benefit from preconditioning and the situations in which preconditioning could be applied. In the present part of this review, we will consecutively discuss these three issues. Considering pharmacological preconditioning, special emphasise will be put on drugs that are used frequently in internal medicine.

## DOES PRECONDITIONING OCCUR IN THE HUMAN HEART?

Analogous to the previously discussed animal studies, the evidence that ischaemic preconditioning also occurs in humans has been derived from various experimental models, which are summarised in *table 1*. The most important difference between animal studies and human studies on this subject concerns the endpoint that is used to estimate ischaemic injury. Also, the design of the experimental protocol often differs substantially. In animal models, in contrast to human clinical practice, coronary occlusion is often induced and ended abruptly in otherwise healthy animals. Traditionally, in animal studies, the endpoint is histological infarct size. For obvious reasons, this endpoint cannot be used in human studies. Therefore, several models have been developed in which surrogate endpoints are used to evaluate ischaemic preconditioning in humans, recently reviewed by Tomai *et al.* and Kloner *et al.*[3,4]

*In vitro*, classical as well as delayed preconditioning has been shown in cultured cardiomyocytes, using tryptan blue exclusion as endpoint of simulated ischaemia and reperfusion injury.[5,6] The existence of ischaemic preconditioning has also been demonstrated in isolated human atrial trabeculae, obtained from patients undergoing open-heart surgery. In this model, using electrical field stimulation, recovery of contractile force after simulated ischaemia and reperfusion is used as endpoint.[7] Later it was found that preconditioning in this model is also critically dependent on protein kinase C (PKC) activation and adenosine-triphosphate sensitive potassium channel ($K_{ATP}$ channel) opening and that adenosine $A_1$ and $A_3$ receptor stimulation can mimic preconditioning.[8,9]

These *in vitro* models are good candidates to screen drugs on their potential to mimic or modulate ischaemic preconditioning, but cannot directly be extrapolated to clinical practice. In addition to these *in vitro* models, there are several observations in daily clinical practice that might be explained by ischaemic preconditioning. The so-called warm-up phenomenon refers to the naturally occurring phenomenon, which is described in more than half of all patients with coronary artery disease, that performance is improved and ischaemia-induced symptoms are attenuated during a second period of exercise, when compared with the first exercise test. Ischaemic preconditioning has been suggested to be one of the possible causes of this phenomenon, particularly because the warm-up phenomenon lasts no longer than 90 minutes.[10] However, because adenosine receptor stimulation does not seem to

**Table 1**

*Overview of the various in vitro and in vivo models of ischaemic preconditioning in humans with main endpoints and limitations*

| METHOD | MAJOR ENDPOINTS | PROBLEMS/LIMITATIONS |
|---|---|---|
| ***In vitro*** | | |
| Cultured cardiomyocytes | Tryptan blue exclusion and lactate/ LDH release | Hypoxia instead of ischaemia<br>Isolated cells, no infarct size |
| Isolated atrial trabeculae | Recovery of contractile function | Hypoxia instead of ischaemia<br>No direct measurement of cellular death<br>Endpoint determined by cell death and stunning |
| ***In vivo*** | | |
| Warm-up phenomenon | Exercise tolerance | Role of ischaemic preconditioning as mediator controversial |
| Preinfarction angina | Clinical outcome after myocardial infarction | Confounded by more rapid thrombolysis |
| Repeated PTCA | ST-segment elevation, anginal pain, myocardial lactate extraction | ST-segment change determined by sarc$K_{ATP}$ channels<br>No direct measurement of cellular death<br>Possible collateral recruitment |
| Aortic clamping before CABG | Postoperative troponin/CK-MB release, postoperative recovery | Confounded by perioperative drugs, which affect preconditioning<br>No direct measure of cellular death |
| $^{99m}$Tc-Annexin A5 scintigraphy | Targeting of annexin A5 | Skeletal muscle instead of myocardium |

be involved in warm-up and because involvement of $K_{ATP}$ channels is uncertain, a role for ischaemic preconditioning in warm-up remains controversial.[10,11] Another naturally occurring phenomenon that could be explained by ischaemic preconditioning is the possible infarct size sparing effect of preinfarction angina. Many patients with acute myocardial infarction have experienced angina in the hours or days preceding the infarction. Several studies have shown that indeed the presence of preinfarction angina, especially within 24 hours before infarction, is associated with improved clinical outcome after acute myocardial infarction, including death and the incidence of heart failure,[12,13] with reduced CK release[12,14] and with a smaller area of necrosis as assessed by nuclear imaging.[15] Also, Solomon *et al.* recently suggested that angina reported during the three months preceding myocardial infarction protects against left ventricular remodelling.[16] However, not all studies showed this association.[17] Moreover, Andreotti *et al.* showed that preinfarction angina is associated with a more rapid reperfusion of the infarct-related artery following thrombolysis, which is an attractive alternative explanation for the beneficial effect of angina.[18] This finding is in accordance with the observations that preinfarction angina only protects in patients treated with thrombolysis and not those treated with coronary angioplasty.[19] In conclusion, although there is strong evidence that preinfarction angina renders the myocardium more resistant to a subsequent myocardial infarction, the role of ischaemic preconditioning in this association remains controversial.

In addition to the above-mentioned naturally occurring forms of preconditioning, there are also two models in which active interventions are able to trigger preconditioning and which are therefore better suited to effectively study the modulation of this protection by external factors such as drugs. Firstly, in clinical practice, percutaneous transluminal coronary angioplasty (PTCA) offers the opportunity to electively and selectively apply ischaemia to a well-defined myocardial region. In theory, the first coronary occlusion in a series of occlusions could offer increased resistance to subsequent occlusions. Using this model, several studies showed that ST-segment shift on electrocardiography and subjective anginal pain are decreased during the second coronary occlusion, as well as wall motion abnormalities and lactate production,[20-22] although some studies showed no protection.[23,24] Subsequently, the finding that the nonselective adenosine receptor antagonist aminophylline could block this protection[25] and that intracoronary infusion of adenosine[26] as well as bradykinin[27] followed by a short period of wash-out before the first inflation could mimic preconditioning further strengthened the probability that indeed ischaemic preconditioning was responsible for the increased resistance to the second period of ischaemia. However, these results have to be interpreted with caution for two reasons. First, acute recruitment of collateral vessels is a major possible confounding factor.[28,29] Secondly, the most important surrogate endpoint used in this model of preconditioning is ST-segment elevation on electrocardiography. However, ST-segment elevation is determined by opening of sarcolemmal $K_{ATP}$ channels,[30] which, as outlined in the first part of this review, are probably not necessary for ischaemic preconditioning to occur. Recently, it was clearly demonstrated that this parameter is not a good endpoint for preconditioning by showing a dissociation between this parameter and infarct size limitation.[31]

A second of the very few clinical scenarios in which cardiac ischaemia is planned is coronary artery bypass grafting (CABG). In this situation, ischaemic preconditioning can by studied while avoiding the possible confounding of recruitment of collateral vessels by applying global cardiac ischaemia instead of local ischaemia. The evidence that ischaemic preconditioning confers additional protection in CABG and the possible use of preconditioning in clinical practice has recently been comprehensively discussed.[32,33] Yellon's group were the first to show that pretreatment with two three-minute periods of cross-clamping and reperfusion before a ten-minute period of ischaemia and ventricular fibrillation induces better preservation of left ventricular ATP content and reduces postoperative troponin I release.[34-36] Whether ischaemic preconditioning is also able to confer additional protection to ischaemia when other techniques than intermittent cross-clamp fibrillation are used is more controversial. Illes *et al.* found improvement in postoperative cardiac index and reduced requirement for inotropics with one-minute aortic cross-clamping before cold blood cardioplegic arrest.[37] Moreover, Lu *et al.* found a reduction in postoperative CK-MB release and improved recovery of myocardial contractility in patients undergoing valve replacement with the use of cardioplegia.[38] However, other groups were not able to demonstrate beneficial effects of ischaemic preconditioning in the setting of cardioplegic arrest.[39,40] Considering pharmacological preconditioning, some studies have shown that pretreatment with adenosine instead of short periods of ischaemia and reperfusion before CABG is associated with better postoperative ventricular performance[41] and less CK-MB release,[42] whereas others did not show a benefit from pretreatment with a specific A1 receptor agonist[36] or adenosine.[43] The discordant results obtained with ischaemic and pharmacological preconditioning in the setting of open-heart surgery could well be caused by two important possible confounders. First, in this setting anaesthetics are always used concomitantly and, as discussed in a later section, it is known that most anaesthetics influence preconditioning in a positive or negative way. Moreover, there are indications that cardiopulmonary bypass itself is able to precondition the myocardium, leaving little room for additional protection.[44,45]

Although the beneficial effect of ischaemic preconditioning

on the incidence of ischaemia/reperfusion-induced arrhythmias remains controversial in animal models,[46,47] recent studies in man suggest clinical benefit. The incidence of ventricular tachyarrhythmias after declamping in CABG patients was shown to be significantly reduced by preconditioning with two two-minute periods of ischaemia and reperfusion.[48]

In conclusion, there is a wealth of evidence that ischaemic preconditioning also occurs in humans, but conclusive evidence and large-scale testing of the ability of drugs to mimic or inhibit preconditioning is still hampered by the lack of an optimal and easy-to-use human model. Ischaemic preconditioning is not confined to cardiac tissue, but has also been described for liver, brain and skeletal muscle.[49-51] Also, the mechanisms of ischaemic preconditioning in heart and skeletal muscle show many similarities.[52,53] Recently, our group developed and validated a new model of ischaemic preconditioning in forearm skeletal muscle. Fundamental to this model is that ischaemic exercise (isometric contraction of the finger flexors while the circulation is occluded with an upper-arm cuff) induces translocation of phosphatidylserines from the inside to the outside of cellular membranes of affected cells, which is considered an early marker of apoptosis. This process can be visualised by scintigraphic imaging of the arm and hand after injection of radiolabelled annexin A5, which selectively binds to these phosphatidylserine residues. With this model, we have shown that ten minutes of forearm ischaemia protects against increases in annexin A5 binding induced by a subsequent ten minutes of ischaemic exercise, that infusion of adenosine into the brachial artery of the experimental arm mimics protection (Rongen *et al.*, Circulation, in press), and that protection is inhibited by pretreatment with the adenosine receptor antagonist caffeine (Riksen, *et al.*, submitted). By infusing target drugs into the brachial artery, it is easy to test their influence on ischaemic preconditioning or ischaemia/reperfusion injury *per se*. Apart from a research tool, this model may eventually be used in a clinical setting to individualise pharmacological strategies that are aimed to improve tolerance against ischaemia and reperfusion.

## PHARMACOLOGICAL PRECONDITIONING AND MODULATION OF ISCHAEMIC PRECONDITIONING

The elucidation, mostly from animal experiments, of great parts of the molecular machinery that is responsible for protection by ischaemic preconditioning, has provided us with several rational targets for pharmacological intervention. Various drugs have been shown to be able to mimic ischaemic preconditioning when applied instead of the preconditioning period of ischaemia. On the contrary, several drugs also interfere with ischaemic preconditioning and actually inhibit or reduce protection from ischaemic preconditioning. An overview of drug classes that are able to influence preconditioning is provided in *table 2*. In this section, we discuss human studies when possible. However, if these studies are unavailable, animal studies are used. It is important to realise that large interspecies differences exist with regard to preconditioning and the mechanism of preconditioning and, therefore, data derived from animal studies need to be interpreted with caution. In this section we will highlight several drugs that are already used in daily clinical practice and which have the potential of mimicking or modulating preconditioning. Consecutively, nucleoside uptake inhibitors, ACE inhibitors and AT1 receptor antagonists, HMG-CoA-reductase inhibitors, sulphonylureas, $K_{ATP}$ channel openers, anaesthetics, and alcohol will be evaluated for their potential to modulate ischaemic preconditioning. Additionally, we will be discuss whether known positive or negative effects of these drugs on cardiovascular function or mortality could be explained by their preconditioning modulating effect.

Both animal and human studies have identified adenosine as one of the most important triggers of ischaemic preconditioning. However, because of its very short elimination time, adenosine itself is not suited for administration to serve this goal. Moreover, more stable specific adenosine receptor agonists are not yet available for human use in clinical practice. However, by inhibiting the cellular uptake of endogenous adenosine, dipyridamole is able to increase the extracellular concentration of endogenous adenosine. Indeed, intravenous pretreatment with dipyridamole significantly potentiated the infarct size limiting effect of ischaemic preconditioning in rabbit heart.[54] In humans, intracoronary administration of dipyridamole before balloon inflation during PTCA also reduced anginal pain and ST-segment shift[55] and prevented deterioration of ventricular function during balloon occlusion.[56] In clinical practice, efficacy of dipyridamole, given especially because of its presumed effect on platelet aggregation, has long been the subject of controversy. A recent meta-analysis concluded that in patients with vascular disease, there is no evidence that dipyridamole reduces the risk of vascular death, although in one study in patients after cerebral ischaemia, dipyridamole reduced the risk of further vascular events.[57] This lack of clinical benefit might be due to the fact that dipyridamole is not dosed high enough to adequately increase the endogenous adenosine concentration or because dipyridamole is often coadministered with acetylsalicylic acid, which might itself inhibit delayed ischaemic preconditioning,[58] offsetting the possible beneficial effects of dipyridamole.

Several studies have shown that bradykinin is also involved as a trigger in ischaemic preconditioning. In humans,

**Table 2**
*Drugs with the ability to mimic or inhibit preconditioning*

| MIMICKING PRECONDITIONING | INHIBITION OF PRECONDITIONING |
|---|---|
| **Adenosine receptor agonists**<br>Adenosine[9,26] | **Adenosine receptor antagonists**<br>Theophylline, aminophylline, bamiphylline[7,25,157] |
| **Nucleoside transport inhibitors**<br>*By increasing endogenous adenosine*<br>Dipyridamole[56] | |
| **$K_{ATP}$ channel openers**<br>Nicorandil[158]<br>Diazoxide[91] | **$K_{ATP}$ channel blockers**<br>Glibenclamide[79,91] |
| **Opioid agonists[108]**<br>Morphine[107] | **Opioid receptor antagonists**<br>Naloxone[109] |
| **$\alpha_1$-adrenergic receptor agonists**<br>Phenylephrine, norepinephrine[159,160] | **$\alpha_1$-adrenergic receptor antagonists**<br>Phentolamine[161] |
| **$\beta_1$-adrenergic receptor agonists**<br>Isoproterenol[162] | **$\beta_1$-adrenergic receptor antagonists[163]** |
| **$B_2$-bradykinin receptor agonists[27]** | |
| **ACE inhibitors**<br>*By increasing bradykinin concentration*<br>Captopril, lisinopril[64] | |
| **Angiotensin II receptor antagonists**<br>Losartan[66] | |
| **Volatile anaesthetics**<br>Isoflurane, halothane, sevoflurane, enflurane, Desflurane[102] | **Intravenous anaesthetics**<br>R-ketamine, thiopental and pentobarbital[105] |
| **Nitric oxide donors**<br>Nitroglycerin[77] | |
| **Statins**<br>Pravastatin[71] | |
| **Ethanol[116]** | |
| **Corticosteroids[164]** | **COX-2 inhibitors**<br>*Inhibit only delayed preconditioning*<br>High-dose ASA,[58] celecoxib[165] |

*References preferentially indicate human studies; if not available, animal studies are referred to.*

bradykinin is able to mimic ischaemic preconditioning in the model of repeated PTCA.[27] Analogues to adenosine, direct bradykinin receptor agonists are not yet available for clinical human use. However, angiotensin-converting enzyme (ACE) inhibitors are known to inhibit the breakdown of bradykinin, thus increasing the concentration of endogenous bradykinin (*figure 1*).[59] Considering preconditioning of the myocardium, animal studies have demonstrated that pretreatment with ACE inhibitors reduces infarct size,[60] potentiates the acute[61] as well as delayed[62] infarct size limiting effect of subthreshhold ischaemic stimuli and attenuates myocardial stunning.[63] Moreover, selective bradykinin B2 receptor antagonists could inhibit these beneficial effects of ACE inhibitors.[61,63] Similar results were obtained in human atrial trabeculae, obtained during CABG, in which postischaemic recovery of contractile function was significantly increased by pretreatment with captopril and lisinopril in combination with a subthreshold ischaemic preconditioning stimulus. These beneficial effects were again completely prevented by a specific bradykinin B2 receptor antagonist.[64] These potentiating effects of ACE inhibitors on ischaemic preconditioning could be one of the mechanisms responsible for the favourable effects of these drugs on cardiovascular death and the incidence of heart failure in several clinical trials, such as the HOPE trial.[65] Surprisingly, AT1 receptor antagonists, initially presumed not to influence the kallikrein-kinin system, could also limit infarct size in rat[66] and pig[60] hearts and intriguingly, this effect could also be blocked by bradykinin antagonists.[66] This observation is in contradiction with earlier studies, showing inhibitory effects of AT1 receptor antagonism on the effect of ischaemic preconditioning.[67] One explanation for this beneficial effect of AT1 receptor antagonists could be that during blockade of the AT1 receptor, AT2 receptor stimulation by angiotensin II is enhanced (*figure 1*). AT2 receptor stimulation has recently been shown to activate the kallikrein-kinin system and thereby stimulate bradykinin release.[68] Indeed, it was subsequently shown that the

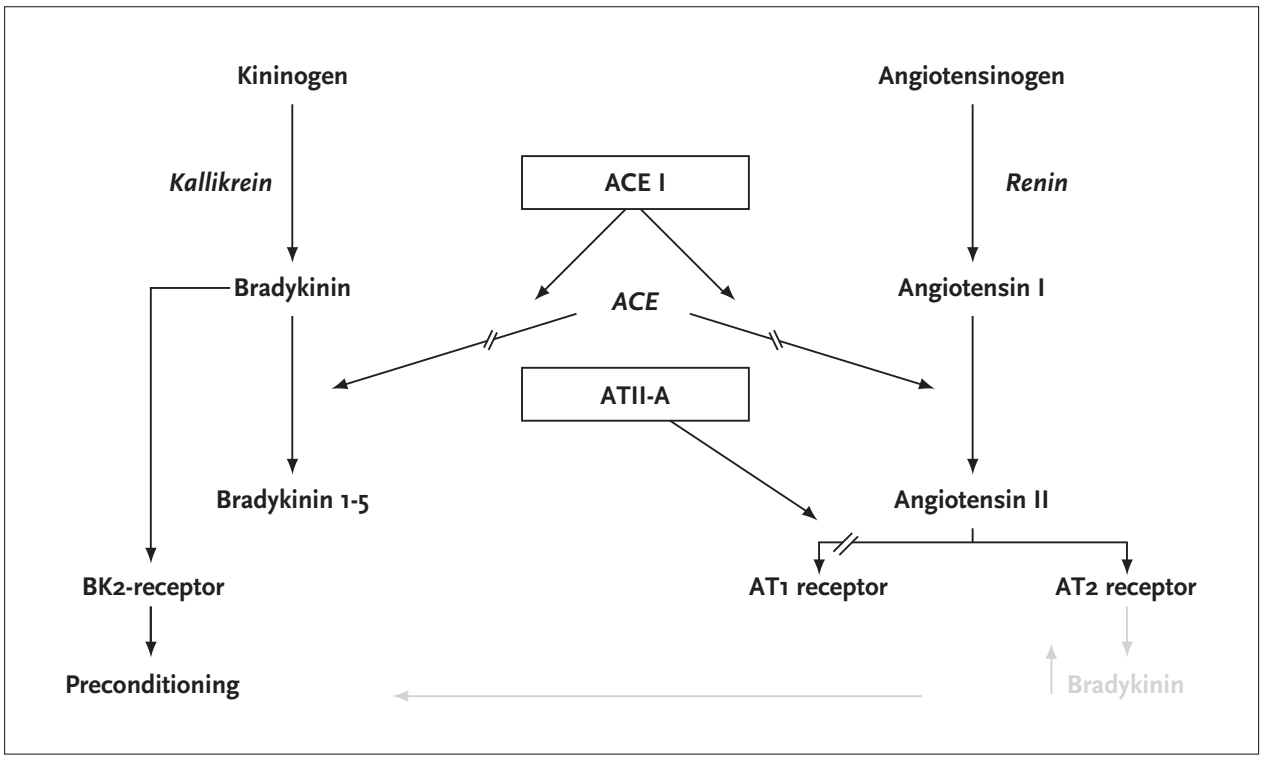


**Figure 1**

*Schematic illustration of the interaction between the renin-angiotensin and the kallikrein-kinin system and the effects of angiotensin-converting enzyme inhibitors (ACE-I) and angiotensin II antagonists (ATII-A)*

**This illustration shows how these drugs mimic ischaemic preconditioning. BK = bradykinin. ACE-I inhibit breakdown of bradykinin, which stimulates bradykinin BK2 receptors. ATII-A only block the AT1-subtype receptor. Subsequent increased stimulation of the AT2 receptor by endogenous angiotensin II could activate the kallikrein-kinin system, also leading to an increased release of bradykinin.**

vascular effects of candesartan are blocked by bradykinin antagonism.[69] This effect might explain the similar effects on mortality of ACE inhibitors and ATII antagonists in patients who are at high risk for cardiovascular events after acute myocardial infarction.[70] It needs to be emphasised, though, that it is very difficult to investigate the preconditioning-mimicking effect of drugs in large clinical trials, because preconditioning would not influence the incidence of cardiovascular events, but rather the outcome, once an event has occurred.

