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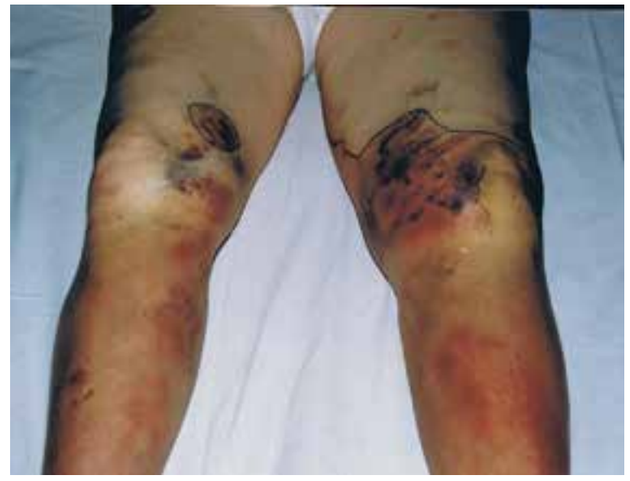

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EDITORIAL

Kidney transplantation in atypical haemolytic uraemic syndrome (aHUS): a cheap way out?

F.J. Bemelman*, I.J.M. ten Berge

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The C5 monoclonal antibody eculizumab is one of the newer biologicals that are the subject of many exciting discussions, not only because of their extraordinary high price, but also because of their high therapeutic potential in diseases in which uncontrolled activation of the complement system plays a central role. As a consequence, the list of difficult to treat inflammatory diseases eligible for treatment with this monoclonal antibody is steadily increasing. Examples are (catastrophic) anti-phospholipid syndrome, the HELLP syndrome, membranoproliferative glomerulonephritis type II, humoral transplant rejection and haemolytic uraemic syndrome (HUS).

HUS is a rare disease, characterised by the occurrence of haemolytic anaemia, thrombocytopenia and acute renal failure, rapidly progressing to end-stage renal disease (ESRD). Histological examination of the kidney reveals, amongst other things, activation and damage of glomerular endothelial cells and consequent thrombotic micro-angiopathy (TMA). Glomerular tufts are destroyed and kidney failure ensues.

In 90% of cases, the disease is caused by a *Shiga* toxin-producing *E. coli* infection. In the remaining cases a dysregulation of the complement system underlies the pathogenesis. Different causes have been discovered: mutations in genes encoding regulatory proteins of the alternative complement activation pathway, such as complement factor H (CFH), complement factor I (CFI), membrane cofactor protein (MCP) or mutations in genes encoding the two proteins of C3 convertase: complement factor B (CFB) or C3; polymorphism in genes involved in complement inhibition; or autoantibodies directed against CFH.[1] Several data point to a second hit: viral infections, some drugs or – in case of a transplanted patient – the occurrence of rejection may all trigger the disease.[2-4]

In general, kidney transplantation offers the best solution for patients with ESRD in terms of morbidity and mortality as compared with other forms of renal replacement therapy. However, for patients with aHUS, kidney transplantation may not be so favourable because the chance of recurrence in the graft may be as high as 80% with an equally high chance of graft loss, depending on the causative mutation.[4,5] Most recurrences occur in the first year after transplantation and are difficult to diagnose before irreversible damage to the graft has occurred, since it is not uncommon that the TMA is confined to the graft.[6-8] Risk factors for recurrence are the type of causative mutation, factors that are harmful for endothelial cells and/or triggers of complement activation which are usually present following transplantation: ischaemia-reperfusion injury, rejection, infection, hypertension and the use of certain drugs, such as calcineurin-inhibitors or mTOR-inhibitors.

Until the introduction of eculizumab, treatment of (recurrent) aHUS consisted of plasmapheresis, which offers a burden to the patient, since after the initial daily treatments, it has to be performed twice to three times a week. Thus, despite transplantation, the patient remains dependent on a haemodialysis-like treatment with all the inherent disadvantages. More importantly, this treatment has limited success, depending on the underlying abnormality. About two-thirds of patients with mutations in genes for factor H, factor I or C3 may respond to treatment with plasmapheresis. In contrast, in patients with MCP deficiency, no differences in remission rates with or without plasmapheresis were shown.[2] In that respect, eculizumab did indeed appear to be a miracle drug, being very effective and making the patient independent of the hospital.[9-12] Its mode of action relies on binding to the complement protein C5, by which cleavage into C5a and C5b is prevented. Blocking the formation of C5b inhibits the subsequent formation of terminal complex C5b-9 (membrane attack complex, MAC). By that, complement-mediated injury is very effectively inhibited. Issues as optimal dosing and length of treatment of

eculizumab are still unanswered. However, at around € 400,000 per patient per year, the drug is prohibitively expensive.

In this issue of the Netherlands Journal of Medicine, Verhave *et al.* describe a strategy to avoid the use of eculizumab following kidney transplantation in aHUS. They aimed to prevent or postpone recurrence of aHUS by a treatment protocol that minimises endothelial damage following kidney transplantation. They accepted only living donors in order to minimise cold ischaemia time and ischaemia-reperfusion injury. In addition, they used low-dose calcineurin inhibition in order to avoid additional insults to the endothelium. Finally, the authors achieved regulation of blood pressure and lipids by treatment with RAAS inhibitors and statins, which may prevent endothelial damage.[13] With a follow-up of 16-21 months, this strategy was successful in four consecutive patients, which is more than might be expected by chance.

If confirmed, these findings offer hope for a selected group of aHUS patients. The data are important because they show that the new drug eculizumab is not always necessary to prevent recurrence of aHUS following kidney transplantation, let alone that every patient would have to be treated prophylactically, as has recently been advocated.[11,14] However, it is unlikely that triggers of complement activation can be completely eliminated in all patients. Rejection occurs in 10-15% of recipients with the best available immunosuppressive drug regimens and may occur beyond the first year after transplantation.[15,16] Rejection incidence may be even higher when specific risk factors such as allo-immunisation, as may occur after pregnancy, transfusion or previous organ transplantation, are present. None of the patients in the report by Verhave *et al.* were immunised; none suffered from a rejection episode. Another known risk factor for the occurrence of aHUS is infection. In that respect, cytomegalovirus (CMV) is in a particular position: first, because it frequently reactivates under immunosuppression and second, because endothelial cells are activated and damaged by CMV infection.[17] Two of the four patients described by Verhave *et al.* were not at risk for CMV infection. Whether CMV reactivation occurred in the other two patients is not mentioned.

Currently, patients with aHUS have to be screened for abnormalities in the complement system before placing them on the waiting list for transplantation, in order to help in predicting their prognosis. When a living related donor is available, this donor should not possess a similar genetic abnormality. This is because it could trigger development of aHUS in the donor himself because of uncontrolled complement activation during the operation procedure.[18] The choice for a living donor to shorten cold ischaemia time and to minimise the risk of endothelial activation should be balanced against the high risk of allograft loss by recurrence of aHUS. Any decision in this respect should be carefully discussed with the individual recipient and the donor in question. When it is decided to wait for a postmortal donor, the use of non-heart-beating donors should be avoided because of the expected long cold ischaemia time and increased risk of ischaemia-reperfusion injury.

Regarding choice of immunosuppressive drug regimen, Verhave *et al.* propose to use calcineurin inhibitors (CNI) in such a dose that rejection episodes can be avoided on the one hand, while minimising endothelial activation on the other hand. Indeed, Artz *et al.* showed that initial use of cyclosporine as part of the immunosuppressive regimen significantly increased the risk of recurrence.[4] However, a recent study by Le Quintrec *et al.* failed to demonstrate a significant relationship between CNI therapy and recurrence of aHUS.[19] In contrast, they showed a significant association between use of mTOR inhibitors, known to induce endothelial cell activation,[20] with the risk of recurrence of aHUS. In view of the demonstrated adverse effects of CNI in some clinical studies[4,21,22] and their known detrimental effects on endothelial cells *in vitro*,[23] the policy as proposed by Verhave *et al.* to use low dosages seems meaningful.

Verhave's suggestion to set up a clinical trial studying the most optimal treatment strategy in patients with aHUS who receive a kidney transplant is to be welcomed. However, such a trial would probably require many more patients than are available, even in a multicentre design. Moreover, in order to get useful answers, the patients should be classified according to their genetic abnormality, corresponding severity of the disease and risk of recurrence. In the meantime, an endothelium-protective approach, i.e. selection of a living donor and avoidance of both triggers of complement activation and drugs potentially activating endothelial cells, is indeed worth following.

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REVIEW

Living kidney transplantation in adult patients with atypical haemolytic uraemic syndrome

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ABSTRACT

Background: Dysregulation of complement activation is the most common cause of the atypical haemolytic uraemic syndrome (aHUS). Many patients with aHUS develop end-stage renal disease and consider kidney transplantation. However, the recurrence rate after transplantation ranges from 45-90% in patients with known abnormalities in circulating complement proteins. It was recently proposed that patients with aHUS should be treated prophylactically with plasma exchange or eculizumab to prevent recurrence after transplantation.

Methods: A case series describing the successful outcome of kidney transplantation *without prophylactic therapy* in four adult patients with aHUS and a high risk of disease recurrence. Patients received a living donor kidney and immunosuppression consisting of basiliximab induction, low-dose tacrolimus, prednisone and mycophenolate mofetil. Patients received a statin, and were targeted to a low blood pressure preferably using blockers of the renin-angiotensin system.

Results: After a follow-up of 16-21 months, none of the patients developed recurrent aHUS. Also, no rejection was observed.

Conclusions: Kidney transplantation in adult patients with aHUS can be successful without prophylactic eculizumab, using a protocol that minimises cold ischaemia time, reduces the risk of rejection and provides endothelial protection. Our data suggest that in patients with aHUS, controlled trials are needed to demonstrate the optimal strategy.