3-Hydroxy-3-methylglutaryl (HMG)-Co-enzyme A (CoA) reductase inhibitors form another class of drugs, widely prescribed in cardiovascular compromised patients, that have been suggested to protect from ischaemia/reperfusion injury. The beneficial effects of HMG-CoA-reductase inhibitors on cardiovascular morbidity and mortality in patients at risk for cardiovascular disease are widely appreciated. Beyond their ability to halt the process of atherosclerosis, mimicking of ischaemic preconditioning has also been suggested to contribute to these major beneficial effects. Ueda *et al.* showed that the infarct size limiting effect of ischaemic preconditioning is blunted in hypercholesterolaemic rabbits and that pravastatin, added to their diet, completely restores this without affecting plasma total cholesterol, HDL and triglycerides.[71] This was explained by the finding that pravastatin also restores the activation of the enzyme ecto-5'-nucleotidase during the preconditioning ischaemia, which is attenuated in the untreated hypercholesterolaemic rabbits. As ecto-5'-nucleotidase converts adenosine monophosphate into adenosine, this could well contribute to the observed effect. Later it was shown that lovastatin and simvastatin also enhance ecto-5'-nucleotidase activity *in vitro*.[72] In the recent study by Lee *et al.*, hyperlipidaemic patients with coronary artery disease were randomised to pravastatin or placebo for three months before PTCA. Patients on pravastatin had less ST-segment shift, anginal pain and myocardial lactate production during the first balloon occlusion than the control group and this protection was abolished by pretreatment with the adenosine receptor antagonist aminophylline, suggesting that the cardioprotection offered by pravastatin was mediated by adenosine.[73] However, the treated patient group also had significantly lower plasma cholesterol levels. Because of these results, it is attractive to speculate that stimulation of ecto-5'-nucleotidase could be one of the

mechanisms that mediate the well-known protection of statins on the cardiovascular system. However, it needs to be stressed that other mechanisms of protection by this class of drugs might be present. Bell *et al.* recently showed very elegantly in mice hearts that administration of atorvastatin during reperfusion after a period of ischaemia significantly reduces infarct size independent of lipid lowering.[74] This protection was achieved by activation of a signalling cascade involving phosphatidylinositol 3-kinase (PI3K), the protein kinase Akt and eNOS. Alternative mechanisms of cardioprotection by statins include inhibition of neutrophil activation and preservation of NO-synthase activity after ischaemia and reperfusion,[75] which could result from inhibition of the mevalonate pathway and subsequent inhibition of the Rho/Rho kinase pathway.[76]

The last drug which has been shown to mimic preconditioning in humans *in vivo* and which acts on the level of the triggers of ischaemic preconditioning is the NO donor nitroglycerin. NO has been implicated especially in delayed preconditioning and this has been tested recently in the model of repeated PTCA.[77] Patients admitted for stable or unstable angina were randomised to receive a four-hour intravenous infusion of nitroglycerin or placebo 24 hours before PTCA. It appeared that nitroglycerin pretreatment, independent of collateral recruitment, rendered the heart resistant against ischaemia, as assessed by ST-segment shift, wall motion and subjective pain.

More distal to the trigger phase of ischaemic preconditioning, opening of mitochondrial $K_{ATP}$ channels is essential for the occurrence of protection by ischaemic preconditioning. Drugs that interfere with $K_{ATP}$ channel opening could therefore theoretically inhibit this protection. Indeed, using recovery of contractile function of human atrial trabeculae as endpoint of ischaemic injury, Cleveland *et al.* showed in an observational study that preconditioning is abolished in patients with type 2 diabetes using glibenclamide or glipizide compared with type 2 diabetics on insulin.[78] Moreover, it was shown that pretreatment with 10 mg of glibenclamide orally before PTCA abolishes ischaemic preconditioning as assessed by anginal pain and ST-segment shift in nondiabetics[79] and in the same model, ischaemic preconditioning was inhibited in type 2 diabetics who were chronically on glibenclamide.[80] Interestingly, in the same model, the newer sulphonylurea glimepiride did not abolish protection by ischaemic preconditioning,[80,81] possibly because it blocks extrapancreatic $K_{ATP}$ channels to a lesser extent than glibenclamide. Finally, Scognamiglio *et al.* showed that type 2 diabetics randomised to the use of insulin have less myocardial dysfunction during dipyridamole stress echocardiography than patients on glibenclamide.[82] However, this model is not well suited for this purpose, because dipyridamole itself is able to provide cardioprotection, as mentioned earlier. Despite the limitations inherent to the human models used, glibenclamide does seem to inhibit ischaemic preconditioning. Does this mean that diabetics who take sulphonylureas are at increased risk for cardiovascular morbidity and mortality? This discussion was opened by the observation in the UGDP study that patients on tolbutamide have an increased cardiovascular mortality rate.[83] In the UKPDS, treatment with metformin decreased mortality, whereas treatment with glibenclamide did not reduce mortality.[84,85] Additionally, various smaller trials have provided conflicting results on the effect of sulphonylureas on cardiovascular morbidity and mortality.[86] In conclusion, there is no convincing evidence that use of sulphonylureas is associated with worse cardiovascular outcome in general clinical practice. Interestingly, in special situations of profound cardiac ischaemia, sulphonylureas may have detrimental effects: diabetics on sulphonylureas did have a higher in-hospital mortality after PTCA for acute myocardial infarction compared with diabetics not on sulphonylureas.[87] However, because most of these latter patients were on insulin, this could also point to a beneficial effect of insulin. At this point, it needs to be realised that insulin, independent of glucose lowering, can reduce myocardial infarction, when administrated early in reperfusion, acting via the Akt prosurvival pathway.[88,89]

In contrast to $K_{ATP}$ channel blocking, pharmacological opening of these channels provides beneficial effects on myocardial ischaemia/reperfusion injury. Indeed, many animal studies have shown that pretreatment with the $K_{ATP}$ channel opener diazoxide mimics the infarct size limiting effect of ischaemic preconditioning.[90] Similarly, ischaemic preconditioning mimicking effects of diazoxide have been shown in the human atrial trabeculae model.[91,92] Very recently, Wang *et al.* demonstrated that patients randomised to pretreatment with an intravenous bolus of diazoxide five minutes before commencing cardiopulmonary bypass for CABG have significantly better improvement of cardiac index after surgery.[93] More clinically oriented research has been done with nicorandil, a hybrid of a nitrate and a $K_{ATP}$ channel opener, registered for use in patients with stable angina. This drug has been shown to reduce infarct size in several animal models via its opening of $K_{ATP}$ channels, both acutely and after 24 hours.[94,95] In humans, pretreatment with an intravenous bolus of nicorandil before PTCA in patients with stable angina appeared to limit ST-segment shift independent of myocardial blood flow.[96,97] However, it needs to be emphasised that ST-segment shift is determined by sarcolemmal $K_{ATP}$ channels, which are influenced by nicorandil but are probably less important in preconditioning, making this parameter highly unreliable for this goal. That these beneficial effects of nicorandil in the experimental setting could indeed also be applied to the clinical

setting is demonstrated by Patel *et al.*[98] They showed that patients with unstable angina who were randomised to nicorandil 20 mg orally twice daily added to an aggressive antianginal treatment with acetylsalicylic acid, β-blockers and diltiazem, suffer less myocardial ischaemia and ventricular arrhythmias in the first 48 hours after admission than the control group. The preconditioning mimicking effect of nicorandil could also have contributed to the results of the Impact Of Nicorandil in Angina (IONA) study, which showed a significant reduction in major coronary events in patients with stable angina and additional cardiovascular risk factors added to conventional antianginal therapy.[99] The role of preconditioning in this study, however, remains speculative.

Another class of drugs that are known for their potential to influence $K_{ATP}$ channels are volatile anaesthetics. Because of the inherent timing before the start of operations and because of the relative ease of administration, this group of drugs would be especially suited to use for early cardioprotection. Indeed, in 1997 it was first described that isoflurane mimics the protective effect of ischaemic preconditioning in rabbits and dogs.[100,101] Today, it is known that most anaesthetics are able to mimic, enhance or inhibit ischaemic preconditioning, which was recently reviewed by Zaugg *et al.*[102,103] and Riess *et al.*[104] In animal studies, protective effects have been demonstrated for isoflurane, enflurane, halothane, sevoflurane and desflurane. Adenosine A1 receptor stimulation, PKC activation and opening of $K_{ATP}$ channels have all been implicated in the mechanism of this protection.[103] On the contrary, various intravenous anaesthetics have been shown to inhibit opening of mito$K_{ATP}$ channels *in vitro* and it was demonstrated that R-ketamine, thiopental and pentobarbital inhibit diazoxide-induced protection in isolated rat ventricular myocytes.[102,105] Recently, a few small trials have investigated the effects of isoflurane, enflurane and sevoflurane preconditioning in patients undergoing CABG. These data provide evidence, although not always significant, that these anaesthetics are able to provide some protection as assessed by postoperative CK-MB and troponin I release and postoperative myocardial function.[102]

A recent randomised study even concluded that sevoflurane preconditioning in CABG patients preserves myocardial as well as renal function as assessed by postoperative plasma levels of N-terminal pro-brain natriuretic peptide and cystatin C, respectively. However, more traditional markers (CK-MB, troponin T and creatinine) were not improved by preconditioning.[106] Finally, considering anaesthesia, it has to be mentioned that opioid receptor agonists, which are frequently used in the perioperative timeframe, are also able to provide cardioprotection by preconditioning in animal models[107] and in isolated human atrial trabeculae.[108] Using the model of repeated PTCA in humans *in vivo*, Tomai *et al.* showed that pretreatment with the opioid-receptor antagonist naloxone completely blocks the protective effect of ischaemic preconditioning.[109] Interestingly, it was recently shown that volatile anaesthetics and opioids may work in conjunction to confer protection against myocardial infarction through potentiation of cardiac $K_{ATP}$ channel opening.[110]

Besides pharmacological agents, compounds present in daily food and drink could also be able to provide protection against ischaemia/reperfusion injury. It is known that moderate alcohol consumption is associated with a decreased risk of cardiovascular disease.[111,112] Moreover, it was found that moderate drinking is associated with increased survival once acute myocardial infarction has occurred.[113] Besides beneficial alterations in lipid metabolism and platelet function, preconditioning of the myocardium by ethanol could contribute to this beneficial effect of alcohol consumption. Indeed, accumulating evidence from various animal models demonstrates that chronic as well as acute ethanol consumption reduces myocardial ischaemia/reperfusion damage by mimicking ischaemic preconditioning.[114-118] Hearts from guinea pigs drinking ethanol for 3 to 12 weeks showed improved functional recovery and reduced myocyte damage after ischaemia and reperfusion.[116] This preconditioning mimicking effect was completely abolished by adenosine A1 receptor blockade during the index ischaemia.[116] Indeed, it has already been shown that ethanol increases extracellular adenosine concentration by inhibiting cellular adenosine uptake,[119] and this mechanism could be involved in the previously described beneficial effect of ethanol. However, in rats, alcohol-induced cardioprotection was not blocked by adenosine receptor antagonists, whereas α-adrenergic antagonism did block this protection, suggesting species-specific signalling.[114] More recently, Miyamae *et al.* showed that chronic ethanol consumption induces a sustained translocation of PKC-ε from the cytosolic to the particulate fraction and that cardioprotection by ethanol is critically dependent on PKC activity during the index ischaemia.[115]

Acute ethanol ingestion shortly before the ischaemic insult, resulting in a concentration similar to that achieved after one to two alcoholic beverages, similarly provided protection by direct activation of PKC-ε.[118] Finally, the infarct size limiting effect of chronic ethanol ingestion in dogs was abolished by administration of glibenclamide during ischaemia, thus providing evidence that opening of $K_{ATP}$ channels is crucial for this protection to occur.[117] In conclusion, chronic as well as acute consumption of alcohol provides protection against ischaemic injury in several animal species via adenosine and α-adrenergic receptor stimulation, PKC-ε translocation and opening of $K_{ATP}$ channels.

## THERAPEUTIC EXPLOITATION

From the evidence outlined in the present paper, it appears that also in the human myocardium, ischaemic preconditioning can significantly increase tolerance to ischaemia and reperfusion. However, in clinical practice, the application of short periods of ischaemia to induce preconditioning is in most circumstances not desirable or feasible. However, several classes of drugs have been described with the potential to enhance, mimic or inhibit ischaemic preconditioning. The prudent use, or avoidance, of these agents may be a more benign approach to elicit cardioprotection in clinical practice.

Because of the relatively tight time boundaries of protection by ischaemic and pharmacological preconditioning, it is essential to apply the pharmacological intervention shortly before the prolonged ischaemic period. However, myocardial ischaemia is seldom planned and accurately predicted. However, two situations in which temporary myocardial ischaemia can readily be predicted are PTCA and CABG. Although routine PTCA carries a small risk for complications, this risk is increased in a high-risk situation, such as unstable angina. Especially in these situations, pretreatment with preconditioning-mimicking drugs could be beneficial. Conversely, the temporary withdrawal of drugs which are known to interfere with preconditioning, such as $K_{ATP}$ blockers or adenosine antagonists, could increase tolerance to ischaemia. Garratt *et al*. have shown in an observational study that diabetics taking sulphonylureas have increased in-hospital mortality after PTCA for acute myocardial infarction compared with diabetics who are not on sulphonylureas.[87] Again, it needs to be realised that this survival benefit could also be caused by the beneficial effect of insulin in the control group. Interestingly, in the setting of PTCA, preconditioning by repeated balloon inflations could also be used to stratify patients for their risk of adverse ischaemic events. Recently, Laskey *et al*. showed that 20% of patients undergoing PTCA fail to manifest ischaemic preconditioning, and that this is significantly associated with an increased risk of death or nonfatal myocardial infarction at one year of follow-up.[120]

A second situation in which cardiac ischaemia is planned and may consequently be preceded by a preconditioning stimulus is CABG, as described above. However, the protective effect of ischaemic or pharmacological preconditioning is still controversial, especially when other techniques than intermittent cross-clamp fibrillation are used.[32,33] It is argued that the protection afforded by cardioplegia and anaesthetics leaves little room for additional protection by preconditioning.[32,33] Moreover, it has been shown that cardiopulmonary bypass alone is able to provide cardioprotection comparable with classic ischaemic preconditioning in sheep hearts.[44] Similarly, in a recent study in humans, preconditioning with ischaemia only offered additional protection during CABG when no cardiopulmonary bypass was used.[45] Thus, preconditioning may only be indicated in settings in which conventional protection is anticipated against be suboptimal, for example in long duration or severe atherosclerosis.[33] Moreover, when considering protection against postoperative pump failure, it needs to be realised that stunning, more than discrete necrosis or apoptosis, might be responsible for this, and that early preconditioning probably does not protect against stunning. Perhaps a more successful, albeit less heroic, approach might be the elimination before surgery of factors with potential inhibiting effects on preconditioning, such as the use of sulphonylureas or caffeine.

Finally, considering anticipated periods of cardiac ischaemia, preliminary evidence exists that ischaemic preconditioning might be beneficial in transplantation. In sheep heart, recovery of systolic function was improved when a short period of ischaemia was applied before the explantation.[121]

Perhaps more benefit from pharmacological preconditioning could be expected when applied to patients at high risk for myocardial infarction, despite adequate conventional treatment. This would particularly concern patients with non-ST-segment elevation acute coronary syndromes, including unstable angina, who are at high risk of progression to complete coronary occlusion. More than 10% will die or suffer a myocardial infarction within six months, with half of these events occurring in the acute phase.[122] Pharmacological preconditioning during this phase could potentially reduce the amount of ischaemic damage. However, as the duration of protection afforded is limited, repeated dosing of the preconditioning drug is necessary to maintain the preconditioned state. Although a 72-hour continuous infusion of an adenosine A1 selective agonist in rabbits was not able to limit infarct size, suggesting receptor downregulation,[123] a more recent study in rabbits showed that repeated bolus injections of an adenosine A1 selective agonist at 48-hour intervals still provides strong limitation of infarct size at day 10.[124] Moreover, consumption of dipyridamole, added to the drinking water for two to six weeks, resulted in an attenuation of ischaemia/reperfusion injury in guinea pigs.[125]

Even more benefit from pharmacological modulation of preconditioning might be expected in large groups of patients with an increased baseline risk for cardiovascular disease, such as diabetics. Sulphonylureas are associated with an unexpected and unexplained small increase in cardiovascular mortality in several trials, as described previously. Reducing the use of sulphonylureas could potentially confer benefit to this patient group, with regards to cardiovascular morbidity and mortality.