KEYWORDS

Atypical haemolytic uraemic syndrome, eculizumab, endothelial activation, kidney transplantation

INTRODUCTION

Haemolysis with fragmented erythrocytes, thrombocytopenia and acute renal failure are the hallmark of the haemolytic uraemic syndrome (HUS), and evidence of severe microvascular damage. Diarrhoea-associated HUS occurs mainly in children, and is caused by Shiga-like toxin producing *Escherichia coli*. In adult patients there is seldom an association with *E. coli* infection, and the term atypical HUS (aHUS) was coined. Recent studies have documented a mutation in proteins involved in complement C3 and complement regulatory proteins (factor H (CFH), factor I (CFI), factor B (CFB), and membrane cofactor protein (MCP)) in more than 50% of the patients. These genetic abnormalities result in unopposed and excessive activation of the complement system leading to endothelial damage. Atypical HUS is a serious disease, with a mortality rate of 25%. The kidneys are most frequently involved, and kidney injury is usually progressive with more than 50% of patients with aHUS developing end-stage renal disease (ESRD). Kidney transplantation is considered the best treatment modality for patients with ESRD. However, patients with aHUS who undergo kidney transplantation have a poor prognosis. They are at high risk for recurrence of aHUS, which is uniformly associated with graft loss.[1,2] Especially patients with a documented mutation in complement regulatory proteins (with the exception of MCP) have a risk of recurrence after kidney transplantation that ranges from 45-90%.[3-5] This process of recurrent aHUS was difficult to treat. Although plasmapheresis and plasma infusion have been used successfully in some patients with aHUS[6,7] many patients did not respond (resistant) or needed continued weekly treatment (plasma-dependent). Prophylactic plasma therapy in kidney transplantation seemed to reduce the risk of aHUS recurrence, however without reaching statistical significance in a retrospective

study.[3] Based on these data, patients with aHUS are often not offered kidney transplantation and remain on dialysis therapy for the rest of their life. The introduction of eculizumab in clinical practice has offered hope for patients with aHUS, particularly for patients who are awaiting a kidney transplantation. Many case reports have documented response to eculizumab in patients with recurrent aHUS after transplantation.[8-10] Also, many patients have been transplanted successfully with eculizumab given prophylactically. This experience has dramatically changed the prognosis of patients with aHUS. Experts now recommend prophylactic treatment with eculizumab for all patients with aHUS who are at medium or high risk for disease recurrence after transplantation.[11] Also, treatment should be continued for at least 12 months (in medium-risk patients) or even lifelong (in high-risk patients).

The use of eculizumab is associated with enormous costs and limiting the use of eculizumab thus becomes utterly relevant. Moreover, in the Netherlands there is still uncertainty as to whether treatment with eculizumab is reimbursed. However, it is unclear if kidney transplantation without eculizumab is a reasonable approach.

Although mutations in complement regulatory proteins are important in the pathogenesis of aHUS, other factors must also contribute since many persons carrying a disease-causing mutation never develop aHUS. Other factors include polymorphisms in complement regulatory proteins or exogenous factors, particularly factors that either induce complement activation or cause endothelial cell injury. Such factors are always present in transplanted patients and include donor graft injury (related to the donor procurement, cold ischaemia time, and reperfusion injury), acute rejection, use of calcineurin inhibitors (CNI), and hypertension- and lipid-mediated vascular injury. Although not tested in clinical trials, protocols that reduce endothelial injury at the time of transplantation and use *rescue* eculizumab therapy in case of active, recurrent disease could be an acceptable solution for renal transplantation in patients with aHUS. We preferred transplantation with kidneys from a living donor, because it limits endothelial damage. We are aware that living donor transplantation in aHUS is usually not performed because of the high recurrence risk and the potential of complement mutation in the living related donor. We hypothesised that a protocol based on living kidney transplantation (which reduces ischaemia time) might allow successful kidney transplantation whilst limiting the use (and thus costs) of eculizumab. The aim of the study was to test if recurrence of aHUS can be prevented or postponed by using a protocol that minimises endothelial damage as much as possible. We describe our experience in four patients.

MATERIALS AND METHODS

Transplantation characteristics

In our centre approximately 110 kidney transplantations are performed each year. Currently, we use triple therapy consisting of tacrolimus 0.1 mg/kg twice daily, prednisone and mycophenolate 1000 mg twice daily as standard immunosuppression. We previously evaluated the outcome of transplantation in aHUS patients[12] and observed both a high recurrence rate as well as a high incidence of especially acute vascular rejections in aHUS patients compared with other patients after renal transplantation. Also, we noted that the use of a standard dose of cyclosporine was related to the development of a recurrence. Overall outcome was dismal. All the above were reasons to temporarily stop transplanting aHUS patients with a high risk of recurrence.

The availability of eculizumab as rescue therapy allowed us to reconsider kidney transplantation in patients with aHUS. Living kidney donors were considered acceptable, and based on our previous experience and theoretical considerations regarding endothelial damage, we developed a protocol directed at both minimisation of risk for rejection by using quadruple immunosuppression with basiliximab induction and low-dose tacrolimus as well as reduction of risk for endothelial injury by preventing exposure to high levels of CNI, aggressive blood pressure lowering and use of statins. Steroids were not withdrawn. Details are given in *box 1* and *figure S1* (supplementary appendix, see http://www.njmonline.nl/all_issues.php).

Box 1. *Kidney transplantation protocol minimising recurrence of aHUS*

Treatment	Goal
Living donor kidney	Minimise endothelial injury
Basiliximab: 20 mg day 1 and 4	Reduce risk of rejection, limit CNI toxicity
Tacrolimus (low dose): 0.03 mg/kg BID, trough level 5 ng/ml	
Mycophenolate mofetil: 1000 mg BID, AUC 40-60 mg/l/h	
Prednisone: Starting at 100 mg day 1-3, thereafter 25 mg/day and taper to 0.1 mg/kg/day at month 3 after transplantation	
Statin	Endothelial protection
Blood pressure target <130/80 mmHg	Endothelial protection
Calcium channel blocker	Limit CNI vasoconstriction
ACE inhibitor	Limit AII toxicity
Close patient monitoring (first three months twice weekly, second three months weekly, than 2-weekly)	Early detection of signs of recurrence aHUS

When compared with our old treatment schedule, the following differences are notable: use of living donor (vs deceased donor), use of basiliximab induction therapy (vs no induction therapy), use of low-dose tacrolimus (vs usual dose cyclosporine), and early start of ACE-inhibitors and statins.

Since 2011 this protocol has been implemented in our hospital. We contacted and screened aHUS patients linked to our centre (n=15) who would be potential transplantation candidates. We transplanted the four for which a living donor was available. While waiting for the results of transplantation in these patients, we decided not to perform transplantations with deceased donor kidneys. As per standard care all patients were informed of the risk of recurrent aHUS. Patients and related donors were aware that in case of disease recurrence treatment would consist of plasmapheresis and eculizumab.

Mutation screening

Mutational screening was performed in the coding regions of the alternative pathway genes complement factor H (CFH), complement factor I (CFI), MCP, C3, and complement factor B (CFB) by means of PCR on genomic DNA and sequence analysis. In addition, we documented the presence of three SNPs in the CFHtgt haplotype (rs3753394 [c.-331C>T], rs3753396 [c.2016A>G; p.Gln672Gln], and rs1065489 [c.2808G>T; p.Glu936Asp])[13] and the MCPggaac haplotype (rs2796267 [c.-652A>G], rs2796268 [c.-366A>G], rs1962149 [c.989-78G>A], rs859705 [c.1127+638G>A], and rs7144 [c.2232C>T]),[14] which are considered high-risk polymorphisms (*table 1*). One patient was screened before transplantation for the presence of autoantibodies against CFH, which was performed as described before.[15]

Potential living related donors were also evaluated for the presence of disease-causing mutations. A living related donor was only accepted for transplantation if a disease-causing mutation was only identified in the recipient and not in the donor.

Table 1. *Patient characteristics*

Patient	Sex	Age primary aHUS episode (years)	Low serum complement C3*	Complement mutation	Presence of three CFH_{tgtgt} haplotype SNPs**	Presence of MCP_{ggaac} haplotype***	Previous Transplant/ Age	Outcome first Tx
A	F	23	Yes	C3 p.Arg161Trp C3 p.Glu1258Ala	rs3753394: heterozygous rs3753396: heterozygous rs1065489: heterozygous	rs2796267: heterozygous rs2796268: heterozygous rs1962149: heterozygous rs859705: heterozygous rs7144: heterozygous	NA	-
B	F	20	No	CFH p.Arg1210Cys	rs3753394: heterozygous rs3753396: heterozygous rs1065489: heterozygous	rs2796267: homozygous rs2796268: homozygous rs1962149: homozygous rs859705: homozygous rs7144: homozygous	LRD/27 years	Recurrent aHUS within 3 months
C	F	50	No	C3 p.Arg161Trp	rs3753394: heterozygous rs3753396: heterozygous rs1065489: heterozygous	rs2796267: heterozygous rs2796268: homozygous rs1962149: homozygous rs859705: homozygous rs7144: homozygous	NA	-
D	M	38	Yes	C3 p.Lys65Gln	rs3753394: not present rs3753396: heterozygous rs1065489: not present	rs2796267: heterozygous rs2796268: heterozygous rs1962149: heterozygous rs859705: heterozygous rs7144: heterozygous	LRD/40 years	Recurrent aHUS within 6 months

CFH = factor H; LRD = living related donor; NA = not applicable. All patients were tested for mutations in CFH, CFI, MCP, C3 and CFB.
***C3 < 750 ng/ml. ****Screened polymorphisms in CFHtgt at-risk haplotype: rs3753394 [c.-331C>T], rs3753396 [c.2016A>G; p.Gln672Gln], and rs1065489 [c.2808G>T; p.Glu936Asp]. *****Screened polymorphisms in MCPggaac haplotype: rs2796267 [c.-652A>G], rs2796268 [c.-366A>G], rs1962149 [c.989-78G>A], rs859705 [c.1127+638G>A], and rs7144 [c.2232C>T].**

RESULTS

In 2011, we transplanted four patients with aHUS using the described protocol. In *table 1* the patient characteristics are presented and in *table 2* the transplantation characteristics. The individual patient characteristics were as follows.

Patient A is a 35-year-old female. In 1999 she was hospitalised with acute kidney failure, accelerated hypertension complicated by an epileptic seizure, and evidence of haemolysis and thrombocytopenia. Family history revealed aHUS in a second cousin on her father's side. Treatment with plasma exchange and high doses of prednisone was without apparent success, although after one year of haemodialysis her kidney function recovered partially (eGFR 15 ml/min/1.73m^2) allowing haemodialysis to be discontinued. Slow deterioration of kidney function necessitated renal replacement therapy and the choice for a pre-emptive kidney transplantation was made. The kidney was donated by the patient's mother, who had no detected complement abnormalities. The CMV status required no valganciclovir prophylaxis (donor negative/receiver negative, D-/R-). The post-transplant course was not complicated by rejection or recurrence of aHUS. She suffered from hair loss due to tacrolimus treatment, rapid reversible kidney dysfunction due to hypotension, and one hospital admission because of hypertension after temporary withdrawal of the ACE-inhibitor. Currently, graft function is excellent (*table 2*).

Patient B is a 29-year-old female. At the age of 20 years, she was diagnosed with ESRD attributed to accelerated hypertension. In 2009 she underwent a pre-emptive kidney transplantation with a kidney from her father. The postoperative course was complicated by a kidney infarction and delayed graft function. Because of a presumed acute rejection, the patient was treated with methylprednisolone and anti-thymocyte globulin, without apparent success. Four months later a transplantectomy was performed and the allograft histology showed thrombotic microangiopathy. At that moment the diagnosis of aHUS was considered, and subsequently confirmed by genetic analysis (*table 1*). She started peritoneal dialysis and switched to haemodialysis because of leakage of peritoneal fluid. We performed a living related kidney transplantation with a kidney from her mother, who did not carry the mutation in complement factor H (CFH). The CMV status was D+/R+. The post-transplant course was uneventful (*table 2*).

Patient C is a female, 54 years of age, with pre-existent hypertension. In 2007 she developed acute kidney failure with clinical evidence of aHUS. Plasma exchange therapy did not improve her kidney function and the patient had to start haemodialysis. She had negative antibodies against CFH. She received the kidney of a living unrelated donor (CMV status: D-/R-). Apart from nausea due to high levels of mycophenolate mofetil in the first week after transplantation, the post-transplant course was uneventful.

Patient D is a 46-year-old man. In 2003 he presented in another hospital with headache and was diagnosed with accelerated hypertension and kidney failure. The kidney biopsy showed a membranoproliferative glomerulonephritis, which could not be classified in the absence of electron microscopy. Treatment with methylprednisolone and cyclophosphamide was not effective. Two years later a living related kidney transplantation was performed. Six months later he developed acute kidney injury caused by thrombotic microangiopathy. The patient was diagnosed with aHUS (confirmed by genetic analysis) and recurrent disease in the kidney allograft. After several years he had to start haemodialysis. We performed a living unrelated kidney transplantation via our cross-over program (CMV status: D+/R+). Two weeks after the transplantation the patient had a deep venous thrombosis in his arm related to the central venous dialyses access. After removal of the catheter he was treated with low-molecular-weight heparin for three months. The patient has had no other complications to date (*table 2*).

DISCUSSION

Our study illustrates that kidney transplantation is feasible in patients with aHUS without using standard prophylactic therapy with eculizumab. Thus far, four patients have been successfully transplanted without evidence of recurrent disease more than 19 months after transplantation. Admittedly, the number of patients is small, and we cannot exclude that the incidence of recurrent aHUS with our protocol may be as high as 50%. Still, our experience suggests that it is acceptable to perform a randomised study to compare a protocol based on prophylactic eculizumab therapy with a protocol based on rescue eculizumab therapy.

Admittedly, the introduction of the C5-inhibitor eculizumab has dramatically changed the prognosis for aHUS patients. Many case reports have reported favourable outcome in patients with aHUS treated with eculizumab. Subsequently, eculizumab was proven beneficial in cohort studies that included patients with plasmapheresis-resistant aHUS, with a cure rate of more than 80%.[16] In 2011 eculizumab was approved by the US Food and Drug Administration and the European Medicines Agency for the treatment of aHUS.

Zuber *et al.* propose to use eculizumab prophylactically in moderate- and high-risk patients who undergo kidney transplantation.[11] The authors suggest that this treatment

Table 2. *Transplantation characteristics and outcome*

Patient	Age at present transplantation (years)	Living donor	HLA mismatch	Donor specific antibodies (Luminex assay)	Follow-up (months)	Creatinine (mmol/l)	Thrombocytes (10^9/l)	LDH (U/l)	Urinary protein
A	35	Related	1-1-1	Neg	21	115	297	196	Neg
B	29	Related	0-1-1	Neg	18	80	273	200	Neg
C	54	Unrelated	1-2-1	Neg	19	112	282	223	neg
D	46	Unrelated	2-2-2	Neg	16	117	209	205	0.1g/l

Lactate dehydrogenase (LDH): normal values <250 U/l.

may be tapered after 12 months in moderate-risk patients, but should be continued in high-risk patients. Treatment according to these guidelines is associated with enormous costs and risks of suppression of complement activation.[17] Our data suggest that prophylactic and long-term treatment with eculizumab is not inescapable in the context of kidney transplantation in adult patients with aHUS. The answer can only be found in a randomised clinical trial. However, our data provide arguments that a protocol that is based on eculizumab rescue therapy may be a reasonable option in selected patients.

Our patients represent the typical patients with aHUS. Their risk of recurrence after kidney transplantation was not low, as estimated following guidelines as recently proposed.[11] Recurrence risk was high in patient B and D because they had a history of aHUS recurrence in a previous graft. In addition patient B had a mutation in CFH that causes diminished binding of CFH to C3b.[18] Patient D had the p.Lys65Gln mutation in C3. This mutation also results in weakening of the C3b-CFH binding.[19] The recurrence risk of patient A and patient C was moderate. They had the p.Arg161Trp mutation in C3. This gain-of-function mutation functionally weakens the C3b-CFH binding.[19,20] In a French cohort of aHUS patients, 12 transplantations were performed in patients with gain-of-function mutations of C3 and five had a recurrence of aHUS in the graft, which results in a 42% recurrence risk.[21]

Thus far, there is no evidence of recurrent HUS in our patients and all are doing well after a follow-up of 16-21 months. We can only speculate why outcome has been beneficial. However, we suggest that several components of our protocol, all directed at limiting endothelial cell injury (use of living donor, aggressive treatment of blood pressure and cholesterol, low dose CNI) and reducing the risk of rejection (by using basiliximab in addition to low-dose CNI, prednisone and high-dose MMF) contributed to this good outcome. Obviously, we cannot determine which factor is most critical in our protocol. Also, we cannot exclude that recurrences may occur with longer follow-up.

Zuber *et al* recently reported nine patients with aHUS who received a kidney transplant and were treated prophylactically with eculizumab.[11] Outcome was excellent in all but one patient who developed arterial thrombosis immediately postoperatively. Based on these data, the authors advocate the use of eculizumab prophylactically in moderate- and high-risk patients, and they advise to continue eculizumab in high-risk patients for an undetermined period of years.

Our data provide support for the notion that prophylactic therapy with eculizumab may not be needed in aHUS. Controlled studies are needed to determine cost-effectiveness, and results of such controlled studies should be used to develop evidence-based guidelines. Certainly, our data are not unique. Øyen *et al*. described seven aHUS patients who were successfully transplanted without aHUS recurrence during a follow-up of more than four years after transplantation. These authors used a CNI-free regimen.[22] Of note, genetic information was not available in these patients. Of the seven patients described by Øyen *et al*. four had a living donor and three patients a deceased heart-beating donor (personal communication). The potential benefit of eculizumab as rescue therapy is illustrated in the abovementioned study of Zuber *et al*. who reported 13 patients who were treated with eculizumab after onset of disease recurrence. Outcome was reported as favourable, especially if treatment was started early after onset of the recurrence (within 28 days). This finding is in agreement with the observations in the clinical trials[16] that early start of eculizumab (within one week) is related to better outcome.

We must admit that our study has limitations. There are only four patients and follow-up is relatively short, averaging 19 months. In the absence of protocol biopsies we cannot exclude subclinical thrombotic microangiopathy; however, none of the patients showed any laboratory abnormalities and their renal function is stable. Notably, our conclusions are only applicable to adults and recipients of a living donor kidney.

We propose that kidney transplantation with kidneys from living donors is feasible in patients with aHUS, and that randomised trials are justified and should be performed to compare the costs, effectiveness, and side effects of a treatment schedule that uses prophylactic eculizumab

with a treatment schedule that merely uses eculizumab as rescue therapy. We also propose that a similar strategy should be evaluated in the setting of non-living donor transplantation.

DISCLOSURES

Meeting Presentation: The results were presented at the 2012 American Society of Nephrology annual meeting in San Diego, California, on 1 November, 2012.
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REVIEW

Cinacalcet for secondary hyperparathyroidism: From improved mineral levels to improved mortality?

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ABSTRACT

Secondary hyperparathyroidism is an almost inevitable complication of advanced kidney failure. The introduction of the calcimimetic cinacalcet for the treatment of secondary hyperparathyroidism in patients on dialysis was based on its ability to reduce elevated levels of parathyroid hormone (PTH). Subsequent clinical studies confirmed the beneficial effects of cinacalcet on biochemical parameters reflecting mineral disturbances and bone disease. In this review we summarise the impact of cinacalcet on biochemical, intermediate and clinical outcomes. We also present previously unpublished mineral metabolism data from 144 Dutch dialysis patients treated with cinacalcet who participated in the pan-European ECHO observational study. Although secondary hyperparathyroidism tended to be more severe in our Dutch cohort, compared with the entire ECHO cohort, cinacalcet was nevertheless effective in reducing PTH in these patients. Two recent clinical studies evaluated, respectively, the efficacy of cinacalcet in improving the intermediate endpoint of cardiovascular calcifications (ADVANCE trial), and its impact on clinical outcomes, including all-cause mortality and cardiovascular events (EVOLVE trial). The ADVANCE trial provided evidence that cinacalcet may indeed improve calcification in both large arteries and cardiac valves. The EVOLVE trial, however, did not meet its clinical primary endpoint (time to all-cause mortality, myocardial infarction, hospitalisation for unstable angina, heart failure or a peripheral vascular event), although secondary and sensitivity analysis suggested a beneficial effect. The clinical implications of these important studies are also addressed in this review.