Very recently, several studies have shown that pharmacological interventions during early reperfusion are also able

to limit infarct size.[126] This approach circumvents the problem that the ischaemic insult is mostly unpredictable, because these drugs can be given at reperfusion rather than before the event and are therefore more clinically applicable, but outside the scope of this review on preconditioning. Briefly, in the AMISTAD trial, it was shown that adenosine as an adjunct to thrombolysis results in a significant reduction of infarct size.[127] Also, infarct size limitation has been shown for insulin,[88,89] atorvastatin,[74] 5'-(N-ethylcarboxamido) adenosine and bradykinin,[128] all via activation of the PI3K/Akt pathway during reperfusion. Also, cyclosporine limits infarct size when administered during reperfusion by inhibiting opening of the mitochondrial permeability transition pore (MPTP).[129] Additional studies have to be performed to show whether this approach could offer clinical benefits.

A final word of caution regarding the potential therapeutic benefits of preconditioning concerns the reported effects of ageing and disease on ischaemic preconditioning.
In the literature, it is repeatedly mentioned that the protective effect of preconditioning may be lost in aged myocardium, in which cardioprotection is undoubtedly more relevant, although there is still no consensus on this subject. Studies on isolated hearts show that the effect of preconditioning is decreased in aged rats,[130-133] but not in aged rabbits[134,135] In humans, a similar controversy exists in the various models of ischaemic preconditioning.[136-139] Decrease in norepinephrine release during the preconditioning episode,[130] attenuated activation of $K_{ATP}$ channels[137] and failure of adequate translocation of PKC isoforms[132] have all been implicated in this reduced protective effect of preconditioning in the aged heart.
A similar controversy exists as to whether protection by preconditioning is still present in the diseased heart, especially concerning diabetes and hypercholesterolaemia, the very conditions in which cardioprotection is particularly important. Although some studies indeed show protection by ischaemic preconditioning in diabetic rats,[140] most studies in rabbits and dogs demonstrated that diabetes abolishes protection by ischaemic preconditioning.[141-143] In dogs, it appeared that both streptozotocin-induced diabetes and hyperglycaemia by dextrose infusion inhibit the infarct-sparing effect of preconditioning, probably due to impaired activation of mito$K_{ATP}$ channels.[142,144,145] Similarly, in an observational study both preconditioning by ischaemia and by pretreatment with diazoxide was abolished in atrial tissue taken from patients with type 1 diabetes using insulin and from patients with type 2 diabetes on sulphonylureas but it was not abolished in patients with diet-controlled diabetes.[146] Finally, it is reported in the literature that the protective effect of preinfarction angina is diminished in patients with diabetes.[16,147] The lack of protection afforded by ischaemic preconditioning in patients with diabetes could well contribute to the consistently shown worse outcome after myocardial infarction in these patients compared with patients without diabetes.[148,149]
Considering hypercholesterolaemia, there is less evidence from the literature. There are studies that show preserved protective effects of ischaemic preconditioning[150,151] as well as studies that show reduced protection by ischaemic preconditioning in hypercholesterolaemic rabbits.[71]
Considering evidence in humans, it was recently shown that in patients with high plasma cholesterol, the preconditioning by repeated PTCA is reduced as compared with patients with normal cholesterol levels.[152] Considering other risk factors for atherosclerosis, little is known about the influence of smoking, hypertension and hyperhomocysteinaemia on the effect of ischaemic preconditioning. Regarding hypertension, it has been shown that protection is still present in spontaneously hypertensive rats and in hypertrophied myocardium from saline loaded rats.[153,154] When interpreting these data on aged and diseased hearts, one has to bear in mind that the exact signalling mechanism involved in preconditioning is dependent on the nature of the preconditioning stimulus.[155] Because the effects of ageing or disease might be limited to specific triggers, such as adenosine,[156] it is conceivable that failure to precondition these hearts is influenced by the choice of the preconditioning stimulus.

In summary, there is a wealth of both *in vitro* and *in vivo* evidence that ischaemic preconditioning also occurs in humans. Since the description of this phenomenon, several classes of drugs have been described which are able to mimic, enhance or inhibit ischaemic preconditioning. The use or avoidance of these drugs before procedures known to induce myocardial ischaemia or in patients at risk for myocardial infarction in general could theoretically reduce ischaemia and reperfusion injury and improve outcome. We recently developed a minimally invasive technique to monitor ischaemic tolerance in humans *in vivo*. Future clinical trials with this technique are needed to address the question whether this method can be used to individualise pharmacotherapy in order to optimise resistance to ischaemia-reperfusion and outcome in patients who are particularly vulnerable to ischaemic cell death: patients at risk for arterial thrombosis and patients with heart failure.

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REVIEW

# Antiretroviral therapy in previously untreated adults infected with the human immunodeficiency virus type 1: established and potential determinants of virological outcome

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## ABSTRACT

**The aim of highly active antiretroviral therapy (HAART) for patients chronically infected with the human immunodeficiency virus type 1 is to achieve maximal and durable viral suppression. Maintaining the blood plasma HIV-1-RNA concentration (pVL) <50 copies/ml is currently considered appropriate for this goal. With the current treatment options, the percentage of previously untreated patients who achieve a pVL <50 copies/ml after one year of initial HAART is about 70%. Characteristics of the host, virus, drugs and the treatment team have been associated with the virological response to initial HAART. Adjusting the initial HAART regimen and patient management to a risk profile based on these factors is possibly helpful in improving the virological response to HAART. Adherence to a potent and well-tolerated HAART regimen is likely to be the most relevant factor for virological success. The additive value of the other factors needs to be clarified.**

## INTRODUCTION

The standard antiretroviral therapy (ART) first given to individuals infected with the human immunodeficiency virus type 1 (HIV-1) is highly active antiretroviral therapy (HAART). HAART is not well defined and is considered to consist of 'a combination of at least three selected antiretroviral drugs with good tolerability and efficacy'. The primary aim of initial ART is to achieve a maximal and durable viral suppression. Maintaining the blood plasma HIV-1-RNA concentration (plasma viral load, pVL) below a detection limit of 50 copies/ml is currently recommended to achieve this goal,[1-5] because it is associated with less virological failure than a level above 50 copies/ml and it may possibly prevent the emergence of drug resistance despite ongoing low-level (residual) viral replication.[6-12] If virological failure to the initial HAART regimen occurs, subsequent therapy is usually less effective due to accumulation of drug-resistance-associated mutations and cross-resistance amongst antiretroviral agents within the same class and generally the subsequent therapy is also more of a burden for the patient.[1,3,13-21] A good initial HAART regimen is therefore of great importance.

The aim of this review is to stress the importance of maximal and durable viral suppression, give an overview and update of the virological response to initial HAART and give insight into which factors have been associated with the virological response to initial HAART. Considering these factors could be of help in identifying patients with high and low risk for virological failure, and choosing an initial HAART regimen and the most appropriate moment to start therapy. To provide a better insight for physicians who are not familiar with antiretroviral therapy, a brief

historical overview and global perspective of ART is given. For this review PubMed and the Cochrane Library were searched. The search terms were "antiretroviral therapy", "highly active antiretroviral therapy", "HIV", "subtype", "clade", "HIV type 2", "pharmacogenetics" and "review". A criterion for selection was those articles which involved initial HAART. Also the reference list of articles selected in this way was screened and specific articles were added by the authors if they were not identified by the search strategy.

## ANTIRETROVIRAL THERAPY IN HISTORICAL AND GLOBAL PERSPECTIVE

In June 1981, the world first became aware of the acquired immunodeficiency syndrome (AIDS).[22-24] Major scientific breakthroughs achieved during subsequent years are summarised in *table 1* and in *table 2* an overview is given of the antiretroviral drugs and their date of licensing by the USA Food and Drug Administration (FDA).[23-25] Now, in the year 2004, HIV can still not be cured with the available HAART regimens and there is still no preventive vaccine available.[26]

In countries where HAART could be widely applied, the HIV-1-related morbidity and mortality has decreased tremendously since its introduction in 1996 and the treatment has appeared to be cost-effective[15,27-33] Basis for clinical improvement is a lasting adequate virological suppression which leads to immunological recovery.[34-36] However, HAART is available to only a minority of the HIV-infected population and knowledge of its efficacy is based mainly on HIV-1 subtype M (major) subtype B. According to estimates of December 2003 about 40 million adults and children throughout the world are infected with HIV, of whom less than 2 million (<5%; those living in high-income countries) have access to HAART.[37] In the year 2000 it was estimated that HIV-1 subtype B accounted for only 12% of new HIV-1 infections.[38]

Thus, in contrast to current advances one must be aware that HAART is scarcely available in countries with limited resources, and that in these countries other HIV-1 subtypes are more prevalent. This may have therapeutic consequences, such as susceptibility to antiretroviral drugs, response to HAART, and rate and pattern of the emergence of drug resistance.[38-43]

## WHAT IS THE VIROLOGICAL RESPONSE TO CURRENT HAART?

A HAART regimen is currently considered adequate when after three to six months a pVL of <50 copies/ml is achieved and this level of viral suppression is maintained.[1-3] In case of a baseline pVL >1,000,000 copies/ml it may take longer than six months to reach the concentration of <50 copies/ml.[44]
In *table 3* an overview is given of the virological response rates reported in published prospective studies in chronic

**Table 1**
*Historical overview of antiretroviral therapy*

| | |
|---|---|
| 1981 | Recognition of first AIDS cases[22,188-192] |
| 1983 | Identification of HIV-1 as the cause of AIDS;[193-196] description of first AIDS cases in the Netherlands[197-201] |
| 1985 | FDA approval of first commercial blood screening test[202] |
| 1986 | Identification of HIV-2[203] |
| 1987 | Introduction of antiretroviral therapy: zidovudine[204] |
| 1994 | Reduction of HIV-1 transmission from mother to child[205-207] |
| 1995 | Availability of standardised (commercial) HIV-1-RNA assay,[208] which gave better understanding of HIV-1 viral dynamics[86,92,209-216] |
| 1996 | Release of first protease inhibitors and introduction of HAART[25,217-219] |
| 1997 | Ritonavir-induced pharmacokinetic enhancement of other PIs, which made twice-daily dosing of many PIs possible;[220-223] Recognition of a long-lived HIV cellular reservoir[224-233] |
| 1998 | After induction therapy maintenance with two NRTIs or one or two PIs or one NRTI and one PI is insufficient;[8,234,235] The virological response is more sustained when a blood plasma viral load nadir of 50 copies/ml is achieved;[6-12] First descriptions of the lipodystrophy syndrome;[236-242] Recognition of strong improvement of survival in HAART era[15,27-31] |
| 1999 | A high degree of drug adherence is needed to achieve a proper viral suppression;[151-156] Demonstration of residual replication during HAART, which made clear that treatment with the current kind of HAART would be for life[229-231] |
| 2000 | HAART can be relatively safely deferred until the CD4 count is nearing 200 cell/mm$^3$[4,31,50,60,61,243-246] |
| 2001 | Current guidelines to initiate HAART in HIV-1 infected adolescents and adults[1-3,62-64] |

*AIDS = acquired immunodeficiency syndrome, FDA = USA Food and Drug Administration, HAART = highly active antiretroviral therapy, HIV = human immunodeficiency virus, NRTI = nucleoside analogue reverse transcriptase inhibitor, PI = protease inhibitor.*

**Table 2**
*Licensed antiretroviral drugs and their date of approval by the Food and Drug Administration (FDA) of the USA*[23-25]

| CLASS AND GENERIC NAME | TRADE NAME | DATE OF FDA APPROVAL |
|---|---|---|
| **Nucleoside-analogue reverse transcriptase inhibitor** | | |
| Zidovudine | Retrovir | 19 March 1987 |
| Didanosine | Videx | 9 October 1991 |
| Zalcitabine | Hivid | 19 June 1992 |
| Stavudine | Zerit | 24 June 1994 |
| Lamivudine | Epivir | 17 November 1995 |
| Zidovudine + lamivudine | Combivir | 26 September 1997 |
| Abacavir | Ziagen | 17 December 1998 |
| Didanosine (enteric coated) | Videx EC | 31 October 2000 |
| Zidovudine + lamivudine + abacavir | Trizivir | 14 November 2000 |
| Emtricitibine | Emtriva | 2 July 2003 |
| **Nucleotide-analogue reverse transcriptase inhibitor** | | |
| Tenofovir | Viread | 26 October 2001 |
| **Nonnucleoside reverse transcriptase inhibitor** | | |
| Nevirapine | Viramune | 21 June 1996 |
| Delavirdine | Rescriptor | 4 April 1997 |
| Efavirenz | Stocrin, Sustiva | 17 September 1998 |
| **Protease inhibitor** | | |
| Saquinavir (hard gel capsule) | Invirase | 6 December 1995 |
| Ritonavir | Norvir | 1 March 1996 |
| Indinavir | Crixivan | 13 March 1996 |
| Nelfinavir | Viracept | 14 March 1997 |
| Saquinavir (soft gel capsule) | Fortovase | 7 November 1997 |
| Amprenavir | Agenerase | 15 April 1999 |
| Lopinavir + ritonavir | Kaletra | 15 September 2000 |
| Atazanavir | Reyataz | 20 June 2003 |
| Fosamprenavir | Lexiva | 20 October 2003 |
| **Fusion inhibitor** | | |
| Enfuvirtide | Fuzeon | 13 March 2003 |

HIV-1-infected, previously untreated adults and adolescents. The HAART regimens mostly used and studied are those of two nucleoside-analogue reverse transcriptase inhibitors (NRTIs) with either a (boosted) protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI) or a third NRTI. As will be discussed later, the latter type of regimen is nowadays considered insufficient for initial ART. *Table 3* demonstrates that the studies with initial HAART are very heterogenous. This table and others[45,46] show that according to an 'intent-to-treat' analysis between 20%[47] and 88%[48] of patients achieve a pVL of <50 copies/ml at 48 weeks of therapy or later with an average of 50%. This may be about 70% with once-daily-dosed regimens and regimens with a low pill burden.[46,49] The 'intent-to-treat' analysis gives insight into the overall success of a certain HAART regimen.

It should be considered that in general clinical practice, the virological response rate to the first HAART regimen could be less than in prospective, randomised studies.[50] Also, as the knowledge and treatment possibilities evolves and patient management and therapy have improved (*tables 1* and *2*) there is a historical bias in the effectiveness figures of HAART regimens. One study showed that between 1996 and 1998 the rate of virological failure after one year of therapy decreased from about 50% to about 15% in an unselected population of an open clinic, but hereafter did not decrease further.[4] This decrease in virological failure could be related to increased experience and the introduction of boosting of PIs with low-dose ritonavir which resulted in a reduction of the dosing frequency and pill burden and an increase in plasma drug levels of PIs (*tables 1* and *2*).

**Table 3**
*Overview of published prospective studies in which HAART is initially used*

| REF | RECRUITMENT PERIOD | FU | HAART REGIMEN | N | CD4 | BASELINE VL | OT | ITT | % VL <50 C/ML +ΔCD4 | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|
| [247] | 7/'94-7/'96 | 52 w | AZT-3/DDI-2/NVP | 51 | 395 | 17,732 | ns | 51 (<20 c) | 139 | |
| [47] | 1/'95-5/'96 | 48 w | AZT-3/DDI-2/NVP | 32 | 37.5 | 5.8 log | ns | 37.5 (<400)* | 101 | *VL: NASBA |
| | | | | ns | ns | <250,000 | ns | 45.5 (")* | ns | |
| | | | | ns | ns | (250,000 | ns | 20.0 (")* | ns | |
| [78] | 11/'95-4/'97 | 52 w | AZT-3/3TC/IDV | 52 | 281 | 4.7 log | 60 | 46 (<20 c) | 178 | |
| [248] | 7/'96-12/'97 | 52 w | AZT-250/3TC/IDV | 35 | 267 | 5.01 log | ns | 66 | 168 | |
| | | | D4T/3TC/IDV | 34 | 313 | 5.21 log | ns | 59 | 232 | |
| | | | D4T/DDI-2,1/IDV | 37 | 277 | 5.00 log | ns | 48 | 166 | |
| [79] | 12/'96-1/'98 | 48 w | D4T/3TC/IDV | 101 | 408 | 4.59 log | 85 | 49* | 142 | *VL: RNA-pcr |
| | | | AZT-3,2/3TC/IDV | 103 | 391 | 4.47 log | 73 | 47* | 110 | <28 d AZT, DDI, D4T, DDC |
| [80] | 12/'96-7/'97 | 48 w | D4T/DDI-2/IDV | 102 | 422 | 31,623 | 70 | 41* | 214 | *VL: RNA-pcr |
| | | | AZT-3,2/3TC/IDV | 103 | | | 69 | 35* | 142 | <4 w AZT, DDI, D4T, DDC |
| [9] | ns | 48 w | AZT-3/3TC/NFV | 99 | 284 | 231,884 | 83 | ns (<400) | ns | |
| | | | AZT-3/3TC/NFV-500 | 97 | 307 | 308,075 | 56 | ns (<400) | ns | |
| [48] | 1-4/'97 | 52 w | D4T/3TC/RTV | 33 | 640 | 35,481 | ns | 88 (<20 c) | 131 | |
| [69] | 1-9/'97 | 48 w | AZT-2/3TC/EFV | 154 | 350 | 60,256 | 90 | 64 | 201 | 3TC-, NNRTI-, PI-naive |
| | | | AZT-2/3TC/IDV | 148 | 341 | 61,660 | 79 | 43 | 185 | |
| | | | EFV/IDV | 148 | 344 | 56,234 | 75 | 47 | 180 | |
| [70] | 2/'97-11/'98 | 48 w | AZT-2/3TC/APV-1200 | 116 | 442 | 4.61 log | 79 | 34 | 128 | |
| [81] | 5/'97-10/'98 | 48 w | DDI-1/D4T/IDV | 100 | 417 | 4.3 log | 80.3 | 55.0 | ns | |
| | | 96 w | | 100 | 417 | 4.3 log | 79.0 | 44.0 | 238 | |
| | | 48 w | DDI-1/D4T/NVP-1 | 89 | 394 | 4.3 log | 80.7 | 53.9 | ns | |
| | | 96 w | | 89 | 394 | 4.3 log | 81.8 | 55.1 | 139 | |
| | | 48 w | DDI-1/D4T/3TC | 109 | 396 | 4.2 log | 58.7 | 45.9 | ns | |
| | | 96 w | | 109 | 396 | 4.2 log | 50.9 | 28.4 | 233 | |
| [249] | 6/'97-10/'98 | 52 w | D4T/3TC-2/IDV | 32 | 708 | 4.38 log | ns | 84 (<200 c) | 186 | |
| | | | | | | | ns | 72 (<5 c) | ns | |
| [82] | 8/'97-6/'98 | 48 w | CBV/ABV/placebo | 282 | 359 | 4.85 log | 69 | 40 | 107 | . |
| | | | | 180 | ns | <100,000 | 57 | 45 | ns | |
| | | | | 102 | ns | >100,000 | 76 | 31 | ns | |
| | | | CBV/IDV/placebo | 280 | 360 | 4.82 log | 82 | 46 | 93 | |
| | | | | 176 | ns | <100,000 | 74 | 46 | ns | |
| | | | | 103 | ns | >100,000 | 88 | 45 | ns | |
| [250] | 9/'97-12/'99 | 52 w | AZT-250/3TC/NVP | 20 | 448 | 4.52 log | ns | 73 | 172 | |
| | | | D4T/3TC/IDV | 22 | 398 | 4.62 log | ns | 68 | 201 | |
| | | | D4T/DDI-2/IDV | 23 | 357 | 4.74 log | ns | 80 | 190 | |

**Table 3 continued**

| REF | RECRUITMENT PERIOD | FU | HAART REGIMEN | N | CD4 | BASELINE VL | OT | ITT | % VL <50 C/ML +ΔCD4 | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|
| [71,251] | 11/'97-'98 | 52 w | D4T/DDI-1/NVP-2 | 60 | 415 | 4.59 log | 67 | ns | 180 | |
| | | | D4T/DDI-1/NVP-1 | 40 | 412 | 4.87 log | 53 | ns | ns | |
| [72] | 11/'98-8/'99 | 12 m | CBV/NFV-2 | 70 | 351 | 65,806 | 79.2 | 65.2 (<20 c) | 173 | |
| | | | | ns | ns | >100,000 | ns | 46.1 (<20 c) | ns | |
| | | | CBV/NVP | 72 | 361 | 59,698 | 70.7 | 50.0 (<20 c) | 162 | |
| | | | | ns | ns | >100,000 | ns | 57.1 (<20 c) | ns | |
| [101] | 11/'98-7/'00 | 48 w | CBV/ABV | 98 | 387 | 4.2 log | 79 | 57 | 110 | |
| | | | CBV/NFV-3 | 97 | 449 | 4.1 log | 80 | 58 | 120 | |
| [73] | ns | 48 w | D4T/3TC/LPV-r | 100 | 334 | 4.89 log | ns | 78* | >213 | *VL: bDNA. Different doses LPV-r |
| [252] | ns | 204 w | D4T/3TC/LPV-r | 100 | 338 | 4.89 log | 97 | 70 | 440 | Follow-up |
| [83] | 1/'99-1/'01 | 48 w | ABV/D4T/DDI-1 | 60 | 190 | 5.0 log | ns | 43 (<20) | 140 | |
| | | | AZT/3TC/SQV/RTV | 60 | 152 | 5.0 log | ns | 69 (<20) | 140 | SQV/RTV 400/400 b.i.d. |
| | | | AZT/3TC/NFV-2/NVP | 60 | 144 | 5.1 log | ns | 62 (<20) | 185 | |
| [253] | 3-10/'99 | 48 w | D4T/3TC/KLT/Placebo | 326 | 232 | 5.01 log | ns | 67 | 207 | |
| | | | D4T/3TC/NFV-3,2/Placebo | 327 | 232 | 4.98 log | ns | 52 | 195 | |
| [74] | 3-12/'99 | 48 w | DDI-1/D4T/NFV-3 } AZT-2/3TC/NFV-3 } | { 511 | 411 | 4.69 log | ns | 32 | 157 | |
| | | | | | 411 | 4.74 log | ns | 32 | 189 | |
| [254] | 3/'99-1/'01 | 48 w | D4T/DDI-1/NVP-1 | 36 | 353 | 23,952 | 88 | 64* | 119 | *VL: bDNA |
| | | | D4T/DDI-1/EFV | 31 | 416 | 22,789 | 100 | 74* | 117 | |
| [255] | 11/'99-3/'00 | 12 m | DDI-1/3TC/EFV | 40 | 164 | 5.4 log | ns | 77 | 184 | |
| [85] | ns | 48 w | DDI-1/3TC-1/EFV | 75 | 251 | 5.09 log | ns | 77 | 208 | |
| [49] | ns | 52 w | DDI-1/3TC-1/EFV | 34 | 184 | 5.21 log | 88.9 | 77.4* | 194 | *VL: bDNA |
| | | | CBV/EFV | 34 | 175 | 5.22 log | 85.7 | 77.4* | 183 | |
| | | | CBV/NFV-2 | 34 | 169 | 5.16 log | 60 | 50* | 165 | |
| [76] | 02/'00-06/'01 | 48 w | D4T/3TC/NVP-1 | 220 | 200 | 4.7 log | ns | 70 | 170 | |
| | | | D4T/3TC/NVP-2 | 387 | 170 | 4.7 log | ns | 65.4 | 160 | |
| | | | D4T/3TC/EFV | 400 | 190 | 4.7 log | ns | 70 | 160 | |
| | | | D4T/3TC/NVP-1/EFV | 209 | 190 | 4.7 log | ns | 62.7 | 150 | |
| [256] | 04/'00-10/'01 | 48 w | 2NRTIs/IDV/RTV | 40 | 80 | 230,957 | 74 | 50 | 167 | IDV/RTV 400/100 mg b.i.d. |
| [103] | 03/'01-11/'02 | 48 w | TZV/placebo | 309 | 234 | 4.85 log | ns | 61 | 174 | |
| | | | CBV/ or TZV/EFV | 765 | 242 | 4.86 log | ns | 83 | 173 | |
| [84] | ns | 48 w | 2-3NRTIs/IDV/RTV | 57 | 50 | 308,000 | 96 | 40 (<80 c)* | 149 | *VL: NASBA |
| | | | | | 42 | <100,000 | ns | 40 (<80 c)* | ns | IDV/RTV 800/100 mg b.i.d |
| | | | | | 15 | >100,000 | ns | 38 (<80 c)* | ns | 2 or 3 NRTIs |

**Table 3 continued**

| REF | RECRUITMENT PERIOD | FU | HAART REGIMEN | N | CD4 | BASELINE VL | OT | ITT | % VL <50 C/ML +ΔCD4 | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|
| [257] | ns | 12 m | D4T/DDI-2/NVP | 47 | 681 | 4.41 log | 79<br>53 | 68 (<200 c)<br>45 (<5 c) | 132<br>ns | |
| | | | D4T/DDI-1/NVP-1 | 47 | 700 | 4.34 log | 85<br>46 | 73 (<200 c)<br>40 (<5 c) | 154<br>ns | |
| [258,259] | ns | 72 w | 2NRTI's/IDV/RTV | 93 | 195 | 210,000 | 94.6 | 59.5 (<80 c)* | 265 | *VL: NASBA<br>IDV/RTV 400/400 mg b.i.d. |
| [260] | ns | 48 w | DDI-1/D4T/ATZV-200 | 104 | 331 | 4.75 log | 33 | 28 | 220 | Phase II trial |
| | | | DDI-1/D4T/ATZV-400 | 103 | 357 | 4.65 log | 42 | 36 | 221 | |
| | | | DDI-1/D4T/ATZV-500 | 110 | 361 | 4.74 log | 52 | 42 | 208 | |
| | | | DDI-1/D4T/NFV-3 | 103 | 341 | 4.79 log | 48 | 39 | 185 | |
| [261] | ns | 48 w | D4T/3TC/ATZV-400 | 181 | 294 | 4.74 log | 40 | 35 | 234 | Dose-finding study |
| | | | D4T/3TC/ATZV-600 | 195 | 302 | 4.73 log | 41 | 36 | 243 | |
| | | | D4T/3TC/NFV-2 | 91 | 283 | 4.73 log | 39 | 34 | 211 | |

*REF = reference. FU = follow-up period in weeks (w) or months (m). N = number of patients included. CD4 = CD4+ cell count, preferentially the median. VL = viral load = blood plasma [HIV-1 RNA] copies/ml in $log_{10}$ or linear count, preferentially the median. % VL <50 c/ml = Percentage with viral load <50 c/ml with reverse transcriptase PCR from Roche Pharmaceuticals. Between brackets VL is indicated if other limit of detection is used. OT = on treatment. ITT = intention to treat. +Δ CD4 = increase of CD4+ T-cell count from baseline. ns = not stated. * = other HIV-1 RNA assay, see remarks.*

*ABV = abacavir 300 mg b.i.d. AZT-3 = zidovudine 200 mg t.i.d. AZT-2 = AZT 300 mg b.i.d. AZT-250 = AZT 250 mg b.i.d.*

*CBV = combivir (zidovudine/lamivudine 300/150 mg) b.i.d. DDI-2 = didanosine 200 or 125 mg b.i.d. DDI-1 = DDI 400 or 250 mg q.d.*

*D4T = stavudine 30 or 40 mg b.i.d. 3TC = lamivudine 150 mg b.i.d. 3TC-1 = 3TC 300 mg q.d. 2-3NRTIs = 2 or 3 nucleoside-analogue reverse transcriptase inhibitors.*

*TZV = trizivir (zidovudine/lamivudine/abacavir 300/150/300 mg) b.i.d.*

*EFV = efavirenz 600 mg q.d. NVP = nevirapine 200 mg b.i.d. NVP-1 = NVP 400 mg q.d. APV-1200 = amprenavir 1200 mg b.i.d.*

*ATZV-200, 400, 500, 600 = atazanavir 200 or 400 or 500 or 600 mg q.d. IDV = indinavir 800 mg q.d.. LPV-r = lopinavir-ritonavir b.i.d.*

*KLT = Kaletra(r) (lopinavir/ritonavir 400/100 mg) b.i.d. NFV-3 = nelfinavir 750 mg t.i.d. NFV-2 = NFV 1250 mg b.i.d. NFV-500 = NFV 500 mg t.i.d.*

*RTV = ritonavir 600 mg b.i.d.. SQV = saquinavir.*

## BASELINE CHARACTERISTICS THAT CAN INFLUENCE VIROLOGICAL RESPONSE

In ART-naive individuals with a chronic HIV-1 infection several factors present at baseline have been associated with the virological response to HAART. These factors can be classified as being related to the host (genetic make-up, adherence), the virus (drug-resistant virus, HIV-1 subtype), the drug, the treatment team or a combination of these. Some factors have been more extensively investigated because they are more easily available, for example the CD4 count, pVL, blood plasma drug levels and patient adherence. The impact of these determinants on virological response is more clear and established. On the other hand, HIV pharmacogenetics, which can elucidate factors at baseline that are associated with proper drug concentrations, toxicity and virological response, is still in its infancy.[51-58] Next, we will describe the factors associated with the virological response to initial HAART starting with the two most extensively investigated factors.

### Low $CD4^+$ T lymphocyte cell count

A low $CD4^+$ T lymphocyte cell count (CD4 count) is usually associated with a high pVL. This makes it unclear which of these two is mostly associated with the virological response. Retrospective cohort studies have shown that patients with a baseline CD4 count <200 cells/$mm^3$ have a worse virological response and more progression to HIV-related morbidity than patients with a higher baseline CD4 count.[4,31,50,59,60] The question is whether this difference in response is due to a difference in biology (host-virus-drug interactions) or in behaviour, for example that patients with a low CD4 count are probably more difficult to treat (initially). Deferring initial HAART until the CD4 count is between 200 and 350 cells/$mm^3$ is associated with a minimally increased risk of AIDS compared with starting at a CD4 count >350 cells/$mm^3$, depending on the pVL, as the risk of AIDS is higher when the pVL is higher.[4,31,50,60,61] As a result, since 2001 most guidelines advise adolescents and adults with a chronic HIV-1 infection to start HAART when their CD4 count is nearing 200 cells/$mm^3$ [1-3,62-64] in order to postpone possible long-term side effects of HAART, such as lipodystrophy syndrome and cardiovascular diseases.[65-68]

### High blood plasma viral load

For ART consisting of two NRTIs and either a (boosted) PI or an NNRTI, there is conflicting evidence from studies (*table 3*) as to whether the risk of virological failure is increased[9,47,49,69-77] or not[49,69,72,78-85] if the pVL at baseline is high. On theoretical grounds there could be an increased risk of virological failure when the pVL is very high. The virological response after HAART is initiated in ART-naive patients biphasic with a rapid decay of the pVL in the first two weeks and a slower decay thereafter. Given that there is a constant first- and second-phase viral decay rate after initiation of HAART, more time is required to reach the level of <50 copies/ml with a higher baseline pVL,[44,71,73,85] thus increasing the window for early development of drug resistance.[4,86-90] It is estimated that per HIV-1 replication cycle one to two base-pair transcriptional errors occur.[19,91,92] Thus, there could be an increased risk of an (early) emergence of strains with one or more drug-resistance mutations if the pVL at baseline is high, especially when drugs with a low genetic resistance barrier (drugs for which only one or two specific mutations in the reverse transcriptase or protease gene are needed to cause significant resistance), such as lamivudine, atazanavir, nelfinavir and the NNRTIs, are components of a triple-drug regimen.[90,93,94]
Adding a fourth active drug in the initial phase of therapy if the pVL is high (e.g. >300,000 copies/ml) is defendable and some guidelines do recommend this strategy.[64]
A triple-class, five-drug regimen (three NRTIs, a (boosted) PI and an NNRTI) is more potent than a two-class, three-drug regimen (two NRTIs and a PI), thus giving rise to the question as to what the optimum potency of a HAART regimen should be.[95-97] However, more drugs implicates more toxicity. An alternative is to use three drugs with a high genetic resistance barrier in the initial phase.

It has now become obvious that when the baseline pVL is high, (certain) convergent regimens consisting of three NRTIs as initial therapy are virologically inferior to divergent regimens consisting of two NRTIs and either a (boosted) PI or NNRTI (*table 3*).[1,3,81-83,98-101] Possibly this inferiority is also the case at low pVL. Some studies, such as a study with tenofovir, lamivudine and abacavir once daily as initial therapy,[93] a study with zidovudine, lamivudine and abacavir,[102,103] and a study with didanosine, lamivudine and tenofovir[94] were even prematurely aborted because of more virological failure. In one very small study with subjects with sustained control of pVL there was more viral evolution in the lymph nodes with subsequent development of drug resistance mutations in the subjects who were treated with dual or triple NRTIs alone compared with the divergent regimens.[104] What needs to be clarified is whether switching to a triple NRTI regimen during proper virological suppression in the absence of previous suboptimal treatment or resistance is associated with more virological failure or not[99,100,105-107] and whether these convergent NRTI regimens can still be applied when the baseline pVL is (very) low.

### Early virological response

It has been demonstrated that the first phase pVL decay rate after initiation of HAART might be predictive for the potency of the regimen and the virological response at two to three months.[108,109] Similarly a decrease in pVL by 1 $\log_{10}$

at week 4 or a pVL of <500 copies/ml at week 4 or 8 of therapy has been associated with a more favourable virological response at 24 or 48 weeks of therapy.[9,110]

**Primary infection with drug-resistant virus**

Of great concern for virological response to initial HAART is the presence of (archived) drug-resistant virus at baseline.[111,112] The prevalence of HIV-1 viruses with one or more RT and/or protease resistance-associated mutations in recently infected individuals has increased in some parts of the world to 20%.[111,113-117] In Europe this is about 10%.[118,119] Thus, depending on the local prevalence, initial HAART needs to be optimised based on empiricism or on genotypic resistance testing. The International AIDS Society – USA Panel recommends a baseline drug resistance test for an HIV-1 infection that was acquired less than two years previously in areas with a drug resistance prevalence of more than 5%.[120]

**SI and NSI phenotype of the virus**

In the early asymptomatic phase of HIV-1 infection nonsyncytium-inducing (NSI) (macrophage or CCR5-tropic) virus variants predominate. In the later course of the infection syncytium-inducing (SI) (T-cell or CXCR4-tropic) variants emerge. This switch in phenotype predominance coincides with a faster progression of the infection.[121,122] *in vitro*, zidovudine preferentially inhibits NSI variants, while didanosine preferentially inhibits SI variants.[123,124] For lamivudine and the protease inhibitor ritonavir an equal inhibition of NSI and SI variants was observed.[122,123] For the other NRTIs and the NNRTIs the effect on NSI and SI has not been compared. The difference in inhibition of NSI and SI variants by zidovudine and didanosine is probably due to differences in intracellular phosphorylation to the active triphosphate.[122,123] Zidovudine and stavudine are preferentially phosphorylated in activated cells, while didanosine, lamivudine and zalcitabine are preferentially phosphorylated in resting cells.[125-127] Activated CD4+ cells express more CCR5 receptors and resting cells express more CXCR4, thus giving a rationale for the divergent inhibition of NSI and SI variants by zidovudine or stavudine and didanosine or lamivudine or zalcitabine, respectively.[122,123] This difference in inhibition is probably of no importance when current HAART is applied, but may be relevant for the use of CCR5 blockers.[128,129]

**HIV-1 subtype**

Based on genetic divergence in the env, gag and pol region, HIV-1 is phylogenetically divided into an M (major), O (outlier) or N (non-M, non-O or new) subtype or clade.[38,39,41] These three subtypes are further subdivided into several subtypes or clades. The O and N subtypes are rare and mainly restricted to West Africa.[41] The M subtype accounts for >90% of reported HIV/AIDS cases.[39] M subtypes that have been identified until now are A, B, C, D, F, G, H, J, K, AE and other recombinants, called circulating recombinant forms (CRFs).[38,39,41] In Europe, the Americas, Australia and New Zealand subtype B is the most prevalent, while the non-B subtypes are mainly prevalent in the other continents. However, due to travel and migration within the Western world these differences in prevalence are changing.[41,130-133]

Within the M subtype inter-clade variation in env is 20 to 30%, in pol about 10% and in gag much less than 10%.[38,39,134] The pol gene encodes for reverse transcriptase, RNase, protease and integrase. These inter-clade variations are or can be accompanied by differences in biological behaviour, susceptibility to antiretroviral drugs, response to HAART, and rate and pattern of emergence of drug resistance.[38,39,41-43] Parallel to this, the HIV-1 subtype outlier is naturally resistant to NNRTIs due to different amino acids at RT position 181.[39,41] Also, HIV-2 is naturally resistant to the NNRTIs because in the wild-type virus amino acid substitutions associated with drug-resistance mutations in HIV-1 (at position 181, 188 and 190) are already present. Furthermore, there is a faster emergence of the multi-NRTI-resistance mutation Q151M and a rapid emergence of genotypic drug resistance (D30N) to the PI nelfinavir in HIV-2.[38,39,135-139]

Clinical studies with antiretroviral therapy have predominantly been done in populations with the B subtype (*table 3*). Prospective studies comparing the virological response among HIV-1 subtypes are lacking. Three retrospective studies with a limited number of patients showed no difference in response to HAART between B and non-B subtypes.[140-142] Thus, the impact of the different HIV-1 M subtypes on the virological response to initial HAART needs to be established.

**Trough concentration of PIs and NNRTIs in blood plasma and the intracellular concentration of triphosphorylated NRTIs**

Retrospective and prospective studies have shown a correlation between the blood plasma (trough drug concentration of PIs and NNRTIs and the virological response.[17,143-147] Minimally effective drug concentrations have been defined, and nowadays therapeutic drug monitoring, the proactive regular measurement of drug concentrations, is considered a standard during PI and NNRTI treatment in some countries.[1,3,143-145,148] Likewise, a positive association was found between the intracellular concentration of triphosphorylated NRTIs and the virological response.[149,150]

**Adherence to therapy**

Proper adherence to the dosing interval and administration requirements of the drugs is one of the most important

factors for a durable virological success. Adherence to therapy of at least 90 to 95% is needed with HIV-1 to properly suppress the virus for a prolonged time.[151-156] Factors that influence adherence to therapy are dosing frequency,[157,158] pill burden,[45,49] acute and long-term toxicity of the drugs,[16,159-161] and sociocultural factors.[162] Before initiating HAART it is essential that the patient is willing and cooperative in taking the drugs and that he/she is well informed, instructed and aware of the drug intake requirements, adverse effects and consequence of nonadherence.