KEYWORDS

Cinacalcet, CKD-MBD, dialysis, secondary hyperparathyroidism, ECHO

INTRODUCTION

Secondary hyperparathyroidism is a frequent complication of chronic kidney disease (CKD).[1] Levels of parathyroid hormone (PTH) increase as CKD progresses, thus there is a high prevalence of secondary hyperparathyroidism among patients with end-stage renal disease.[2] Hypocalcaemia, vitamin D deficiency, and hyperphosphataemia, all hallmarks of CKD, are physiological stimuli for PTH secretion.[3] Moreover, during CKD expression of the vitamin D receptor, the calcium-sensing receptor and Klotho on parathyroid glands declines, thereby abolishing the inhibitory effects of 1,25-dihydroxyvitamin D, calcium and fibroblast growth factor 23 (FGF-23) on PTH production and secretion.[2,4-7] All these factors lead to hyperplasia of the parathyroid glands, which, once established, is generally irreversible.[8] Originally, PTH was considered to be an indicator of renal bone disease, but it is a poor predictor of bone histology, especially in advanced CKD,[9] unless levels of alkaline phosphatase are also considered along with PTH.[10] In end-stage renal disease, PTH was found to be associated with mortality. This association, after multivariate adjustment, showed a U-shaped curve for studies using both cross-sectional data and evolution of values over time,[11-14] with increased mortality at both high and low PTH levels. Unfortunately,

there is no consistency across these studies regarding the optimal level of PTH in end-stage renal disease, and for this reason, the recently published Kidney Disease Improving Global Outcomes (KDIGO) guidelines could only suggest preventing PTH levels from moving outside extreme ranges (2 to 9 times the upper limit of normal range).[15] Apart from the epidemiological association that exists between levels of PTH and mortality in patients with secondary hyperparathyroidism, two additional lines of evidence suggest a contributory role for elevated PTH levels in the causal pathway to adverse clinical events. Firstly, patients with primary hyperparathyroidism (who do not have uraemia), have increased mortality, mainly due to cardiovascular complications,[16] including left ventricular hypertrophy.[17] Secondly, as the PTH receptor is present on cardiomyocytes and vascular smooth muscle cells,[18] elevated PTH levels may lead to changes in the functioning of these cells, including disturbances in calcium channels and energy handling.

Treatment options for secondary hyperparathyroidism aim to prevent hyperphosphataemia and hypocalcaemia, while correcting 1,25-hydroxyvitamin D deficiency.[19,20] These include active vitamin D sterols,[21-24] the calcimimetic cinacalcet,[25-27] (in dialysis patients), combinations of both,[28,29] phosphate binders[15,30] and surgery.[31] Although parathyroidectomy can effectively lower PTH, it is associated with postoperative hypocalcaemia,[32] and can induce prolonged irreversible hypoparathyroidism.[33] The latter is associated with increased mortality.[34] The use of parathyroidectomy is declining,[35] and a detailed discussion is beyond the scope of this review paper.

The present review summarises the subsequent effects of cinacalcet on biochemical endpoints, intermediate endpoints including bone mineral density (BMD) and arterial calcification, and finally on hard clinical endpoints. We also present previously unpublished mineral metabolism data from 144 Dutch dialysis patients treated with cinacalcet who participated in a pan-European observational study. We conclude with a discussion on the implications of these new data for clinical practice.

CINACALCET

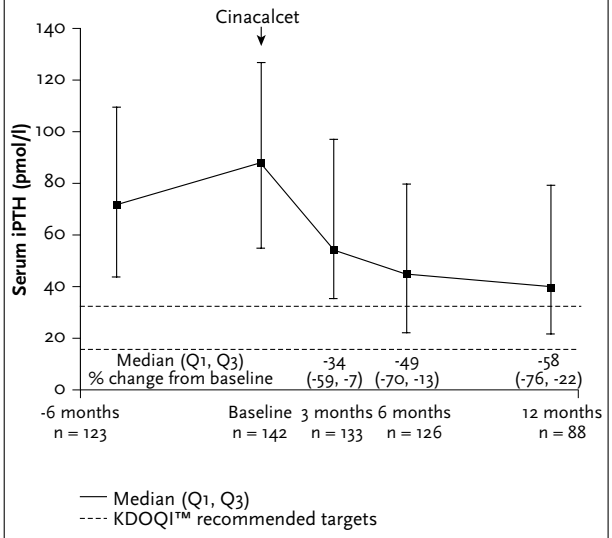
The calcimimetic agent cinacalcet hydrochloride (Mimpara®, Amgen Inc, Thousand Oaks, CA, USA) was approved in the Netherlands in 2005 for the treatment of secondary hyperparathyroidism in patients with stage 5 CKD on dialysis. This class of drugs acts by increasing the sensitivity of the calcium-sensing receptor to extracellular calcium ions, inhibiting the release of PTH.[36,37] In phase III clinical trials, cinacalcet improved achievement of target levels for the metabolic abnormalities associated with chronic kidney disease-mineral and bone disorders (CKD-MBD).[25,26,38] As the controlled setting of prospective clinical trials can differ substantially from everyday clinical practice, it was encouraging that similar levels of improvement in calcium, phosphorus, and especially PTH, were achieved in the ECHO observational study (Evaluation of the Clinical Use of Mimpara® in Haemodialysis and Peritoneal Dialysis Patients, an Observational Study).[27,39]

DUTCH EXPERIENCE: THE ECHO STUDY

The ECHO study was a one-year observational study among European dialysis patients receiving cinacalcet (n=1865). Detailed descriptions of the methodology used have been published previously.[27] In short, all dialysis patients at participating centres who were prescribed cinacalcet at the decision of their treating physician were eligible for inclusion in the analysis. They were followed from six months prior to initiation of cinacalcet, until 12 months afterwards. There was no treatment algorithm provided. The main outcome parameter was the proportion of patients who attained target levels for PTH, phosphate, and calcium.

As there were important differences across the 12 countries that participated in the ECHO study, we conducted a separate analysis of the 144 participants from the

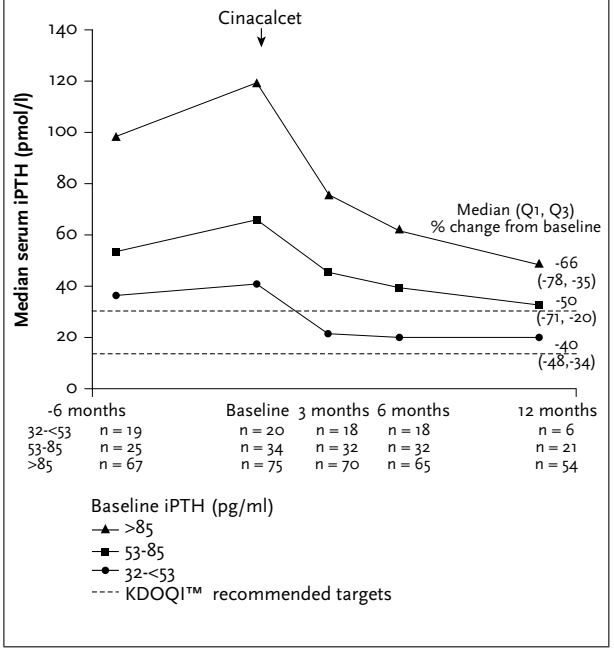
Figure 1. *Evolution of intact parathyroid hormone (iPTH) in Dutch patients treated with cinacalcet in the ECHO observational study. During the observation period KDOQI™ guidelines were followed, aiming at PTH levels between 16-32 pmol/l*



KDOQI™= Kidney Disease Outcomes Quality Initiative; Q = quartile.

Netherlands (80% on haemodialysis, the remainder on peritoneal dialysis). The Dutch participants generally had more severe secondary hyperparathyroidism, as evidenced by higher baseline levels of PTH, compared with the pan-European ECHO cohort as a whole (n=1865).[40] Because of this higher baseline level of PTH, differences in PTH response might have been expected in the Dutch patients. The median age of the Dutch dialysis patients was 59 years (range 19-90), and the median dialysis duration was 52 months. Median PTH was 88 pmol/l (interquartile range 55-127, c.f. normal values of <7-9 pmol/l, depending on the assay used in the Dutch participating centres), despite extensive use of phosphate binders and active vitamin D sterols. Median calcium level was 2.6 mmol/l (IQR 2.4-2.7). As shown in *figure 1*, a relevant decline in PTH was observed. Simultaneously a decline in calcium and a small decline in phosphate were also observed (data not shown), leading to an improved calcium-phosphorus product. Interestingly, baseline PTH levels did not predict responsiveness to cinacalcet (*figure 2*). These data indicate that in everyday practice, patients with severe secondary hyperparathyroidism generally respond well to cinacalcet, even though the majority (73%) were already treated with active vitamin D sterols.

Figure 2. *Magnitude of intact parathyroid hormone (iPTH) reduction according to baseline disease severity in Dutch patients treated with cinacalcet in the ECHO observational study: mild (baseline iPTH 32-<53 pmol/l), moderate (baseline iPTH 53-85 pmol/l) and severe (baseline iPTH >85 pmol/l)*



EFFECT ON INTERMEDIATE ENDPOINTS

Better control of PTH and calcium by cinacalcet does not necessarily imply improved clinical outcome. This improved laboratory profile after initiation of cinacalcet, however, is accompanied by alleviation of both bone disease and arterial calcifications, which are thought to be modifiable entities and are accepted as intermediate endpoints.

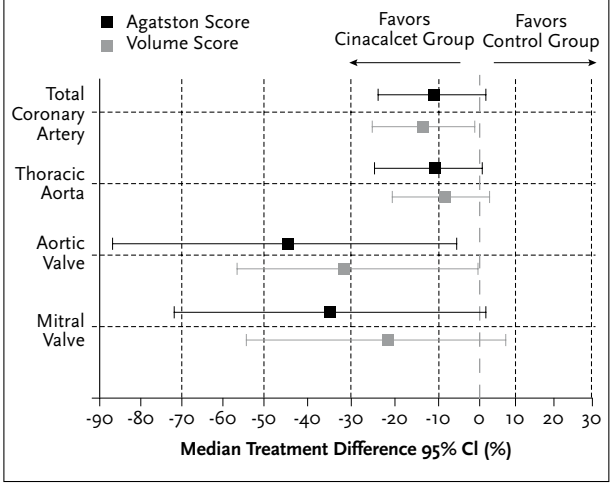
In a small subgroup of a placebo-controlled randomised trial,[26] the effects of 26 weeks of cinacalcet treatment on BMD included improvement in the proximal femur, but not in the lumbar spine.[41] After kidney transplantation this beneficial effect was confirmed for both the femoral neck and lumbar spine.[42,43] As BMD is considered a poor indicator of bone histology in advanced CKD, these results remain difficult to interpret. Ideally, detection of bone histology changes requires sequential bone biopsies. This indeed was performed in a placebo-controlled study in 32 dialysis patients and demonstrated that cinacalcet use for one year led to improved bone turnover, along with a decline in bone-specific alkaline phosphatase.[44] These results contrast somewhat with findings of a very recent pilot study that initiated cinacalcet only in haemodialysis patients with declining BMD and bone-biopsy proven osteitis fibrosa, the bone histological hallmark of hyperparathyroidism. Repeated bone biopsies were not conducted, but ongoing loss of BMD could not be halted in patients treated with cinacalcet.[45]

Another intermediate endpoint that has been studied extensively is vascular and cardiac valve calcification. Based on encouraging results from animal models of uraemia[46] in which calcimimetics such as cinacalcet led to undisputable improvement, the ADVANCE trial was conducted.[47] This open-label randomised trial in 360 dialysis patients compared a treatment regimen of fixed low doses of active vitamin D plus cinacalcet with a regimen of flexible doses of active vitamin D (both known to lower PTH). After one year of treatment, the cinacalcet-treated group showed decreased calcification (using two scoring systems, based on electron beam CT scanning) in the coronary arteries, aorta, and mitral and aortic valves, although some of these changes, including the predefined primary endpoint, did not reach statistical significance (*figure 3*).

EFFECTS ON CLINICALLY RELEVANT ENDPOINTS: THE EVOLVE TRIAL

It is well appreciated that a simple model of cause-effect in pathophysiology seldom explains observed complications in the clinical setting. Furthermore, the assumption that pharmacological intervention aimed at modifying the assumed cause can prevent complications is even

Figure 3. *Percentage change from baseline to week 52 in calcification scores at four different anatomical sites in patients treated with cinacalcet (n=115) and a control group treated with flexible doses of vitamin D sterols (n=120) in the ADVANCE study. The box represents the median treatment differences; the line represents 95% confidence intervals. Raggi P, et al. The ADVANCE study: a randomized study to evaluate the effects of cinacalcet plus low-dose vitamin D on vascular calcification in patients on hemodialysis. Nephrol Dial Transplant. 2011, 26(4):1327-39. Reprinted by permission of the European Dialysis and Transplant Association - European Renal Association.*[47]



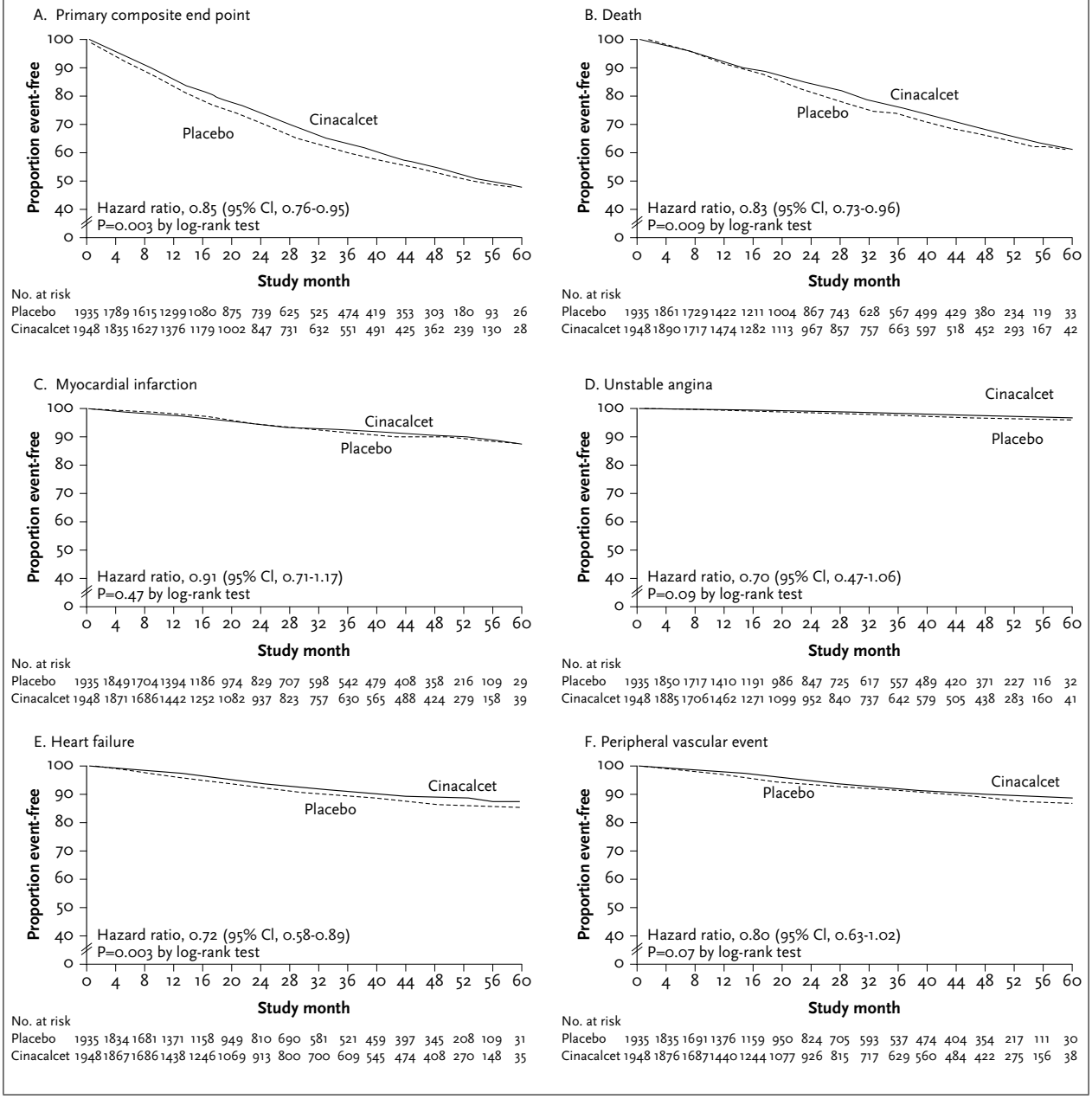
more likely to be a misconception. A recent meta-analysis published while the EVOLVE trial was ongoing (see below) concluded that there is consistency among trial findings regarding the ability of cinacalcet to reduce PTH. This may subsequently reduce the progression rate of calcification, but existing literature does not support any impact of cinacalcet on mortality,[39] with the exception of some post-hoc analyses.[48] The randomised double-blind, placebo-controlled EVOLVE trial prospectively studied the impact of cinacalcet on a composite endpoint in haemodialysis patients. This comprised time to all-cause mortality, myocardial infarction, hospitalisation for unstable angina, heart failure or a peripheral vascular event.[49] After analysing according to the intention-to-treat principle, the study did not show a significant effect on its primary composite endpoint (hazard ratio [HR] 0.93, 95% confidence interval 0.85-1.02, p=0.11, *figure 4*). A prespecified secondary analysis adjusting for baseline covariates associated with the primary endpoint (including age: there was a one-year difference between treatment groups) revealed a nominally significant improvement in achievement of the primary endpoint in the cinacalcet group (HR 0.88, 95% confidence interval 0.79-0.97). The study was hampered by several problems. The two most important issues were the high dropout rate in both treatment groups and the number of patients who were prescribed cinacalcet as a component of their regular treatment. As expected, the latter event occurred mostly in the placebo group; in an attempt to correct for this issue, lag censoring was performed, with data being censored six months after discontinuation of the investigational product. (The period of six months was chosen as the anticipated duration of any effect of altered mineral metabolism on extraskeletal calcification.) This analysis also showed a nominally significant effect on the primary endpoint in favour of the cinacalcet group (HR 0.85, 95% CI 0.76-0.95).

IMPLICATIONS FOR EVERYDAY PRACTICE

The failure of the EVOLVE trial to meet its primary endpoint requires reconsideration of the assumptions on which the study was based. Although the ability of cinacalcet to reduce PTH is undisputed,[26,39] its potential to slow or halt calcification, although likely, did not translate into a straightforward improved clinical outcome. An important consideration when trying to explain this finding is that in the ADVANCE study, the primary endpoint was a change in calcification scores.[47] However, calcification scores were extremely elevated in both groups at baseline, suggesting that considerable vascular damage may already have occurred, and calcification had therefore already become a non-modifiable risk factor by the time patients were enrolled in the EVOLVE study. Another possibility is that vascular calcification (as detected by electron beam CT or multi-slice CT) is just a marker of severe underlying vascular pathology.[50,51] If cinacalcet improves the marker (i.e. calcification), but not the underlying vascular disease (for instance atherosclerosis of the intima, elastin degradation or apoptosis of smooth muscle cells in the media), then it is not surprising that no clinical improvements were detected in EVOLVE, since the vascular processes that precede final calcification are the actual culprits for cardiovascular events. An additional factor that may explain the failure of the EVOLVE study to meet its primary endpoint is the fact that many aspects of clinical treatment were not predefined in a treatment algorithm, leading to important differences between the cinacalcet and placebo groups. For instance, the use of active vitamin D sterols, which have been associated with improved survival in dialysis patients,[52] was lower in patients randomised to cinacalcet.

It is important to realise that the minimal PTH level required for inclusion in the EVOLVE trial (31.8 pmol/l, based on the older KDOQI guideline) is within the range that can be considered acceptable according to

Figure 4. *Kaplan-Meier curves comparing cinacalcet treatment and placebo for the primary composite endpoint (panel A) and the five components of the composite endpoint (panels B-F) in the EVOLVE trial. 'From N Engl J Med, Effect of cinacalcet on cardiovascular disease in patients undergoing dialysis, Chertow GM, et al., 367:2482-94. Copyright © 2012 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.*[49]



the current KDIGO guideline.[15] However, the potential impact of this difference in upper PTH threshold is probably limited. The median baseline PTH for EVOLVE patients allocated to cinacalcet was 74 pmol/l (percentile 10-90: 39-182 pmol/l); therefore, the majority could also be considered candidates for treatment according to the current clinical guidelines. Moreover, additional analyses revealed that cinacalcet treatment postponed the first clinical event by approximately five months. This potential gain in event-free time has to be weighed against potential adverse effects and costs of the intervention. Finally, sensitivity analysis showed a difference in effects of cinacalcet treatment on the primary endpoint based on age, suggesting that those above the age of 65 years derived the most benefit.