Frequent consultations and monitoring of drug levels, pVL and adherence in the early phase of therapy might be useful for this purpose.

**Pharmacodynamic interaction between antiretroviral drugs**
Stavudine and zidovudine have a proven antagonistic effect.[3,163,164] The cause of this antagonism is unclear, but theoretically it could be due to steric hindrance at the enzyme active site or due to interference in the metabolic pathway of the drug. The virological inferiority of a regimen consisting of only three NRTIs could also be due to antagonism or a low genetic barrier to resistance.[93,94] On the other hand, genotypic resistance to lamivudine (mutation M184V) can (partially) reverse resistance to zidovudine and improve the virological response to tenofovir.[19,165] Possibly, resistance to NRTIs causes (*in vitro*) hypersusceptibility to NNRTIs and *vice versa*.[20,166-171] Another example of increased susceptibility that can occur is the presence of the N88S protease gene mutation and improved virological response to amprenavir.[172] Whether such *in vitro* hypersusceptibilities result in clinical benefit remains to be proven.[171]

**Genetic make-up of the patient**
Certain polymorphisms of chemokines (SDF-1 3'a, G protein b3 subunit 825T), chemokine receptors (CCR5-delta32, CCR2-V64I, CCR5-promotor allele 59029-G), and certain HLA alleles (B57, B27, Bw4, B*5701, B14, C8) are associated with a slower progression of the HIV-1 infection, while CCR5-promotor allele 59029-A and other HLA alleles (B35, Cw4, DQB1*0402) are associated with a faster progression of the infection.[57,58,173-178] Some of these factors are associated with a better (CCR5-delta32) or worse (the combination of wild-type CCR5, wild-type CCR2 and homozygous CCR5-promotor allele 59029-A) response to ART.[53,56-58] Also, a homozygous C/C genotype at base-pair position 3435 in exon 26 of the multidrug resistance transporter 1 (MDR1) gene is associated with a worse virological response to HAART compared with the T/T or C/T genotype.[54,55,57,58,179]

This may have consequences for choosing the type of initial HAART to be used among ethnic groups since the C/C genotype is more prevalent in Afro-Americans and Africans than in Caucasians and Asians.[57,58,180-183]

**Experience and knowledge of the treating physician**
A better survival was associated with more experience in treating HIV on the part of the physician as well as better adherence to therapy by the patient.[184] These two factors even outweighed the worse outcome if HAART was started at a CD4 count below 50 cells/mm$^3$.[184] Although not evaluated, the improved outcome was probably partly due to a better virological response because of a better instruction and management of the patient by the more experienced physician.

## EPILOGUE

The aim of initial HAART is to achieve maximal and durable viral suppression which is currently a pVL of <50 copies/ml. With current knowledge, patient management and treatment possibilities virological success after one year of initial HAART has improved to about 70%. So, there is much room for further improvement and the challenge is how to achieve this. Adjusting therapy to a virological failure risk profile could be a useful strategy. As an example, a patient with a high pVL (e.g. >300,000 copies/ml), a low CD4 count (e.g. <100 cells/mm$^3$) and a low socioeconomic status (e.g. poor housing) could be given a four-drug divergent regimen as initial HAART or a three-drug divergent regimen with a high genetic barrier to resistance, and such a patient should be more intensively monitored.
How the clinical relevance of the several determinants of virological response compare with each other needs to be established. However, likely adherence to a potent and well-tolerated HAART regimen is the most basic and relevant factor for virological success and the other factors probably modulate this response.

## SUGGESTIONS FOR FURTHER STUDIES

What needs to be further evaluated is the importance of other factors, what are early markers for long-term virological response, whether switch to a triple NRTI regimen is virologically safe, whether once-daily-dosed regimens have a virological advantage over twice-daily-dosed regimens, how regimens with a low, middle and high genetic resistance barrier compare with each other, how the virological response in non-B subtypes compare with subtype B, and what level of virus suppression is needed to prohibit virological failure. As long as HIV cannot be cured, this last factor will really define what should be considered as HAART. However, improving adherence to therapy is likely to be the most relevant measure to achieve a durable virological success.[185]
Since the risk of HIV-related morbidity and mortality is

substantially increased as long as the CD4 count is below 200 cells/mm$^3$, strategies to raise the CD4 count more rapidly above this threshold (e.g. type of HAART regimen, interleukin-2) should also be explored.[186] Chronic hyperactivation of the immune system might be an important cause of CD4 cell loss.[187] Considering the toxicity of HAART and the importance of a high level of adherence to HAART, it is also worthwhile to evaluate inhibition of this hyperactivation.

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# A randomised study on the efficacy and safety of an automated Tru-Cut needle for percutaneous liver biopsy

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## ABSTRACT

**Background: We studied whether the theoretical advantages of a spring-loaded liver biopsy needle exist in clinical practice and if so if they are dependent upon the experience of the physician performing the biopsy.**

**Methods: In a stratified randomised study we enrolled 215 consecutive patients to compare the safety and efficacy of a new automatic biopsy gun (Acecut) with that of a standard Tru-Cut needle.**

**Results: A total of 464 biopsies were performed. The endpoints of the study were number of needle passes needed per patient, tissue yield of each needle pass and post-biopsy complications. The performance of the automatic needle was superior and more consistent with respect to tissue yield compared with the Tru-Cut needle (median yield 100% and 80%, respectively; p<0.001). The difference was most marked for inexperienced physicians. There was no difference between the two needles in the number of passes needed. More post-biopsy pain and post-biopsy use of analgesics were observed in the automatic needle group (p=0.04).**

**Conclusion: The automatic Tru-Cut needle offers an advantage, particularly for physicians with no or limited experience in liver biopsies. However more post-biopsy pain and post-biopsy use of analgesics were observed in the automatic needle group.**

## INTRODUCTION

Several new types of automatic spring-loaded Tru-Cut liver biopsy needles have recently been introduced. These devices are commonly referred to as 'biopsy guns'. The potential advantage of a biopsy gun is the shorter duration of the actual biopsy procedure which could reduce the number of complications. In addition, since the difficult Tru-Cut movement is automated, one would expect the tissue yield to be larger and needle performance more constant. However, whether these theoretical benefits are of importance in clinical practice may depend upon the experience of the physician performing the biopsy.

### Aims of the study

We initiated a randomised study of the standard Tru-Cut liver biopsy needle and an automatic spring-loaded Tru-Cut device to compare tissue yield, the quality of the biopsy specimen obtained and post-biopsy complications. A secondary aim was to test the hypothesis that a possible advantage of the automated device would be most apparent among inexperienced operators.

## MATERIALS AND METHODS

The study population consisted of a cohort of all consecutive patients referred for percutaneous liver biopsy to the Department of Hepatogastroenterology between October 1994 and October 1996. The unit is a tertiary referral centre for liver diseases and liver transplantation.

### Randomisation procedure

A computer-generated randomisation procedure was followed. Patients were randomly allocated to one of two

groups using consecutively numbered sealed nonopaque envelopes. Patients were stratified according to the experience of the operator. A physician was considered inexperienced if he had performed less than 50 Tru-Cut liver biopsies.

**Biopsy needles**

For this study we used a 14 gauge x 11.4 cm Tru-Cut needle (Baxter Healthcare Corporation, Deerfield, Ill, USA) as standard needle. This needle has a biopsy specimen notch of 20 mm. This was compared with a needle biopsy gun (Acecut, TSK Laboratory, Japan) with a 14 gauge x 11.5 cm needle and a 15 mm biopsy specimen notch.

**Biopsy procedure**

Preparation for the biopsy was identical for the two groups. After informing the patient about the procedure, a mid-axillary biopsy site was selected and checked by ultrasonography. The actual biopsy procedure was performed without ultrasound guidance. If desired by the patient premedication, consisting of intravenous midazolam (Dormicum) in a bolus dosage of 5 mg, was given. Dosages of 1 to 2.5 mg were administered to patients over 65 years of age who had cardiopulmonary or other diseases considered to increase the risks associated with intravenous administration of benzodiazepines. Oxygen saturation and heart rate were monitored by pulse oximetry. After skin disinfection and liberal local anaesthesia with 1% lidocaine the randomisation envelope was opened by the endoscopy nurse and the appropriate biopsy needle was presented. Our standard procedure is to obtain two biopsy specimens; if the biopsy was less than 15 mm long, the biopsy pass could be repeated a maximum of four times.

After the biopsy procedure the wet biopsy specimen was placed on a plate of paraffin and the length was measured using a micrometer. All patients undergoing this procedure as outpatients were observed for three hours in the day-care facility with standard checks on post-biopsy pain, pulse rate and blood pressure. A patient with pain was evaluated by the operator who could prescribe a bolus of 50 or 100 µg intravenous fentanyl citrate (Fentanyl). Our policy is to administer fentanyl early in the event of post-biopsy pain. At the end of the three-hour observation period the occurrence of pain was scored by the physician as none, mild or severe. This was further documented by the prescription of fentanyl. At the end of the three-hour observation period the patient was again seen by the physician and either sent home or admitted for further observation. Any admission was scored as a complication. When an in-patient underwent the biopsy procedure, the occurrence of pain, use of fentanyl and complications were recorded by the attending ward physician the day after the procedure. All data were registered on standard forms.

**Study endpoints**

The study endpoints were:

- the number of passes performed and total biopsy length/number passes;
- the cumulative length of the liver tissue obtained and the quality of the material obtained (fragmented *vs* a coherent biopsy specimen);
- the number of passes with insufficient material defined as less than 10 mm (insufficient pass), no tissue yield (failed passes) or no tissue yield at all (failed procedure);
- post-biopsy complications.

**Statistical evaluation**

The aim was to include at least 50 patients in each physician stratum. Power calculations were not performed since no data were available on the performance of the automatic needle. We assumed that in a study of 100 patients no clinically significant differences would be missed. Since recruitment in the inexperienced physician group was slower than expected, a total of 215 patients were enrolled. Equality of the medians was tested by the two-sample Wilcoxon rank-sum test. The relationship between the type of physician who took the biopsy, needle type and tissue yield was evaluated using multiple regression analysis. Whether the differences between needle types depended on the physician was tested using appropriate interaction terms. Dichotomous parameters were tested by Fisher's exact test.

## RESULTS

**Patients**

In total, 215 patients were randomised. Five patients were excluded from the study because the physician decided to change the biopsy procedure to either an ultrasound-guided biopsy (of the left liver lobe) (n=2) or a laparoscopic biopsy (n=3). In one case the data sheets were lost. In total 209 patients could be evaluated. The groups were well matched for demographic, clinical and laboratory variables (*table 1*).

**Physicians**

Four experienced and three inexperienced physicians participated in this study. In 159 cases the biopsy procedure was performed by an experienced physician (78 automatic needle and 81 Tru-Cut). In 50 cases the biopsy procedure was performed by an inexperienced physician (24 automatic needle and 26 Tru-Cut).

**Biopsy length**

No differences were found in either the number of passes needed for each needle type or the number of insufficient or failed passes. There were no failed procedures.
To correct for the maximum tissue yield possible, all data

**Table 1**
*Patient characteristics and features at entry*

| | AUTOMATIC NEEDLE | HAND-OPERATED NEEDLE |
|---|---|---|
| Number of patients | 102 | 107 |
| Age | 41 (19-72) | 45 (17-68) |
| Sex (female/male) | 39/63 | 38/69 |
| Aetiology of the liver disease | | |
| Hepatitis B | 47 | 52 |
| Hepatitis C | 24 | 20 |
| Primary biliary cirrhosis | 4 | 5 |
| Primary sclerosing cholangitis | 4 | 1 |
| Autoimmune hepatitis | 2 | 2 |
| Alcoholic liver disease | 2 | 3 |
| Post-transplantation | 4 | 8 |
| Cryptogenic | 15 | 16 |
| Evaluation of haemostasis | | |
| Number of patients with prolonged prothrombin time | 1 | 3 |
| APTT (normal 25-40 seconds) | 29 (20-40) | 28 (23-46) |
| Platelets (normal 140-360.$10^9$/l) | 202 (58-660) | 202 (41-381) |
| Bleeding time (normal <240 seconds) | 170 (60-355) | 142 (60-420) |

*Continuous data are presented as median plus ranges.*

were analysed as percentage of the specimen notch used per biopsy pass. This percentage was significantly higher for the automatic needle compared with the Tru-Cut needle (100 *vs* 80%; p<0.001) (*table 2*).
The difference in mean use of the needle notch did not differ between individual physicians in one group. A clear trend (p=0.109) was found towards an increased benefit of the automatic biopsy device among inexperienced physicians compared with the experienced ones (*table 3*).

**Complications**
Three patients (all randomised to the automatic needle group) suffered complications in this study. Two patients were admitted because of pain and discharged the next day without complaints. One patient with documented intraperitoneal bleeding received two units of red blood cells. The difference between the two groups was not statistically significant.

After a biopsy procedure the pain experienced by the patient as interpreted by the physician was more severe in the automatic needle group (p=0.012). This paralleled the increased prescription of fentanyl in the automatic needle group (p=0.04). The difference in post-biopsy pain was independent of the experience of the physician (p=0.08 and p=0.05 for the experienced and inexperienced groups, respectively) (*table 4*).
The overall incidence of major complications in this series was 0.6% (3/464). The incidence of minor complications (pain with administration of analgesics) was 13.5% (63/464).

## DISCUSSION

When performing a liver biopsy the first decision to be made is the choice between the cutting needle (Tru-Cut system) and the aspiration needle (Menghini type). With both needle types adequate tissue samples can be obtained while there is an advantage for Tru-Cut needles in diagnosing cirrhosis.[1] In an experimental animal model using direct comparison the Tru-Cut needle performed better compared with aspiration needles with regard to tissue yield and specimen quality.[2] Although the overall incidence of post-biopsy bleeding in nonmalignant liver disease is low (0.4 per 1000 for fatal bleeding and 1.6 per 1000 for nonfatal bleeding), the incidence of severe post-biopsy bleeding is higher for cutting needles compared with aspiration needles.[3,4] In the experimental model as well as in autopsy studies it has been shown that an automatic biopsy device produces adequate tissue samples.[5] The aim of our study was to compare in everyday clinical practice the performance of an automatic with a hand-operated Tru-Cut needle. Our hypothesis was that the impact of an automatic biopsy device would be the greatest among inexperienced physicians whereas no clear advantage would found for experienced physicians. This study shows that the use of an automatic Tru-Cut needle device is superior to a standard Tru-Cut needle as far as tissue yield is concerned. Lindor demonstrated that this is also true when only experienced operators participate.[6]
Are these differences clinically relevant? Obviously adequate tissue specimens can be obtained with both needles. The tissue yield of the automatic needle seems to be more con-

**Table 2**
*Results of biopsy procedures*

| | AUTOMATIC NEEDLE | HAND-OPERATED NEEDLE |
|---|---|---|
| Needle passes | 2 (1-4) | 2 (1-4) |
| Number of patients (n) | | |
| Needle passes (%) | | |
| 1 pass | 5 (4.9) | 2 (1.9) |
| 2 passes | 77 (75.5) | 78 (72.9) |
| 3 passes | 17 (16.7) | 24 (22.4) |
| 4 passes | 3 (2.9) | 3 (2.8) |
| Number of failed needle passes (n) | | |
| First biopsy | 3 | 8 |
| Second biopsy | 4 | 7 |
| Third biopsy | 0 | 5 |
| Fourth biopsy | 0 | 0 |
| Number of failed procedures (no material obtained) | 0 | 0 |
| Biopsy length (mm) | | |
| Cumulative biopsy length as percentage of biopsy notch (%) | 100 (25-140) | 80 (21-138) |
| Cumulative biopsy length | 30 (15-56) | 35 (17-73) |
| Median biopsy length per pass | 15 (5-20) | 16 (6-24.5) |
| Biopsy quality if biopsy obtained | | |
| Good-quality first biopsy (%) | 94.8 | 94.8 |
| Good-quality second biopsy (%) | 92.3 | 86.4 |
| Good-quality third biopsy (%) | 94.7 | 85.7 |
| Good-quality fourth biopsy (%) | 100 | 100 |

*Continuous data are presented as median plus ranges. Quality data relate to the percentage of biopsies yielding good quality tissue.*

**Table 3**
*Liver tissue yield expressed as percentage of the biopsy notch in relation to the experience of the operator*

| | AUTOMATIC NEEDLE | HAND-OPERATED NEEDLE |
|---|---|---|
| Inexperienced operator | 100% (36-123) | 69% (30-137) |
| Experienced operator | 100% (25-140) | 85% (21-122) |

*Data are presented as median plus ranges.*

**Table 4**
*Complications and post-biopsy sequelae*

| NEEDLE TYPE | AUTOMATIC NEEDLE | HAND-OPERATED NEEDLE |
|---|---|---|
| Patients on midazolam pre-medication (%) | 82 | 84 |
| Post-biopsy pain (n (%)) | | |
| None | 45 (47.4) | 70 (68.6) |
| Mild | 33 (34.7) | 20 (19.6) |
| Severe | 17 (17.9) | 12 (11.8) |
| Post-biopsy fentanyl (n) | | |
| None | 56 | 78 |
| 50 µg | 35 | 17 |
| 100 µg | 4 | 6 |
| Admissions (n) | 3 | 0 |

sistent which is particularly beneficial for operators who perform liver biopsies sporadically. Our data support a recommendation that those who will not be performing frequent liver biopsies in the future should learn to use an automatic device while the type of needle is of less relevance for those working in specialised liver units.
We started using ultrasound routinely for all patients in 1983. This approach has since been shown to reduce the incidence of post-biopsy complications.[6] However the occurrence of post-biopsy pain and use of analgesics were higher in the automatic needle group. Although it seems logical to attribute more blunt tissue trauma and more pain to the spring-loaded device, the study design with regard to the occurrence of pain should be interpreted with caution: the scoring of post-biopsy pain and the decision to use fentanyl were left to the physician in charge and not to more patient-related measurements. In the combined Mayo Clinic – Barcelona study 207 patients were biopsied with an automatic Tru-Cut needle under ultrasound guidance and 216 hand-held Tru-Cut biopsies under ultrasound guidance were performed.[6] The occurrence of post-biopsy pain was 40.6% for the automatic group and 34.3% for the hand-operated group. The data seem to support the idea that post-biopsy pain increases with the use of a spring-loaded biopsy needle. Since compliance with repeated liver biopsies is essential for the treatment and follow-up of patients with chronic liver disease, this is an important aspect which should not be disregarded easily. We can only speculate on the exact cause of post-biopsy pain with a spring-loaded biopsy needle. One explanation may be that because some operators keep close contact with the upper side of the rib during the procedure, the spring-loaded device causes more blunt trauma to the periostium.

Our data support the recommendation that physicians who perform liver biopsies infrequently should learn to use an automatic Tru-Cut biopsy device. A follow-up study with a 20 mm specimen Tru-Cut biopsy device would be worthwhile, especially to address the issue of an increase in post-biopsy pain following the use of a spring-loaded biopsy gun.

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ORIGINAL ARTICLE

# Vascular graft infection in aortoiliac and aortofemoral bypass surgery: clinical presentation, diagnostic strategies and results of surgical treatment

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## ABSTRACT

**Objectives: Evaluation of the prevalence, diagnostic procedures and clinical outcome of infections of aortoiliac and aortofemoral vascular grafts presented in our centre.**
**Design: Retrospective study.**
**Materials: All patients who underwent a surgical aortoiliac or aortofemoral revascularisation between 1991 and 2001.**
**Methods: Evaluation of several hospital databases.**
**Results: 32 cases of aortoiliac and aortofemoral vascular graft infection with varied clinical presentation were found. Enteral bleeding was the first clinical manifestation in 31% of the cases, inguinal swelling, wound, or fistula in 59% and fever or sepsis in 6.3%. In 3% the cause was unknown. The vast majority (84.5%) of the infections presented three or more months after surgery (late infections). In cases of enteral bleeding, endoscopy procedures only revealed the diagnosis in 55%. Diagnostic algorithms including an abdominal CT scan appeared to have a sensitivity of 94% for establishing an accurate diagnosis. Remarkably, no specific risk factors for graft infection could be demonstrated. Furthermore, a 30-day survival of 20% or less was observed in early graft infections, whereas late infections managed with extra-anatomical bypasses appeared to have a better survival rate of up to 70%.**
**Conclusion: Endoscopy in cases of enteral bleeding and CT scanning overall were shown to be very useful for establishing the diagnosis. Clinical outcome and survival after treatment remain poor.**

## INTRODUCTION

The infection of a vascular graft is a rare complication in bypass procedures with an estimated incidence of 0.5 to 2.5%. However, the mortality and morbidity rates due to this complication are high (25 to 75%).[1,5,6,10] The identification of a graft infection entails a potentially complicated treatment for both the patient and the surgeon. Of all graft infections, those of vascular prostheses in the aortoiliac or femoral region almost always lead to serious life-threatening situations.[2]

It is known that groin infections predominate as the most common site of contamination.[10-14] Aortofemoral grafts have higher infection rates than aortoiliac grafts.[3]

Although graft infections may manifest with clear symptoms (especially the infections of the femoral graft component), the actual diagnosis can be notoriously difficult, due to subtle and nonspecific signs and symptoms. Several studies have evaluated the available diagnostic techniques and have shown that computed tomography (CT scan) is a clinically valuable technique to detect infectious complications with a high sensitivity and specificity.[2,3,4,7,9-11] However, a possible pitfall in using the CT scan is that the absence of substantial perigraft fluid or air collections does not exclude a graft infection. Especially during the postoperative period of 12 weeks an infection cannot be distinguished from a retroperitoneal haematoma.

Although improvement has been made in clinical management of graft infections, current therapeutic options are still accompanied by high morbidity and mortality rates.[2,6,10-12,14] An accurate and timely identification of a graft infection

** A.F.H. Stalenhoef was not involved in the handling and review process of this paper.

is needed to prevent unnecessary intervention or complications because of late intervention.[6,7,14]
To explore the prevalence of graft infections and the results of diagnostic procedures, we evaluated all registered cases of aortoiliac and aortofemoral graft infections in our hospital over the past ten years. We also looked at the outcome of therapeutic management. In all cases we gathered information on the indication for revascularisation and circumstances at the time of implantation, the way in which a graft infection emerged, the diagnostic algorithms used to demonstrate the infection, and the management and outcome.

## METHODS

In order to collect all cases we consulted three different databases over the last ten years (from 1991 to 2001): the hospital information system, the database of the infectious disease consultation service and the surgical archives.
In the vascular surgical archives (lists of performed procedures, with indication), search terms were: 'infectious complications' in combination with 'aortoiliac or aortofemoral or aortic vascular graft', 'extra-anatomical bypass', 'aorta' and/or 'vessel infection', 'prosthesis infection', 'axillo-(bi)femoral bypass', 'infected central vessel prosthesis', 'pseudoaneurysm', 'removal of a central vessel prosthesis'. Medical records of these patients were collected and thoroughly studied. The cases in which the aortoiliac or aortofemoral graft was infected were included. Infected 'crossover' prostheses were excluded. The same method was used on the infectious diseases consultation service, in which the diagnosis 'infection of aorta and/or vessel graft' was formulated. In the hospital system we looked for the registered complications (infection) of performed central bypass surgery.
The following data were gathered from the medical records of all included cases: clinical presentation of infection, time between initial bypass surgery and graft infection, indication for bypass surgery, type and place of the infected graft, presence of risk factors for infection (*table 5*), sequence and results of used diagnostic procedures, time between presentation and diagnosis, method of treatment, complications and outcome of treatment and cultured microorganisms on removed grafts. All data was analysed with the help of a database programme.

## RESULTS

### Prevalence

Between 1991 and 2001, 964 procedures for central blood vessel reconstruction were performed in our Centre. Altogether, 32 cases with an aortoiliac or aortofemoral graft infection were found. Most patients (n=27) emerged after three months or more and were therefore categorised as 'late infections' (84.5%). The initial clinical presentation of late infections could be divided into three groups, 'enteral bleeding', 'malaise/fever' and 'palpable mass', as shown in *table 1*. All cases of 'early graft infection' (presentation within three months after operation) presented with inguinal fistula or wound. In these various presentations, enteral bleeding indicates an aortoenteric fistula, which can be considered as a subset of aortic graft infection. The characteristics of the patients are shown in *table 2* and the different sites of infection are summarised in *table 3*. To evaluate the diagnostic procedures used, as well as the management and outcome of the included cases, we analysed all relevant and available data as provided by the medical records.

**Table 1**
*Clinical presentation of patients (n=32) with infection of aortoiliac or aortofemoral vascular graft*

| CLINICAL PRESENTATION | NUMBER OF PATIENTS (%) |
|---|---|
| **Early central graft infections** | |
| Inguinal fistula or wound | 5 (15.6) |
| **Late infections** | |
| Enteral bleeding | 10 (31.3) |
| Malaise, fever | 2 (6.3) |
| Palpable mass<br>In abdomen (n=3)<br>In the groin (n=4)<br>With fistula/wound in groin (n=7) | 14 (43.8) |
| **Unknown*** | 1 (3.1) |

**No data on the initial clinical presentation were available for this patient, who was operated immediately because of an infected aortic graft.*

### Diagnostic techniques

The various diagnostic procedures used are presented per group of patients in *table 4*. Cases with 'enteral bleeding' (n=10) were predominantly admitted to the Department of General Internal Medicine. The severity of the bleeding varied from massive (with haemodynamic instability or coma) to minimal blood loss over a longer period. Apart from the one case in which no investigations were carried out because of massive bleeding (immediate operation), endoscopy was the first diagnostic technique used (*table 4*). Only a small blood clot may represent an aortoenteric fistula. However, in some cases the fistula itself or even the prosthesis was observed during endoscopy. Six out of eleven endoscopies revealed a fistula or a visible prosthesis in the enteric tract (sensitivity of 55%). Repeated endoscopy

**Table 2**
*Age, gender, graft position and indication for revascularisation in 31 patients with graft infection*

| | ESTABLISHED INFECTION | | | | ALL |
|---|---|---|---|---|---|
| | LATE INFECTION | | | EARLY INFECTION | |
| | ENTERAL BLOOD LOSS | MALAISE, FEVER, WITHOUT OTHER SIGNS | SWELLING IN ABDOMEN OR GROIN | | |
| Men:women | 9:1 | 0:2 | 11:3 | 4:1 | 24:7 |
| Age (mean ±SD) | 70 | 72 | 68 | 69 | 70 |
| **Location of prosthesis** | | | | | |
| Aorta prosthesis | 5 | 1 | 0 | 2 | 8 |
| Aortoiliac prosthesis | 5 | 1 | 12 | 2 | 20 |
| Iliacoiliac | 0 | 0 | 2 | 0 | 2 |
| Iliacofemoral | 0 | 0 | 0 | 1 | 1 |
| **Indication for revascularisation** | | | | | |
| Intermittent claudication | 4 | 0 | 11 | 1 | 16 |
| Critical ischaemia | 0 | 0 | 1 | 1 | 2 |
| Aneurysm | 5 | 1 | 0 | 2 | 8 |
| Ruptured aneurysm | 1 | 1 | 1 | 1 | 4 |
| Infected aneurysm | 0 | 0 | 1 | 0 | 1 |

**Table 3**
*Observed infections*

| | | LATE INFECTIONS | EARLY INFECTIONS |
|---|---|---|---|
| **Aortic graft/aortic part of graft** | Nonaneurysm | 10 | 0 |
| | Aneurysm | 2 | 0 |
| **Iliac part of the graft** | Nonaneurysm | 13 | 5 |
| | Aneurysm | 2 | 0 |

appeared to be useful in eventually finding a fistula or prosthesis in another patient. In the cases in which endoscopy did not reveal the diagnosis, a CT scan was then performed (*table 4*), always after determination of infection parameters (ESR, CRP and/or white blood cell count). The results of three out of the four CT scans performed indicated a graft infection (fluid collection, hypodense tissue and/or gas bubbles surrounding the prosthesis) (sensitivity of 75%). Thus, in more than half of the graft infections presenting with enteral bleeding, the diagnosis could be made within two or three days. The period of time until diagnosis increased in the cases in which an endoscopy did not demonstrate the site of the bleeding.

All cases with inguinal pain, fistula or wound were treated immediately by a vascular surgeon. Infection of the graft was already suspected by evaluation of the medical history. In most cases, taking the body temperature was the first diagnostic investigation, followed by determination of infection parameters and culturing wound smears. As shown in *table 4*, various diagnostic routes were used for establishing the diagnosis. Although in some cases the diagnosis could be made on the basis of physical examination alone, the diagnostic techniques used were primarily related to the clinical presentation (inguinal pain, swelling, a fistula or a wound). In most cases, abdominal or inguinal ultrasound was undertaken to evaluate signs of infection (fluid around the graft or abscess formation, or a pseudoaneurysm in case of swelling). An abdominal CT scan was performed, predominantly to evaluate the extent of the infection. In some cases a diagnostic puncture from a fluid collection or abscess was carried out. Angiographies were performed in cases of swelling of the groin which were suspected of being a pseudoaneurysm (infected or not). All the CT scans (n=9) demonstrated fluid collections, or showed the presence of a (pseudo)aneurysm (sensitivity of 100%). In two of nine patients, the revascularisation procedure had been performed less than three months previously, so the fluid detected by CT scan or ultrasonography may also have been a consequence of the procedure itself. The ultrasound investigations, frequently

**Table 4**
*Diagnostic procedures in individual patients suspected of graft infection*

| USED DIAGNOSTIC MODALITIES | NUMBER | SENSITIVITY (%) | MEAN TIME PERIOD TO DIAGNOSIS (DAYS) |
|---|---|---|---|
| **Internal medicine department** | | | |
| *Enteral bleeding* | | | |
| Endoscopy only | 4 | *Duodenoscopy* | 1-3 |
| Endoscopy (three times), bleeding scan | 1 | 6 of 11 (54%) | |
| Endoscopy, coloscopy, CT (one also had a DSA) | 3 | | |
| Endoscopy, echo, CT | 1 | *CT scan* | |
| No diagnostic process (direct operation for massive bleeding) | 1 | 3 of 4 (75%) | |
| *Illness, fever, septicaemia* | | | |
| US (twice), CT (twice), sigmoidoscopy, coloscopy, IgG scan | 1 | *CT scan*<br>2 of 2 (100%) | 5-14[1] |
| CT, leucocyte scan | 1 | | |
| **Surgical department** | | | |
| *Early infection (wound after operation)* | | | |
| CT | 1 | *CT scan* | 7[2] |
| Echo, CT, CT-guided puncture | 1 | 3 of 3 (100%) | |
| CT (twice), leucocyte scan | 1 | | |
| No diagnostic process | 2 | | |
| *Late infections; inguinal fistula/wound* | | | |
| US, CT, with:<br>US-guided puncture and leucocyte scan<br>Leucocyte scan<br>CT-guided puncture, IgG scan | <br>1<br>1<br>1 | *CT scan*<br>9 of 9 (100%)[3] | 105[4] |
| US, US-guided puncture, CT | 1 | | |
| US, angiographic view | 1 | | |
| US, MRI | 1 | | |
| CT, fistulogram | 1 | | |
| CT (three times), IgG scan | 1 | | |
| Fistulogram (three times), CT | 1 | | |
| Angiography | 1 | | |
| No diagnostic process | 3 | | |

*CT = abdominal computed tomography; US = abdominal ultrasound investigation. [1]CT scan performed on the first day already showed signs of graft infection. [2]Initial blood cultures were taken and antibiotic therapy was started. Additional investigations were performed later. [3]Two CT scans in this group were within three months after the (second) operation and therefore do not indicate infection. Two CT scans were performed because of an initial aneurysm, and not because of suspected infection of a prosthesis. [4]In the outpatient clinic.*

preceding the final CT scan, all indicated the presence of fluid or infiltrated (hypodense) tissue around the graft. Fistulograms appeared to be helpful in demonstrating the connections between anatomical structure and the graft. The diagnostic period in this group depended primarily on the clinical presentation. In cases of a local inguinal wound infection after bypass surgery an initial treatment with antibiotics in order to prevent ongoing infection, delayed further investigation.

Most diagnostic effort was made in two cases with 'septicaemia without any indications of a central graft infection'. *Table 4* shows the additional diagnostic algorithms used, after standard blood examination for elevated parameters of infection. In these cases, a CT scan was the first diagnostic technique to evaluate the cause of the fever. Noteworthy, after fluid collections surrounding the graft were detected (which occurred in both cases), even more investigations (white blood cell scan and an IgG scan) were performed to establish convincingly the graft infection and decide on surgical intervention.

In summary, 19 CT scans were performed in the 32 included cases, of which 18 demonstrated a fluid collection or other findings due to an infection (sensitivity of 94.7%). In all other cases the graft infection was demonstrated by

**Table 5**
*Management and outcome in the case of proven graft infection*

| TREATMENT | COMPLICATIONS | SURVIVAL[1] |
|---|---|---|
| **Enteral bleeding (n=10)** | | |
| Surgery (n=9) | Exsanguination (n=2) | 2 (20%) |
| Graft removal and closure of enteral defect, bypass:<br>Axillobifemoral (n=8)<br>Axillofemoral with crossover (n=1) | Septicaemia, ARDS, MOF (n=4) | |
| Antibiotics (n=1)[2] | | |
| **Inguinal swelling/wound/fistula (n=19)** | | |
| Drainage, wound exploration, antibiotics[3], surgery (n=14) | **Early complication (n=5)** | 1 (20%) |
| Graft removal (n=13): whole graft (n=9), part of leg (n=4)<br>Extra-anatomic bypass (not done in 4 cases)[4]<br>Axillobifemoral (n=2)<br>Axillofemoral/poplitial (n=2)<br>Axillofemoral and crossover Veneus/Dacron[5](n=1)<br>Crossover Veneus/Dacron[5] (n=4)<br>Replaced bifurcation (Dacron[5]) with local gentamycin (n=1) | Sepsis, MOF (n=3)<br>Amputation (n=2)<br>**Late complication (n=14)**<br>Thrombosis of the bypass (n=5)<br>Sepsis and MOF (n=1)<br>Injury of serosa followed by reoperation (n=2)<br>Fever (n=2) | 10 (71%)[6] |
| **Septicaemia, malaise, without further signs (n=2)** | | |
| Surgery (n=2) | Septicaemia, MOF (n=2) | None |
| Graft removal, extra-anatomic bypass:<br>Axillobifemoral (n=1)<br>Crossover (n=1) | | |

*ARDS = adult onset respiratory distress syndrome, MOF = multiple organ failure. [1]Within 30 days after surgery. [2]Patient refused surgery because of the associated risks, he is still alive and attends the outpatient clinic with persistent episodes of fever. [3]It is not known whether this was done first in all patients; furthermore some of these patients could, unknown to us, have had a later operation in another hospital. [4]E.g. because of lack of possibility for reconstruction. [5]Rifampicine coated. [6]Seven of these patients had persistent fistula or infection of the retroperitoneal remaining part of the prosthesis.*

a combination of diagnostic tests, including white blood cell scans (n=4) and IgG scans (n=3) showing hotspots in the graft region. Of these other tests the endoscopy and ultrasound techniques were useful specifically in subsets of patients.

### Management and outcome

As shown in *table 5*, nearly all cases of enteral bleeding underwent surgery to remove the infected graft. To maintain circulation an extra-anatomic bypass was made. In the two cases of septicaemia without further clinical clues, the same procedure was carried out. The overall survival rate in this group was poor and serious complications occurred (*table 5*).

Patients who presented with a local wound or fistula in the groin were usually first treated by drainage and removal of infected tissue, followed by antibiotics. After a period of time (weeks or months) the removal of the graft was necessary. Patients with an aneurysm were operated in order to treat the aneurysm and to evaluate the graft for infection. Frequently, only a part of the prosthesis was removed and replaced by a rifampicin-coated substitute graft. Furthermore, gentamicin-containing beads were left behind in more than half of the cases, in order to get prolonged local antimicrobial activity. Survival in this group of patients appeared to be better (71% of the patients with an inguinal infected graft (i.e. the late infections) survived). However, a prolonged complication rate was seen in this group (*table 5*). The five patients with an early graft infection (less than three months after implantation) showed a survival rate of only 20%. Culturing of the removed graft revealed predominantly *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas* and *Enterococci species*.

### Predisposing factors

In the 32 cases of aortoiliac en aortofemoral graft infection (*table 6*), none of the supposed risk factors we checked for were present in more than 35% of the patients. Surprisingly, only two out of the 32 patients with a graft infection had diabetes. In our group, five of 32 patients underwent reoperation at the infection site, which can be noted as an acquired risk factor.