CONCLUSION

For almost a decade cinacalcet has been considered to be an important addition to the pharmaceutical armamentarium for treating dialysis patients. It has proven to be valuable in improving laboratory markers, as seen in our Dutch cohort, with possible benefits on intermediate endpoints such as bone disease and vascular calcifications. However, a final verdict on the impact of cinacalcet on clinically relevant outcomes remains elusive and unfortunately there are no ongoing or planned studies that will elucidate this in the near future. The EVOLVE trial, which aimed to answer this question, did not meet its primary endpoint and could only suggest a benefit of cinacalcet. Nevertheless, the EVOLVE data may justify the use of cinacalcet as a component of future multi-targeted intervention trials in dialysis patients.

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Conflicts of interest

M.V. has received research grants from Abbott and Genzyme, and participated in advisory boards for Abbott and Genzyme. N.M. is an employee of Amgen. Dr du Buf-Vereijken, Dr Potter van Loon, Dr Reichert, and Dr Smak Gregoor report no conflicts of interest.

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REVIEW

An upper gastrointestinal ulcer still bleeding after endoscopy: what comes next?

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ABSTRACT

Introduction: Recurrent bleeding from an upper gastrointestinal ulcer when endoscopy fails is a reason for radiological or surgical treatment, both of which have their advantages and disadvantages.
Case: Based on a patient with recurrent gastrointestinal bleeding, we reviewed the available evidence regarding the efficacy and safety of surgical treatment and embolisation, respectively.
Discussion: Transarterial embolisation (TAE) and surgical treatment are both options for recurrent gastrointestinal bleeding when endoscopy fails. Both therapies have serious complications and a risk of rebleeding. Choosing the therapy depends on the capability of the patient to tolerate haemodynamic instability, resuscitation and hypotension.
Conclusion: Choosing between TAE and surgery depends a great deal on the case presented, haemodynamic stability and the skills and tools available at that moment.

KEYWORDS

Gastrointestinal bleeding, failed endoscopy, TAE, surgery

INTRODUCTION

A bleeding upper gastrointestinal ulcer is a potentially fatal disease, and immediate treatment is necessary. The primary treatment of a bleeding ulcer is endoscopic, but with persistent or recurrent bleeding, rescue treatment may be necessary or even life saving. In this report, we describe a patient with recurrent massive bleeding where initial endoscopic treatment failed. Based on a literature review we discuss the treatment options.

CASE

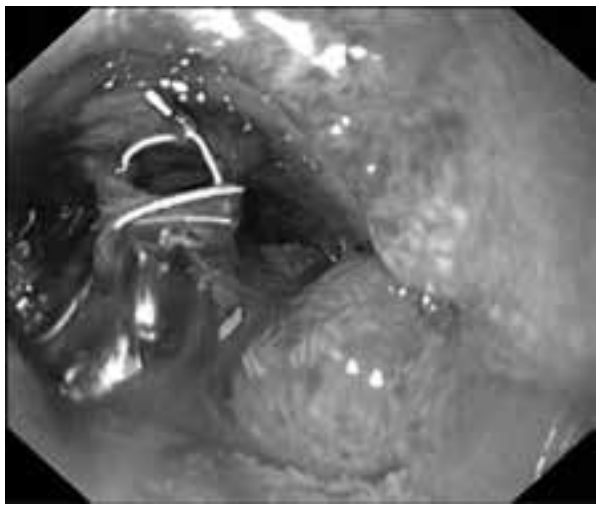
A 58-year-old male was admitted to the GI department of the hospital with gastrointestinal bleeding. He was mentally impaired and his medical history revealed nephrotic syndrome and hypertension. Because of his mental impairment, endoscopy had to be performed under sedation. He was admitted to the ICU where he was intubated. The endoscopy revealed multiple lesions in the bulbus duodeni, one of them being the source of the bleeding. This large semi-circumferential ulcer was coagulated and then the patient started esomeprazole therapy (80 mg iv twice daily). The patient showed no signs of persistent bleeding. After extubation he was haemodynamically stable and was discharged to the ward. He had a rebleed six days later. Endoscopy revealed that the same ulcer was bleeding from a visible vessel, and the treatment was coagulation around the ulcer. The performing endoscopist stated that there where no further options for endoscopic therapy in case of rebleeding. In that case, surgical or radiological consultation should be sought. The patient was discharged to the ward again, in a good condition.
Two days later, the patient had to be resuscitated because of haemorrhagic shock due to recurrent bleeding. The patient could be stabilised initially. The decision was made to do an interventional angiography because he was judged to be stable enough. During the decision-making process, the patient became unstable again with abdominal distension, high pressure ventilation and hypotension.
Despite this, he underwent an interventional angiography. The bleeding site was seen, marked by extravasation of contrast in the lumen of the bowel. The gastroduodenal artery and the proximal gastroepiploic artery were embolised. The procedure was difficult due to the size and anatomy of the lesion, and was further complicated due to ongoing bleeding requiring continuous resuscitation.

But ultimately haemostasis was reached. After the angiography, intra-abdominal hypertension developed reaching a maximum pressure of 40 mmHg. It decreased after several hours to 15 mmHg. The following night systemic hypotension developed without signs of further bleeding. The patient underwent a laparotomy where an ischaemic colon and gall bladder were seen with open arterial blood supply. Both were removed. The operation was complicated by massive blood loss. The next day he showed gastrointestinal blood loss again. An endoscopy revealed a necrotic stomach. The ulcer showed no signs of healing and the radiologically placed coils could be seen intraduodenally (*figure 1*). The patient died shortly after, without further therapy for shock.

DISCUSSION

In upper gastrointestinal bleeding, endoscopy is the diagnostic modality of choice. It has a high sensitivity and specificity for locating and identifying bleeding lesions in the upper GI tract. In addition, once a bleeding lesion has been identified, therapeutic endoscopy can achieve acute haemostasis, by thermal coagulation or haemoclip placement. This prevents recurrent bleeding in most patients. In most cases, endoscopy achieves haemostasis, but 10-30% of the patients have repeated bleeding for various reasons.[1,2] When haemostasis is not (expected to be) achieved with endoscopic techniques, other options are surgery or transarterial embolisation (TAE). Surgery has long been the standard of care but, with the development of intervention radiology, coiling a bleeding artery has gained a prominent role.

Figure 1. *Endoscopic view during the third endoscopy of the bulbus duodeni with the intra-arterial coil visible in the intestinal lumen*



Surgery

Surgery, the classical therapy, is effective in patients with uncontrolled bleeding. The aim of emergency surgery is not to cure the disease but rather to stop the haemorrhage when endoscopic therapy is unavailable or has failed. Generally accepted indications for surgery are failures of endoscopic techniques, haemodynamic instability despite resuscitation, recurrent bleeding after two endoscopic attempts, and continued slow bleeding (three transfusion units per day). It is an option in patients who may not tolerate recurrent or worsening bleeding. High-risk patients may not tolerate prolonged resuscitation, large volume transfusion, or periods of hypotension.[3,4]

Several surgical approaches are possible. In peptic ulcer disease, emergency surgery includes over-sewing the ulcer plus truncal vagotomy and pyloroplasty. Another approach for a gastrointestinal bleed is removing the bleeding site (e.g. performing a (partial) gastrectomy or duodenectomy), or ligating the bleeding vessel with a non-absorbable suture.[5,6] In a multicentre randomised prospective trial, Poxon *et al.* compared minimal surgery (ligating the vessel or ulcer excision) with conventional ulcer surgery (vagotomy and pyloroplasty or partial gastrectomy) for the treatment of a bleeding ulcer. The patients were randomised to undergo either minimal surgery, in which case the artery that supplied blood to the ulcer was ligated or where the ulcer itself was removed, or they underwent conventional surgery, in which case a vagotomy with pyloroplasty or a partial gastrectomy was added to vessel ligation. They found more fatal rebleeding in the minimal surgery group.[7] This finding is supported by the study of Billat *et al.* who found that gastrectomy with ulcer excision is the procedure of choice for emergency surgical treatment, because postoperative bleeding recurrence is lower, and the overall mortality rate and duodenal leakage is the same as with over-sewing and vagotomy.[8] Barkun *et al.* recommend that surgical consultation should to be sought for patients at risk for rebleeding after endoscopic retreatment, because salvage surgery can be required.[9,10] Emergent surgery is associated with mortality rates of up to 36%.[6] Surgical therapy is not always definitive. Recurrent bleeding rates following surgery vary from 3 to 23%, depending on the kind of surgery performed.[7,8]

Interventional angiography

TAE of gastrointestinal bleeding vessels has become the first choice in some centres for patients who do not to respond to medical and/or endoscopic therapy. Before intervention the bleeding locus can be identified. This can be done by clipping during endoscopy, CT angiography or standard angiography.[11]

Depending on the suspected location of the bleeding lesion the coeliac artery and either the superior mesenteric artery or the lower mesenteric artery are selectively filled with

contrast. Extravasation of contrast in the lumen (blush) of the bowel marks the bleeding site. In the absence of a blush, indirect evidence is sought, which includes visualisation of an aneurysm or pseudo-aneurysm, filling of spaces outside the bowel lumen (diverticula), early draining vessels (angiodysplasia), neovascularity (tumours), arterio-venous fistulas and hyperaemia (colitis). Once the bleeding site is identified, the therapy can be delivered. In upper GI bleeding, the therapy can be given in the suspected vessel, even when the bleeding is not seen during angiography, when the bleeding site was identified during endoscopy.

Angiographic therapy consists of infusion of vasoconstrictive medication (vasopressin) at the bleeding site, or embolisation. In embolisation the arterial blood supply to the bleeding site is occluded. Materials used for embolisation can be gelatine sponges, polyvinyl alcohol (in small microspheres or sheets), liquid agents e.g. N-butyl 2-cyanoacrylate (NBCA) or ethylene-vinyl alcohol copolymer (Onyx, Micro Therapeutics, Inc, Irvine, CA). Once delivered, liquid agents solidify, leading to embolisation. The mechanical blocking devices, such as coils, platinum microcoils, balloons and silk threads, induce blood flow reduction and coagulation. These mechanical blocking agents are best suited for patients bleeding from varices, a large visceral artery or the gastroduodenal artery. The coils are placed proximally and distally from the bleeding site to prevent back-bleeding from collateral vessels.