## DISCUSSION

This retrospective descriptive study confirms that although the prevalence of central graft infection is low, it is associated with a high morbidity and mortality rate, in this case series of up to 80%. Furthermore, in case of enteral

**Table 6**
*Factors associated with graft infection*

| | AT RISK (N) | NOT AT RISK (N) | PERCENTAGE | UNKNOWN |
|---|---|---|---|---|
| **Preoperative risk factors** | | | | |
| Length of hospital stay before surgery | 7 | 16 | 30 | 9 |
| No perioperative antibiotics given | 0 | 16 | 0 | 16 |
| **Peroperative risk factors** | | | | |
| Acute surgery within 24 hours | 5 | 27 | 16 | 0 |
| Other interventions during surgery[1] | 4 | 23 | 15 | 5 |
| Duration of surgery (>3.5 hours) | 4 | 15 | 21 | 13 |
| Complication during surgery[2] | 8 | 18 | 31 | 6 |
| Body mass index >25 kg/m$^2$ | 4 | 14 | 22 | 14 |
| **Postoperative risk factors** | | | | |
| Complications[3] | 10 | 16 | 38 | 6 |
| Intensive care >5 days | 5 | 18 | 22 | 9 |
| Postoperative wound infection | 8 | 18 | 31 | 6 |
| **Other risk factors** | | | | |
| Diabetes mellitus | 2 | 30 | 6 | 0 |
| Low resistance to infection[4] | 9 | 21 | 12.5-30 | 0 |
| Illness just before the emerging infection | 6 | 26 | 19 | 0 |
| Surgery just before the emerging infection | 11 | 21 | 34 | 0 |

*[1]Embolectomy (twice), profunda replacement (once), replacement of a bifurcation prosthesis instead of an aortic prosthesis placed in the same procedure. [2]Bloodloss (six times), injury of serosa (twice). [3]Thrombosis (twice), bleeding (twice), necrosis of sigmoid, ARDS (twice), metabolic acidosis (once), inguinal abscess (twice). [4]Use of prednisone, recent radiotherapy, paraproteinaemia, malignancy in recent past.*

bleeding and wounds or swelling in the groin, the diagnosis can be made rapidly with endoscopy and CT scan, and this may prevent an extensive diagnostic process, including an invasive guided puncture for culturing.

Although the clinical presentation of graft infection shown in our study is well known from previous studies,[8,10,11-14] the fact that most patients were diagnosed within two days is remarkable and not been mentioned in other studies. The large number of patients presenting with enteral bleeding may explain this, because in these cases a gastroscopy and a CT scan were carried out on the same day, confirming the diagnosis. This vigorous diagnostic process was also observed in patients who presented with an inguinal fistula or spontaneous wound in the groin.

This retrospective study also clearly demonstrates the great diagnostic sensitivity of computed tomography (94%) in other patients with less clear clinical symptoms, comparable with the observations of Orton *et al.*[5] and Low *et al.*[9] Modral and Clagett also advocated the CT scan in diagnosing late aortic graft infections.[1] They recommend duplex ultrasonography in cases of superficial grafts to show perivascular fluid or pseudoaneurysms. The results of our study are similar to these observations. However, this outcome should be interpreted with caution, because of the retrospective character of these studies, with exclusive inclusion of established infections. Patients with an actual graft infection that could not be determined by the diagnostic methods used may have been lost to follow-up, may have died without the exact diagnosis being established or may have been treated for another supposed diagnosis.

The sequence of the variable diagnostic methods used in our patients is comparable with reported algorithms[5] and proves to be practical and appropriate for this diagnosis. Our study does not provide evidence for the necessity of nuclear IgG or bleeding scans in those cases in which a CT scan has already demonstrated a graft infection.[6]

Our study confirms the statement by Orton *et al.*[5] that in patients with upper or lower gastrointestinal bleeding after an aortic graft, a graft to intestine fistula should be excluded by endoscopy. In our series this technique demonstrated a fistula in 55% of the cases, but could at the same time exclude other causes of the bleeding.

The group of patients who were suspected of having a graft infection but not confirmed after diagnostic investigations was too small to draw conclusions about the specificity of the various diagnostic methods. Moreover, it should be taken into consideration that negative cultures of removed grafts may not fully exclude graft infection, since undetectable micro-organisms may be present.

The cultured micro-organisms of removed grafts in our

study are largely comparable with those observed by others.[3,10,11] Bunts *et al.* observed *Staphylococcus aureus* in 43%, *E. coli* in 17%, *Staphylococcus epidermidis* in 14% and *Pseudomonas* in 10% of the cases of graft infections. More recent studies show similar bacterial infections, although coagulase negative *Staphylococcus epidermidis* has now been emphasised as an important cause of aortic graft infections rather than an innocent bystander.

The survival rates observed in our study should be considered with caution, because we only have follow-up data on patients who did not survive and not on those who may still be alive. Moreover, the overall prevalence of infectious complications may be underestimated if not all the graft infections were referred to our hospital.

Our study did not clarify the possible role of risk factors in the development of graft infection. It is known that graft infection may occur haematogenously or per continuum from surrounding tissue.[10] Some theories suppose that micro-organisms may dwell on the graft from the time of implantation and multiply when the condition of the patient deteriorates. Orton *et al.* state that largely sterile abdominal aortic aneurysms yield positive cultures of the intraluminal clot in 8 to 20% of the cases, but despite this, graft infection does not usually occur.[5] Various graft materials have been studied in an attempt to further prevent infections due to vascular surgery. Graft-to-intestine fistulas may be prevented by closing the aneurysmal aortic wall remnant and the peritoneum over the newly inserted graft. However, this procedure is routinely performed in abdominal aneurysm reconstruction, not in bypass surgery. Other well-accepted risk factors are emergency operations, faulty sterile technique, prolonged preoperative hospital stay, extended operation time and reoperation at the site of infection.[10] The fact that no special risk factors were evident in our study does not mean that these factors do not play a role in the developing of a graft infection. Also, the lack of a control group makes it difficult to estimate the influence of such a factor.

Based on the results of this retrospective study, the best ways of dealing with a patient suspected of having an infected aortoiliac or aortofemoral graft are endoscopy in cases of enteral bleeding and CT scanning in all other cases. Both were shown to be very useful techniques for establishing the diagnosis. In general, the clinical outcome and survival after treatment are still poor. To further evaluate the exact merit of various diagnostic procedures and the role of possible risk factors, prospective studies on a larger scale should be performed.

## ACKNOWLEDGEMENT

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# A patient with long-standing skin lesions

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## CASE REPORT

A 64-year-old man presented with progressive, severely painful, nonpruritic, erythematous superficially necrotic and crusted skin lesions, involving the face, especially the perioral region, the groin, perineum and genital region, and the lower extremities (*figure 1* and *2*). The skin lesions had been present for ten years and had not responded to various topical and systemic treatment regimens, including corticosteroids, antibiotics and antifungal medication. He had lost 15 kg in weight during the last three months. Normochromic, normocytic anaemia, hypoalbuminemia and impaired glucose tolerance were present.

## WHAT IS YOUR DIAGNOSIS?

See page 462 for the answer to this photo quiz.

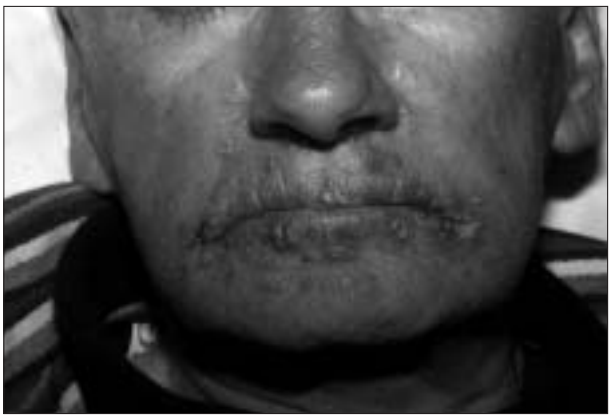

**Figure 1**

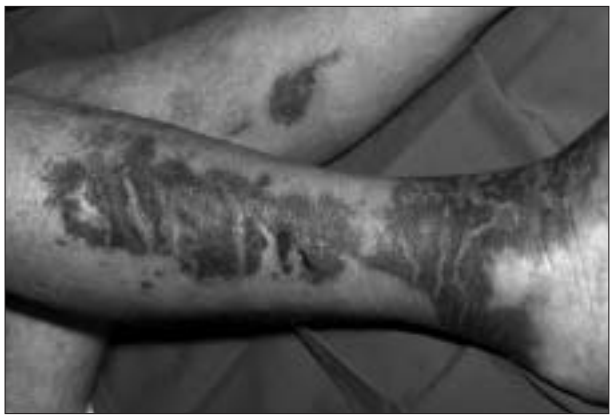

**Figure 2**

**A colour version of this photo quiz can be found on our website www.njmonline.nl.**

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CASE REPORT

# Macronodular adrenocortical hyperplasia in a postmenopausal woman

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## ABSTRACT

**This case report describes the diagnosis of Cushing's syndrome due to macronodular adrenal hyperplasia in an elderly woman who presented with fatigue, muscle weakness and oedema, and recent excessive bruising. Long-standing disease and comorbidity precluded adrenalectomy. Despite treatment with metyrapone and diuretics, the patient died after two months hospitalisation. Postmortal examination revealed overexpression of luteinising hormone (LH) receptors in the adrenal glands, suggesting that the postmenopausal rise in LH may have a role in adrenal hyperplasia and hypercortisolism.**

## CASE REPORT

A 79-year-old woman with a history of ischaemic stroke, pulmonary embolism and a recent deep venous thrombosis of the right leg, was admitted to our hospital because of extensive bruising under anticoagulant use. During the last two years, she had been mentally depressed and walking had been increasingly impaired by fatigue, muscle weakness and leg oedema. Physical examination revealed centripetal adipositas with a buffalo hump (*figure 1*), thoracic kyphosis, muscle atrophy, a thin, easily bruisable skin and generalised oedema. There was no normal diurnal cortisol rhythm, urinary cortisol excretion was high (581 nmol/24 h), a 4 mg overnight dexamethasone suppression test was abnormal (cortisol 535 and 590 nmol/l before and after, respectively) and serum cortisol level was 465 nmol/l after 7 mg dexamethasone intravenously in seven hours. Plasma ACTH levels were repeatedly low (1.2 pmol/l). An abdominal CT scan showed enlarged adrenal glands. Somatostatin receptor scintigraphy, performed in search of ectopic ACTH-producing source(s), showed increased uptake in the pituitary region. Adrenalectomy had to be postponed because of the patient's poor cardiopulmonary condition. Despite treatment with metyrapone and diuretics, she died after two months hospitalisation.

Autopsy revealed huge adrenal glands of 145 and 150 g, containing multiple large nodules (*figure 2*). The tissue showed malignant transformation. This means that on several locations in both adrenals there were broad fibrous bands with a trabecular pattern, areas with a diffuse growth pattern including nests of more compact cells with eosinophilic cytoplasm and vesicular nuclei, with significant cellular polymorphism. Focally there was vascular invasion. In one of the adrenal glands capsular invasion was seen as well as solitary areas with necrosis. The mitotic activity was 3 per 50 high-power fields.

Interestingly, islands of ACTH-positive cells were present in the adrenal glands. Stains for glucose-dependent insulinotropic peptide (GIP), vasopressin, serotonin and β-HCG were negative. However, staining for luteinising hormone (LH) was strongly positive, indicating adrenal expression of LH receptors. The somatostatin scan appeared false-positive due to an olfactory meningioma (diameter 3 cm), which was positive on somatostatin staining. There was no evidence of infection. Taken together, this patient died of Cushing's syndrome due to ACTH-independent macronodular adrenal hyperplasia (AIMAH).

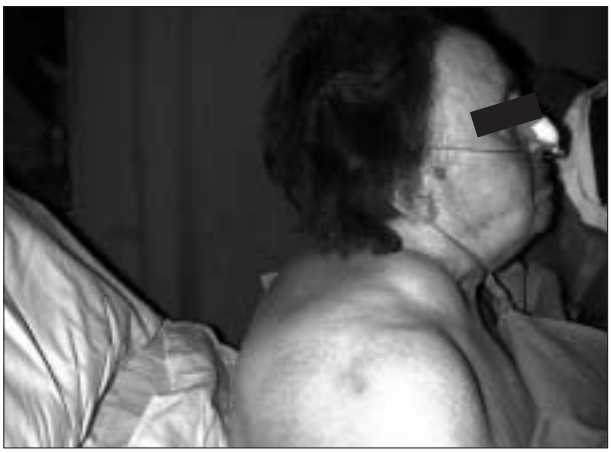

**Figure 1**
*Patient with an apparent buffalo hump and thoracic kyphosis*

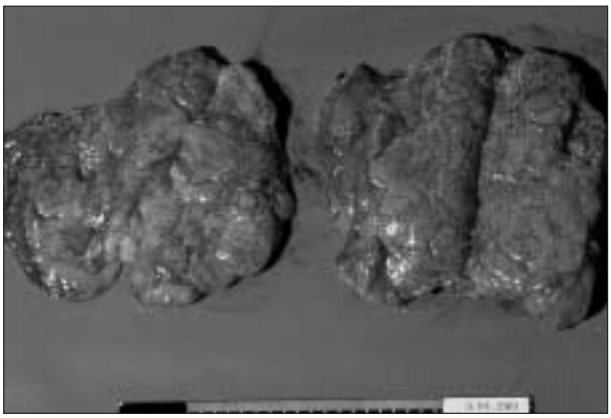

**Figure 2**
*Adrenal glands of the patient on autopsy, weighing 145 and 150 g and containing multiple large nodules, consistent with bilateral macronodular adrenocortical hyperplasia*

## DISCUSSION

Bilateral macronodular hyperplasia is a rare (<1%) cause of Cushing's syndrome. The disease is characterised by enlarged adrenal glands weighing from 24 to >500 g, containing multiple nodules >5 mm, and may be associated with McCune-Albright syndrome. The pathogenesis of AIMAH is unknown. The diagnosis, usually made in elderly patients, is often delayed (from 1 to 20 years). In our case, obvious signs of hypercortisolism were present years before diagnosis. This illustrates that recognition of cortisol excess may be difficult in the elderly, possibly because symptoms are easily considered 'age-related'. Laparoscopic bilateral adrenalectomy is the treatment of choice for AIMAH. However, when long-standing disease and comorbidity preclude immediate surgery in elderly patients, medical treatment with steroidogenesis inhibitors such as metyrapone can be a temporary alternative.

The presence of ACTH-positive cells in the adrenals suggests that intra-adrenal ACTH production may be responsible for the autonomous cortisol production. Two recent cases describe the expression of ACTH receptors and production of ACTH in adrenocortical adenomas and AIMAH,[1,2] regulated by the ACTH precursor proopiomelanocortin (POMC) gene. Locally produced ACTH has paracrine effects on cortisol secretion and adrenocortical cell proliferation, and does not lead to elevation of plasma ACTH.[2] This observation shows that Cushing's syndromes with suppressed plasma ACTH levels may be dependent upon ACTH production within adrenocortical tissue. In this case, the term 'ACTH-independent' is inappropriate.[2] It was recently dertermined that aberrant expression and function of adrenal receptors for various hormones cause the secretion of cortisol in several cases with AIMAH.[3,4] These aberrant receptors include ectopic receptors for gastric inhibitory polypeptide (GIP), LH, HCG or catecholamines and abnormally active eutopic receptors, such as vasopressin V1 and serotonin 5-hydroxytryptamine$_4$ (5-HT4) receptors. In our patient, there was adrenal overexpression of LH receptors. Cortisol secretion can be controlled by LH, as described in three postmenopausal and one premenopausal women with AIMAH.[5-7] Two patients responded to suppression of LH with leuprolide acetate therapy.[5,6] We hypothesise that LH-controlled adrenal hyperplasia might be a process that especially develops after menopause, induced by the rise in serum LH.

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# Leuprolide acetate therapy in LH-dependent Cushing's syndrome: *in vivo* and *in vitro* observations

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## INTRODUCTION

In Cushing's syndrome, there is an excess of cortisol secretion due to hyperfunction of the adrenal cortex. Hypersecretion of cortisol may be ACTH dependent or independent. The latter is present in an adrenal adenoma/carcinoma or bilateral macronodular adrenal hyperplasia. In some of these patients cortisol secretion is driven by stimuli other than ACTH, such as gastric inhibitory polypeptide (GIP), vasopressin, catecholamines or luteinising hormone (LH).[1] LH-responsive Cushing's syndrome was first described by Lacroix *et al.*[2] In their patient *in vivo* investigations demonstrated that cortisol secretion was LH driven, but *in vitro* investigations were not performed.

Recently, *in vitro* studies in two patients with LH-responsive Cushing's syndrome demonstrated LH receptor mRNA in hyperplastic adrenal cells.[3] Definitive proof that cortisol secretion was LH dependent could not be provided, because both patients refused LH-suppressive therapy. We now report another patient with LH-responsive Cushing's syndrome. Our patient responded to GnRH agonist therapy with a profound decrease in 24-hour urinary cortisol excretion, which proves that in this patient cortisol secretion was indeed LH driven. *In vitro* studies demonstrated LH receptor mRNA expression in the adrenal cells.

## CASE REPORT

A 48-year-old woman presented with an 18-month history of fatigue, weight gain of 15 kg, muscular weakness and emotional instability. She reported an absence of menses for 2.5 years. She had had four uncomplicated pregnancies with no signs of transient Cushing's syndrome and no abnormal weight gain. She was not taking any medication. Physical examination revealed a woman with classical clinical features of Cushing's syndrome (hypertension, moonface, buffalo hump, truncal obesity, atrophy of the skin and easy bruising). Endocrine evaluation demonstrated ACTH-independent hypercortisolism. A computed tomography showed bilateral macronodular adrenal hyperplasia. *In vivo* stimulation tests (*table 1*) showed increases in cortisol production after administration of GnRH (Etipharma, Assen, the Netherlands, 100 μg iv), recombinant human LH (rhLH, Serono, Rome, Italy, 300 IU im), and cisapride (Janssen-Cilag, Tilburg, the Netherlands, 10 mg orally). No cortisol response was seen after administration of recombinant human FSH (rhFSH, Organon, Oss, the Netherlands, 300 IU im) and there were no abnormal cortisol responses to meals.

LH-responsive Cushing's syndrome associated with bilateral macronodular adrenal hyperplasia was diagnosed and treat-

**Table 1**

*In vivo cortisol responses to GnRH, rhLH, cisapride and rhFSH (plasma cortisol values are expressed as μmol/l)*

| | BASELINE VALUE | PEAK VALUE |
|---|---|---|
| GnRH (100 μg iv) | 0.54 | 0.77 |
| RhLH (300 IU im) | 0.23 | 1.30 |
| Cisapride (10 mg po) | 0.57 | 2.90 |
| RhFSH (300 IU im) | 0.48 | 0.55 |

ment was started with the GnRH agonist leuprolide acetate, 3.6 mg sc every four weeks. Serum LH levels declined rapidly from high postmenopausal values to lower than the detection limit of the assay after two months. This was accompanied with a decline in 24-hour urinary cortisol excretion, which became normal six months after starting treatment with leuprolide acetate (*figure 1*). However, the complaints and symptoms of Cushing's syndrome only partially disappeared and 24-hour urinary cortisol excretion was again above normal eight months after starting leuprolide acetate. Subsequently the patient underwent a laparoscopic bilateral adrenalectomy. Histological examination of the removed adrenal glands revealed macronodular adrenocortical hyperplasia. The size of the adrenals was 6 cm (right side) and 9 cm (left side).

The results of the *in vitro* studies showed a significant increase in cortisol production after administration of ACTH1-24 and metoclopramide (*table 2*). Remarkably, the response of cortisol to LH was absent *in vitro*.

LH receptor mRNA expression was demonstrated in adrenal tissue although in low concentration (ratio of LH receptor mRNA to γ-actin mRNA was 0.68; this ratio was 0.58 and 1.07 in two control patients, respectively).