Complications of embolisation include complications from the angiography itself (haematomas, arterial thrombosis, dissection, embolism, formation of pseudo-aneurysm) and bowel infarction. In their systematic review, Mirsadraee *et al*. found complications from embolisation in 5-9% of the patients, with ischaemia and infarction accounting for the majority of the complications.[12] They occur even though the GI tract has a rich collateral blood supply. Risk factors for these complications include previous surgery, pancreatitis, radiation therapy and concurrent vasopressin infusion. In their study, which included 95 patients with GI bleeding, Yap *et al*. found complications to be technical (migration of coils from the gastroduodenal artery into proper hepatic artery (3%), non-target embolisation of splenic artery (1%)), and they found four patients (4%) to have post embolisation ischaemia, all of the upper GI tract.[13]

Angiography with TAE for persistent or recurrent peptic ulcer bleeding is a less invasive alternative to surgery. Initial success rates for patients with acute peptic ulcer bleeding have been reported from 52% up to 98%, with recurrent bleeding rates ranging from 10% up to 38%.[13,14]

Indications for interventional angiography for acute non-variceal upper gastrointestinal bleeding have been suggested in a consensus statement from the American College of Radiology[15]:

- Endoscopy is the best initial diagnostic and therapeutic procedure
- Surgery and transcatheter arteriography/intervention are equally effective following failed therapeutic endoscopy, but transcatheter arteriography/intervention should be considered particularly in patients at high risk for surgery
- Transcatheter arteriography/intervention is less likely to be successful in patients with impaired coagulation
- Transcatheter arteriography/intervention is the best technique for treatment of bleeding in the biliary tree or pancreatic duct

TAE versus surgery in the literature

TAE and surgery have only been compared in retrospective studies of patients with peptic ulcer bleeding that could not be controlled endoscopically. No randomised trials have been performed and will probably never be done.

Ripoll *et al*. analysed the outcome of 70 patients with refractory peptic ulcer bleeding. Although the patients in the TAE group were older and had more comorbidity, the incidence of rebleeding (29 *vs* 23%) and mortality (26 *vs* 21%) was similar to the surgical group.[16]

Eriksson *et al*. found a trend towards lower 30-day mortality in the TAE group (3%) compared with the surgical group (14%) ($p<0.07$). However, the patients in the TAE group were older and had more comorbidity. The repeat bleeding frequency after TAE was slightly higher (25 *vs* 18%). There were no complications related to TAE, and TAE could prevent unnecessary resection of the upper gastrointestinal tract. Although the study has several limitations, they suggested that TAE might be superior to surgery.[17]

Wong *et al*. found TAE to be a safe procedure, with no ischaemic complications, although there is a high rate of recurrent bleeding. They state that TAE should at least be considered as an alternative to surgery. The high percentage of recurrent bleeding is supported by the data of Yap *et al*. who found rebleed percentages of up to 38%.[13,18]

Loffroy *et al*. state that embolisation is effective in patients for whom surgery is not a realistic option, even when extravasation is not visualised by angiography. The radiologist should be well informed about the patient's condition, the procedure should take place shortly after onset of bleeding, and coagulation disorders should be corrected. The choice of embolic agent in relation to the characteristics of the bleeding vessel is important.[19]

TAE versus surgery; the clinical balance

No decisive data were found in the literature. Therefore the choice for an individual patient has to be based on other arguments. Some of these are patient based. In

older fragile patients, in patients with massive bleeding leading to deep hypotension, surgery might be preferable because the bleeding is, at least perceived to be, more quickly controlled. In rather stable patients or patients with previous abdominal surgery, TAE might be the first choice. Other arguments are institutional. Performing upper gastrointestinal tract surgery for benign reasons has diminished in frequency and not every surgeon is equally experienced. TAE also requires skills that not every radiologist possesses. The limited possibilities for resuscitation and monitoring in the angiography room during procedures that are sometimes lengthy can defer the choice to the operating theatre.

CONCLUSION

Our patient died of ischaemic complications after ongoing haemodynamic instability. An extensive literature search did not reveal convincing evidence for an alternative therapy. The initial treatment of an upper gastrointestinal bleed is endoscopy and an attempt at local control of bleeding. If endoscopic treatment fails there are two options: TAE or surgery. The available evidence from a limited number of retrospective studies suggests that there is a similar outcome in TAE and surgery to control gastrointestinal bleeding. Recurrent bleeding rates might be higher in patients treated with TAE, but complications might be more frequent in patients treated with surgery. As for surgery, there are different techniques for the procedure without any one proving superior. There seems to be no place for minimal surgery in this setting. The best way to proceed in patients with upper GI bleeding that cannot be controlled endoscopically is determined by patient related and institutional arguments.

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ORIGINAL ARTICLE

No effect of atorvastatin and simvastatin on oxidative stress in patients at high risk for cardiovascular disease

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ABSTRACT

Background: Statins are thought to have anti-atherogenic effects beyond cholesterol lowering. One such mechanism may involve reduction of oxidative stress. The aim of our study was to investigate and to compare the oxidative stress lowering capacity of atorvastatin with that of simvastatin in patients at high risk for cardiovascular disease using conventional markers and sensitive markers measured by highly specific techniques such as liquid chromatography tandem mass spectrometry.

Methods: We included 30 statin-naive patients with diabetes mellitus, and/or obesity, and/or hypertension (12 male, 18 female, mean age 44.8±11.1 years), and randomised them to receive either atorvastatin 10 mg or simvastatin 40 mg daily to obtain an equimolar cholesterol reduction. Blood and urine samples were obtained at baseline and at 1, 6 and 12 weeks.

Results: Low-density lipoprotein (LDL) cholesterol and coenzyme Q10 decreased significantly in both groups. Simvastatin caused a faster initial LDL cholesterol lowering than atorvastatin (p=0.01), but the overall effect after 12 weeks of atorvastatin and simvastatin was similar. Plasma myeloperoxidase and malondialdehyde did not change during the study period in the two groups. Urinary F2-isoprostanes decreased gradually and significantly in the atorvastatin group but not in the simvastatin group, but the between-group difference was not significant. Urinary 8-hydroxy-2-deoxyguanosine did not change in the two groups.

Conclusion: This study suggested that an important role of oxidative stress lowering as possible pleiotropic effect of atorvastatin and simvastatin is questionable.

KEYWORDS

8-OHdG, cholesterol, F2-isoprostanes, oxidative stress, statins

INTRODUCTION

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, or statins, are widely used for lowering plasma low-density lipoprotein (LDL) cholesterol and have a clear role in the primary prevention of cardiovascular disease (CVD) mortality and major events.[1] It was initially assumed that the reduction of cholesterol by statins was the only mechanism responsible for their beneficial effect. However, subgroup analysis of large clinical trials indicated that subjects in statin-treated arms have less cardiovascular events than subjects in placebo-controlled arms with similar serum cholesterol levels.[2] In addition, several studies have demonstrated a rapid improvement in vascular function with atorvastatin which might not solely be accounted for by the achieved initial cholesterol reduction.[3,4] Hence, it has been suggested that statins may have additional anti-atherogenic effects such as improving endothelial function, attenuating vascular

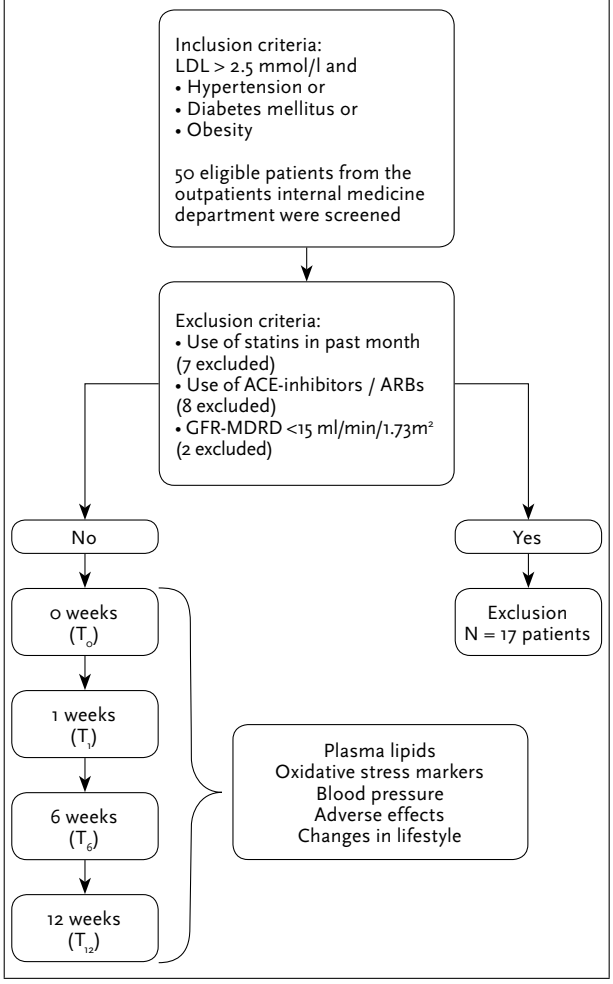
and myocardial remodelling, stabilising atherosclerotic plaques, and inhibiting vascular inflammation and oxidation.[5] One of these so-called pleiotropic effects of statins may be a reduction of oxidative stress even before the lipid-lowering effect becomes apparent and this mechanism is thought to be, at least partly, responsible for the beneficial effects which seem to occur very early in the course of the therapy.[6] Atorvastatin and simvastatin are frequently used statins and their oxidative stress lowering potential has been examined in several studies. The effects of atorvastatin versus simvastatin on oxidative stress have been examined in patients with coronary heart disease,[7] type 2 diabetes mellitus,[8] in women with polycystic ovary syndrome,[9] in patients on haemodialysis,[10] and in hyperlipidaemic subjects.[11] In these studies malondialdehyde (MDA), total peroxides, and auto-antibodies against oxidised LDL were used as markers of oxidative stress. Although MDA has been used as a marker of oxidative stress for decades, most commonly used methods to measure MDA are insufficiently sensitive and specific.[12] In addition, it is also known that lipid peroxides cannot be used as a universal marker of oxidative stress.[13] Since neither of the above-mentioned studies[7-11] examined specific oxidative stress markers, the anti-oxidative properties of atorvastatin and simvastatin remain principally unelucidated. F2-isoprostanes and 8-hydroxy-2-deoxyguanosine (8-OHdG) measured by mass spectrometry (MS) have gained recognition as better and more specific markers of oxidative lipid and DNA modifications, respectively.[14,15] Therefore, the objective of this study was to compare the oxidative stress lowering capacity of atorvastatin with that of simvastatin in a sample of patients with increased oxidative stress using sensitive markers measured by highly specific techniques on top of conventional markers.