**Table 2**

*In vitro cortisol responses of cultured adrenal adenoma cells to GnRH, rhLH, rhFSH, $ACTH_{1-24}$ and metoclopramide (two-hour stimulated cortisol values, expressed as the percentage of control, untreated cells)*

| TEST SUBSTANCE | | CORTISOL VALUE (% CONTROL) |
|---|---|---|
| Control | | 100 ± 8 |
| GnRH | 10 nM | 92 ± 7 |
| | 100 nM | 96 ± 1 |
| RhLH | 10 mIU/ml | 99 ± 7 |
| | 50 mIU/ml | 100 ± 10 |
| | 100 mIU/ml | 95 ± 3 |
| | 1000 mIU/ml | 100 ± 16 |
| RhFSH | 10 mIU/ml | 87 ± 2 |
| | 50 mIU/ml | 92 ± 10 |
| | 100 mIU/ml | 90 ± 11 |
| | 1000 mIU/ml | 101 ± 6 |
| $ACTH_{1-24}$ | 16 pg/ml | 227 ± 4° |
| | 32 pg/ml | 267 ± 31° |
| | 64 pg/ml | 275 ± 32° |
| | 128 pg/ml | 310 ± 42° |
| | 615 pg/ml | 400 ± 30° |
| Metoclopramide | 0.1 μM | 184 ± 20[a] |
| | 1 μM | 372 ± 24° |
| | 10 μM | 454 ± 8° |

*Data are expressed as mean ( n = 3) ± SD; [a]p<0.01 vs control, °p<0.001 vs control. For methods see Feelders et al.[3]*

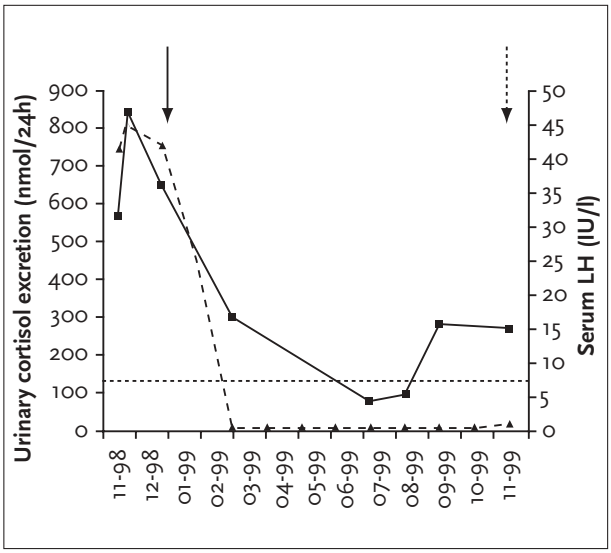


**Figure 1**

*Urinary cortisol excretion ( ▪ ) and serum LH levels ( ▲ ) before and during treatment with leuprolide acetate (3.6 mg sc every 4 weeks)*

**The first injection was given on 14 January 1999 (*arrow*) and the last one on 4 November 1999. Bilateral adrenalectomy was performed on 26 November 1999 (*dashed arrow*). The horizontal dashed line represents the upper limit of normal for 24-hour urinary cortisol excretion.**

## DISCUSSION

In patients with ACTH-independent Cushing's syndrome due to an adrenal adenoma/carcinoma or bilateral macronodular adrenal hyperplasia, cortisol secretion can be stimulated by hormones other than ACTH due to aberrant expression of one or more G-protein-coupled receptors.[1] Until now such aberrant stimulation of cortisol has been demonstrated for GIP, vasopressin, 5 hydroxytryptamine (5HT7 receptor), β-adrenergic receptor agonists and angiotensin II. Increased expression of eutopic receptors can also cause cortisol hypersecretion; this mechanism explains the stimulation of cortisol secretion in patients with ACTH-independent Cushing's syndrome by vasopressin, LH/human chorionic gonadotropin (HCG), 5 hydroxytryptamine (5HT4 receptor) and leptin.[1]

In our patient with LH-dependent Cushing's syndrome we demonstrated, in line with the data of Lacroix *et al.*,[2] that suppression of enhanced LH levels *in vivo* by leuprolide acetate led to suppression of cortisol levels. We can not explain the unexpected absence of a response of cortisol to LH *in vitro*. We hypothesise that stimulation of LH receptors needs, besides LH, a cofactor that is lacking *in vitro*. Furthermore, in our patient adrenalectomy was performed

after seven months of treatment with leuprolide acetate, which may have influenced the *in vitro* results, if suppression of LH levels causes downregulation of LH receptor expression. In our patient *in vitro* investigations demonstrated a cortisol response to ACTH and metoclopramide (that exerts its action via the 5HT4 receptor), in line with the results of Feelders *et al.*[3]

Only very few patients with LH-responsive Cushing's syndrome and ACTH-independent macronodular adrenal hyperplasia have been described so far. In the first patient, described by Lacroix *et al.*,[2] administration of the long-acting gonadotropin-releasing hormone (GnRH) analogue leuprolide acetate led to suppression of endogenous LH and normalisation of cortisol production. In this patient cisapride and metoclopramide also stimulated plasma cortisol. Feelders *et al.*[3] described two women with LH-responsive Cushing's syndrome and ACTH-independent macronodular adrenal hyperplasia. Both patients had aberrant responses to GnRH, LH, hCG, cisapride and metoclopramide. mRNA encoding the LH receptor was slightly higher in the macronodular adrenals from these patients than in normal adrenals.

In our patient with ACTH-independent macronodular adrenal hyperplasia and Cushing's syndrome with aberrant responses to GnRH, LH and cisapride *in vivo* and to metoclopramide *in vitro* suppression of endogenous LH with leuprolide acetate only partially improved hypercortisolism. This may be due to persistent aberrant stimulation of cortisol secretion via 5HT4 receptors, but may also indicate the presence of either other, unidentified, aberrant receptors or another mechanism that regulates cortisol secretion. Many questions with respect to Cushing's syndrome variants secondary to aberrant hormone receptors remain unanswered. Nevertheless, the phenomenon of cortisol stimulation via receptors other than the ACTH receptor opens new possibilities to treatment of ACTH-independent Cushing's syndrome.

## ACKNOWLEDGEMENTS

Dr A.P.N. Themmen, Erasmus Medical Centre, Rotterdam, the Netherlands, is gratefully acknowledged for the LH receptor mRNA analyses.

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CASE REPORT

# HIV infection presenting with duodenal tuberculosis

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## ABSTRACT

**Extrapulmonary tuberculosis is a protean and often difficult to recognise infection. Gastrointestinal tuberculosis is a rare condition that mainly occurs in immunodeficient people. We report a case of duodenal tuberculosis, which presented with gastrointestinal symptoms, anaemia and hyponatraemia, in a patient with previously undiagnosed HIV infection.**

## INTRODUCTION

Tuberculosis, one of the oldest infections known to affect humans, is today still one of the leading causes of death globally. The disease occurs worldwide, although it is found primarily in developing countries, where poor sanitation contributes to its spread.[1,2] Pulmonary manifestations predominate in most tuberculosis cases, yet in up to one-third of patients other organs are affected. Gastrointestinal involvement may be present as a part of the multiorgan disease process or, less commonly, as primary gastrointestinal tuberculosis. We report a patient, with previously undiagnosed HIV infection, who presented with anaemia and duodenal tuberculosis.

## CASE REPORT

A 23-year-old Nigerian male presented with a four-week history of fever, malaise, nausea, vomiting, anorexia and a 15 kg weight loss. His past medical record was unremarkable except for malaria. He was cachectic but not acutely ill, temperature was 38.3°C, and lymph nodes (Ø 2 cm) were felt in both sides of his neck, while the rest of the examination was normal. Blood analysis showed albumin 1.8 g/dl, lactic dehydrogenase 728 IU/l, gamma-glutamyl transpeptidase 100 IU/l, alkaline phosphatase 799 IU/l, C-reactive protein 126 mg/dl, IgG 3604 mg/dl, IgM 770 mg/dl, iron 23 µg/dl, total iron binding capacity 495 µg/dl, transferrin saturation 9%, ferritin 1317 ng/ml, haemoglobin 8.4 g/dl, mean corpuscular volume 76 fl, erythrocyte sedimentation rate 120 mm in the first hour, D-dimer 622 ng/ml, sodium 120 mEq/l, and osmolality 280 mOsm/kg, while all other results, including haemoglobin electrophoresis, haptoglobin, reticulocyte count, vitamin B12, folic acid, and thyroid hormones, were normal. Urine analysis disclosed abundant red blood cells, sodium 31 mEq/l, and osmolality 369 mOsm/kg, while all other results were normal.

The electrolyte disorder was classified as the syndrome of inappropriate ADH and the patient was treated with water restriction. Cultures of blood and urine, acid-fast bacilli of sputum, and serology of multiple infections were negative, except for anti-HIV, ELISA and *Western blot*, which were positive. CD4 cell count was 159 per $mm^3$, and HIV RNA was 100,000 copies/ml. An electrocardiogram showed no abnormalities. Chest radiographs revealed a tenuous left lower lobe infiltrate. An upper gastrointestinal endoscopy demonstrated severe inflammation and ulceration of the mucosa situated just distal to the duodenal bulb, with the presence of a mucosal bridge (*figure 1*); a biopsy of the affected area revealed a granulomatous and necrotic inflammatory infiltrate, as well as the presence of acid-fast bacilli. Abdominal ultrasound and computed tomography exam-

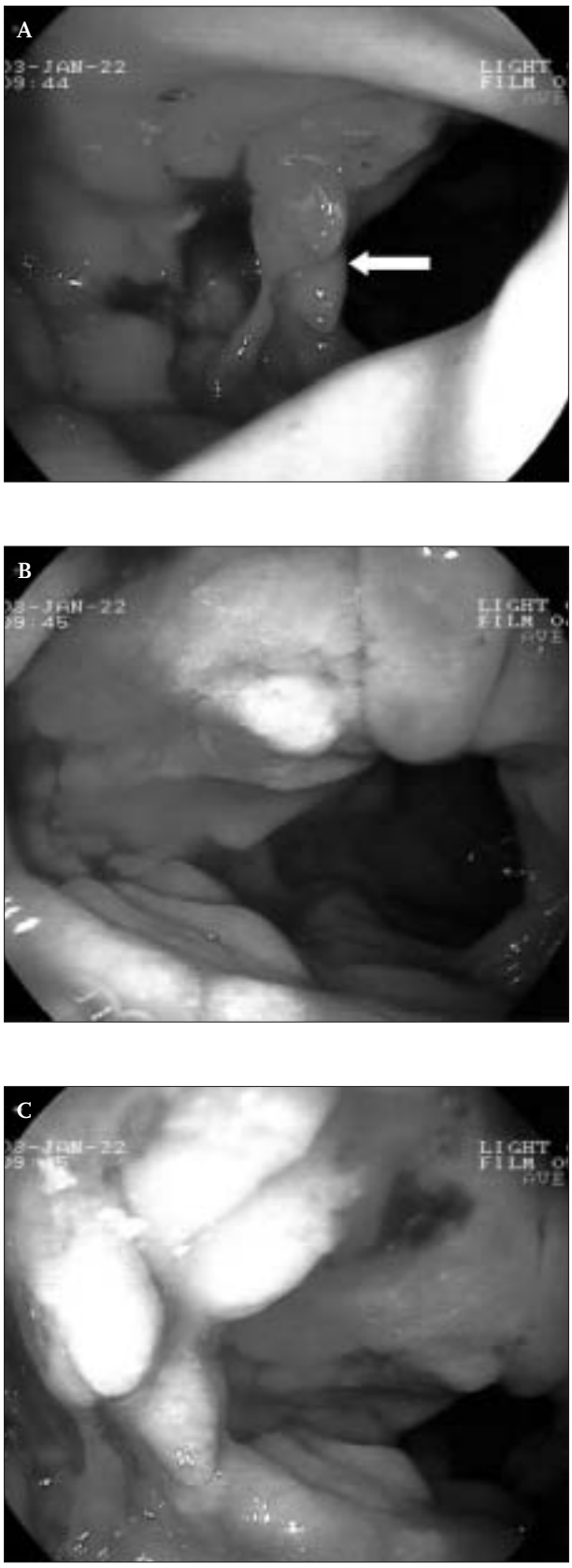


**Figure 1**
*Inflammation and ulceration of the mucosa distal to the duodenal bulb, resembling Crohn's disease, with the presence of a mucosal bridge (arrow in A)*

inations showed multiple lymphadenopathies. A lymph node biopsy revealed a granulomatous and necrotic infiltrate. Suspecting tuberculosis, treatment was instituted with isoniazid, rifampin, pyrazinamide and ethambutol, and the patient's condition slowly improved over the next few weeks. By the second week of therapy *Mycobacterium tuberculosis* grew in the sputum. When the patient had completed eight weeks of antituberculous treatment, antiretroviral therapy was initiated, isoniazid and rifampin were continued, and pyrazinamide and ethambutol were withdrawn. At that time he was completely asymptomatic. He received no corticosteroid therapy.

## DISCUSSION

In this paper we report the duodenal manifestations of tuberculosis in an HIV-positive African patient. Although fever and wasting in a patient of African descent always points in the direction of tuberculosis, a duodenal manifestation was not suspected clinically.

Tuberculosis usually affects the lungs, although extrapulmonary tuberculosis is also prevalent, especially among people with HIV infection.[3] The extrapulmonary sites most commonly involved are the lymph nodes, pleura, genitourinary tract, bones and joints, meninges, and peritoneum. However, virtually all organ systems may be implicated.

The gastrointestinal tract is only rarely affected. Prior to the HIV epidemic, it was seen most commonly in immunecompetent persons with untreated advanced pulmonary disease.[4] Today, it is most commonly observed in association with immunosuppression and, in one series, more than 40% of patients with gastrointestinal tuberculosis had AIDS.[5] Swallowing of sputum with direct seeding and haematogenous spread are the main pathogenetic mechanisms. Any portion of the gastrointestinal tract may be involved, although the terminal ileum and caecum are the most commonly affected sites. Abdominal pain, diarrhoea, obstruction, haematochezia and a palpable mass are common findings at presentation. Fever, weight loss and night sweats are also frequent.[6,7]

Duodenal tuberculosis generally presents with obstructive symptoms. Other nonspecific upper gastrointestinal complaints are also common. The differential diagnosis includes cancer, other infections, and chronic inflammatory conditions. Crohn's disease and intestinal tuberculosis may closely resemble each other, not only macroscopically, but also microscopically. Because the management of both conditions is so different, it is critical to distinguish them, generally with a biopsy of the affected mucosa.[8-10]

Our case of duodenal tuberculosis is another example of close similarity of such infection with inflammatory bowel

disease. Probably, as a result of the inflammatory process, our patient developed a mucosal bridge, a finding that has also been described in Crohn's disease.[11]
Also remarkable in the reported patient is the presentation with severe hyponatraemia, probably resulting from inappropriate secretion of antidiuretic hormone (SIADH), a rare initial presentation of tuberculosis.[12,13] The described case serves as an illustration of the protean and difficult to recognise presentations that tuberculosis may adopt.

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## ANSWER TO PHOTO QUIZ (ON PAGE 453)
## A PATIENT WITH LONG-STANDING SKIN LESIONS

The picture is classical for necrolytic migratory erythema (NME), a hallmark of the glucagonoma syndrome. NME is a skin rash that usually begins in the groin and perineum and may also involve the lower extremities and the perioral area. The exact aetiology of the skin rash is unknown but elevated plasma glucagon levels as well as deficiencies of zinc, amino acids and fatty acids may be involved.[1,2] A severely increased serum glucagon concentration of 380 pmol/l (reference value below 23 pmol/l) was found in our patient. The computed tomography (*figure 3*) showed a large tumour involving the pancreas and the left adrenal gland with calcifications as well as several hepatic lesions suspect for metastases. Somatostatin receptor scintigraphy showed concentration of radiolabelled somatostatin in the pancreas and left adrenal region as well as in the liver. A diagnosis of metastasised glucagonoma was made.

When the tumour is small and limited to the pancreas at the time of diagnosis, radical surgery is possible. However, in the setting of metastatic disease, the treatment is mainly symptomatic and consists primarily of administration of somatostatin analogues, by reducing the levels of circulating glucagon via SST2 receptors, improving the skin lesions and promoting weight gain. However, its effects on tumour growth are often modest. The skin rash may also respond to nutrient supplementation.[3] In our patient treatment was initiated with octreotide and nutritional support. After one month of treatment the skin lesions improved and were no longer painful, and patient regained weight.

Early diagnosis is crucial in case of glucagonoma, in view of the malignant course of the disease. The symptomatology is subtle or aspecific. NME often represents the first sign of disease, but unfortunately many years can sometimes elapse after the presentation of the skin lesions before the diagnosis is made. Thus recognising NME and its association with a glucagonoma is essential.

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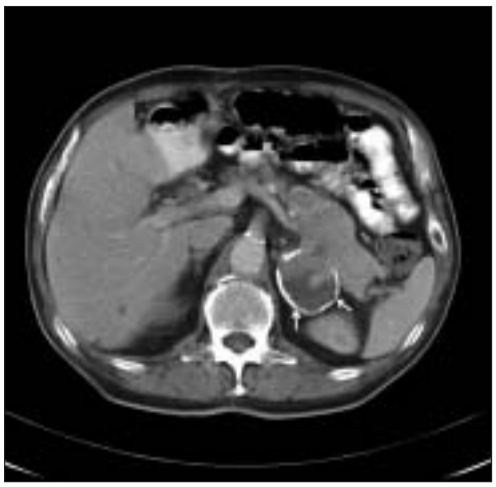

**Figure 3**
*Computed tomography showing the large tumour involving the pancreas and the left adrenal gland (arrow) and lesions in the liver*

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ABOUT THE COVER

# 'Untitled'

**Marion Rutten**

Marion Rutten studied Graphic Arts at several academies. After starting her education in Arnhem, she moved to The Hague where she attended the Royal Academy of Arts. During her studies in The Hague she went to the Norwich school of Art and Design in the UK for a few months.

Although Rutten is in the first phase of her artistic development, one can recognise the power in her sturdy, large prints. Her work shows her talent for drawing coupled with a great expressivity.

By choosing legs as her subject, she enables herself to express movement and balance. The ultimate goal is to distance ourselves from the physically recognisable in her work, and to build a figure only in marking the contours. This technique is similar to the polarisation filter photography of Man Ray.

Rutten exhibits her work in solo as well as in group expositions in our country and abroad. You can order this month's print (20 x 25 cm) (limited edition of 5) at a price of € 175 at Galerie Unita, Rijksstraatweg 109, 6573 CK Beek-Ubbergen, the Netherlands, e-mail: galerie-unita@planet.nl or on our website: www.galerie-unita.com.

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2. Kaplan NM. Clinical Hypertension. 7th ed. Baltimore: Williams & Wilkins; 1998.
3. Powell LW, Isselbacher KJ. Hemochromatosis. In: Braunwald E, Fauci AS, Kasper DL, et al., editors. Harrison's Principles of Internal Medicine. 15th edition. New York: McGraw-Hill; 2001. p. 2257-61.

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