MATERIALS AND METHODS

Subjects

In total 33 statin-naive patients were included with type 1 or 2 diabetes mellitus (according to the American Diabetes Association criteria[16]), and/or obesity (body mass index >30 kg/m^2), and/or hypertension (systolic/diastolic blood pressure >140/90 mmHg). The lowest threshold of plasma LDL cholesterol for inclusion in the study was 2.5 mmol/l. Patients who were being treated with angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers were excluded because of the oxidative stress lowering properties of these agents, as were patients suffering from renal insufficiency defined as an estimated glomerular filtration rate calculated by the modification of diet in renal disease equation (GFR_{MDRD}) below 15 ml/min/1.73m^2 (*figure 1*).

Figure 1. *Flow of participants through the study.*



The study was approved by the Ethics Committee of the VU University Medical Center. Written informed consent was obtained from all participants, before randomising them into two groups. After one baseline measurement (T_0), one group received atorvastatin 10 mg once daily, the other group simvastatin 40 mg once daily. These doses were used to achieve a similar degree of LDL cholesterol lowering. The study was single blinded; the researchers were blinded while the patients and the treating physicians were not. After an overnight fast, blood and urine samples were obtained at baseline and at 1, 6 and 12 weeks (T_0, T_1, T_6 and T_{12} respectively) and side effects of the medication were registered. Urine samples were preserved at -20°C until analysis and EDTA-plasma samples were aliquoted and stored at -80°C.

Biochemical analyses

Total cholesterol, HDL cholesterol and triglycerides were measured by standard enzymatic methods (Roche, Germany). Plasma LDL cholesterol was calculated according to Friedewald's formula.[17] The total concentration of coenzyme Q10 (sum of oxidised and

reduced forms) was determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) as described before.[18] The intra- and inter-assay coefficients of variation (CV) were 2.1% and 3.2%, respectively. A sandwich ELISA (Mercodia, Uppsala, Sweden) was used to determine myeloperoxidase (MPO) concentrations in EDTA plasma, with intra- and inter-assay CVs of 3.9% and 5.0%, respectively.[19] Total, i.e. free and protein-bound, plasma malondialdehyde (MDA) was measured by high performance liquid chromatography (HPLC) and fluorescence detection after alkaline hydrolysis and reaction with thiobarbituric acid, as previously described.[20] The intra-run and inter-run CVs of the MDA procedure were 3.5% and 8.7%, respectively. Urinary F2-isoprostanes (8-iso PGF 2α)[21] and 8-OHdG[22] concentrations were determined using LC-MS/MS. The intra- and inter-assay CVs for F2-isoprostanes were 6.8% and 8.4%, respectively, and the intra- and inter-assay CVs for 8-OHdG were 4.1% and 5.3%, respectively. To adjust for differences in analytic dilution in the urine samples, F2-isoprostanes and 8-OHdG concentrations were divided by their urine creatinine concentration. Creatinine was measured by the Jaffé reaction procedure using a commercial reagent (Roche, Germany).

Statistical analysis

In case of normally distributed variables, data are presented as mean and standard deviation (SD) or as median and interquartile range (IQR) in case of non-normally distributed variables. Differences in baseline characteristics between the two treatment groups were tested with Student's t-tests, Mann-Whitney U tests or χ^2-tests, as appropriate. Correlations between variables were expressed as Pearson's correlation coefficients. To assess the longitudinal association of treatment group with oxidative stress makers, generalised estimating equations (GEE) were used with treatment group as the independent variable, adjusted for age and sex. A p-value of less than 0.05 was considered statistically significant. All analyses were performed with SPSS version 20 (IBM SPSS Statistics, Armonk, New York).

RESULTS

After screening, we enrolled 33 subjects for this study and randomly allocated them to the atorvastatin (n=15) or simvastatin group (n=18). In the simvastatin group all data were eligible for analysis. However, in the atorvastatin group two patients were excluded because of missing urine samples and one patient never took the pills because of negative publicity about statins in the media and was therefore excluded from the study. None of the participants reported lifestyle changes such as changes in diet, smoking, medication or exercise during the study. The mean age of the remaining 30 participants was 44.8±11.1 years with 40% males. The baseline characteristics and the medications administered to the subjects are presented in *table 1*. No statistically significant differences were observed in baseline characteristics with respect to age and sex, blood pressure, and serum lipids (all p>0.05). The mean levels of LDL cholesterol, coenzyme Q10, MDA, MPO, and urinary F2-isoprostanes and 8-OHdG measured at baseline and after 1, 6, and 12 weeks are shown in *table 2* and *figure 2*, stratified by treatment allocation. No statistically significant differences were observed in baseline characteristics with respect to oxidative stress markers (all p>0.05). Simvastatin caused a faster initial LDL cholesterol lowering than atorvastatin during the first week (p=0.01). In both groups LDL cholesterol and coenzyme Q10 decreased

Table 1. *Characteristics and medications of the study population at screening and adverse side effects during the study stratified by treatment allocation*

Variable	Atorvastatin (*n*=12)	Simvastatin (*n*=18)	P-value*
Age (years)	44.0 (11.7)	45.3 (11.0)	0.75
Sex (male %)	50	30	0.46
Diabetes (yes %)	75.0†	72.2	0.67
Smoking (yes %)	16.7†	38.9	0.41
Systolic blood pressure (mmHg)	118 (14)	122 (15)	0.56
Diastolic blood pressure (mmHg)	81 (9)	79 (9)	0.52
Total cholesterol (mmol/l)	5.0 (1.0)	5.1 (1.0)	0.94
LDL cholesterol (mmol/l)	3.0 (0.8)	3.0 (0.7)	0.96
HDL cholesterol (mmol/l)	1.52 (0.49)	1.72 (0.67)	0.41
Triglycerides (mmol/l)‡	0.9 (0.8-1.3)	0.9 (0.6-1.3)	0.66
Medication			
- Insulin	8 (67%)	11 (61%)	
- Metformin	1 (8%)	3 (17%)	
- Sulphonylurea derivatives	0 (0%)	2 (11%)	
- Thiazolidinediones	0 (0%)	1 (6%)	
- Thrombocyte aggregation inhibitors	1 (8%)	2 (11%)	
- Coumarin derivatives	0 (0%)	1 (6%)	
- Dihydropirines	2 (17%)	3 (17%)	
- Thiazide diuretics	1 (8%)	2 (11%)	
Adverse effects			
- Gastrointestinal symptoms	2 (17%)	1 (6%)	
- Myalgia	3 (25%)	8 (44%)	

***P-values tested with linear or with logistic regression analyses as appropriate adjusted for age and/or sex; †n=11 (smoking status and diabetes was not recorded in one subject); ‡log-transformed prior to analysis.**

Table 2. *Changes in LDL cholesterol, coenzyme Q10 and oxidative stress markers during the study*

Variable	At baseline	At 1 week	At 6 weeks	At 12 weeks	P-value*	P-value**
LDL cholesterol, mmol/L						
- Atorvastatin group (n=12)	3.0 ± 0.8	2.4 ± 0.5	2.0 ± 0.4	2.0 ± 0.5	**<0.001**	0.15
- Simvastatin group (n=18)	2.9 ± 0.6	1.9 ± 0.5	1.6 ± 0.5	1.7 ± 0.5	**<0.001**	
Coenzyme Q10, µmol/L						
- Atorvastatin group (n=12)	0.89 ± 0.37	0.78 ± 0.26	0.74 ± 0.22	0.67 ± 0.23	**<0.001**	0.84
- Simvastatin group (n=18)	0.93 ± 0.29	0.79 ± 0.25	0.66 ± 0.20	0.62 ± 0.17	**<0.001**	
Plasma malondialdehyde, µmol/l						
- Atorvastatin group (n=12)	7.7 ± 3.3	7.1 ± 3.0	8.0 ± 3.3	8.4 ± 3.1	0.10	0.97
- Simvastatin group (n=18)	7.6 ± 2.8	7.9 ± 3.1	7.9 ± 3.1	7.1 ± 2.8	0.55	
Plasma myeloperoxidase, µg/l						
- Atorvastatin group (n=12)	56.8 ± 15.8	59.2 ± 16.9	55.4 ± 17.3	56.7 ± 14.6	0.64	0.48
- Simvastatin group (n=18)	51.9 ± 13.6	50.5 ± 13.4	48.7 ± 10.9	53.4 ± 16.0	0.32	
Urinary F2-isoprostanes, pmol/mmol creatinine						
- Atorvastatin group (n=11)	96.0 ± 39.2	87.7 ± 40.7	86.0 ± 32.6	80.0 ± 32.2	**0.023**	0.60
- Simvastatin group (n=17)	80.2 ± 42.4	80.4 ± 38.5	86.1 ± 43.6	85.5 ± 33.5	0.26	
Urinary 8-OHdG, nmol/mmol creatinine						
- Atorvastatin group (n=16)	1.34 ± 0.48	1.29 ± 0.43	1.10 ± 0.32	1.25 ± 0.41	0.16	0.08
- Simvastatin group (n=18)	1.67 ± 0.67	1.61 ± 0.65	1.61 ± 0.61	1.41 ± 0.53	0.98	

*** P-value: significance of change during study period; ** P value: significance of difference between the two treatment allocations.**

significantly during the treatment period of 12 weeks with no significant differences between the simvastatin group and the atorvastatin group (p>0.05). LDL cholesterol and coenzyme Q10 were positively and significantly associated at baseline (r=0.52, p<0.01), and at each time point during statin treatment. No significant changes in plasma MPO and MDA were observed during the treatment period or between the two treatment groups (*table 2*). Urinary F2-isoprostanes decreased gradually and significantly in the atorvastatin group but not in the simvastatin group. However, the between-group difference in F2-isoprostane reduction was not significant. There was a trend towards a greater reduction in urinary 8-OHdG in the simvastatin group versus the atorvastatin group, which was not statistically significant (p=0.08).

DISCUSSION

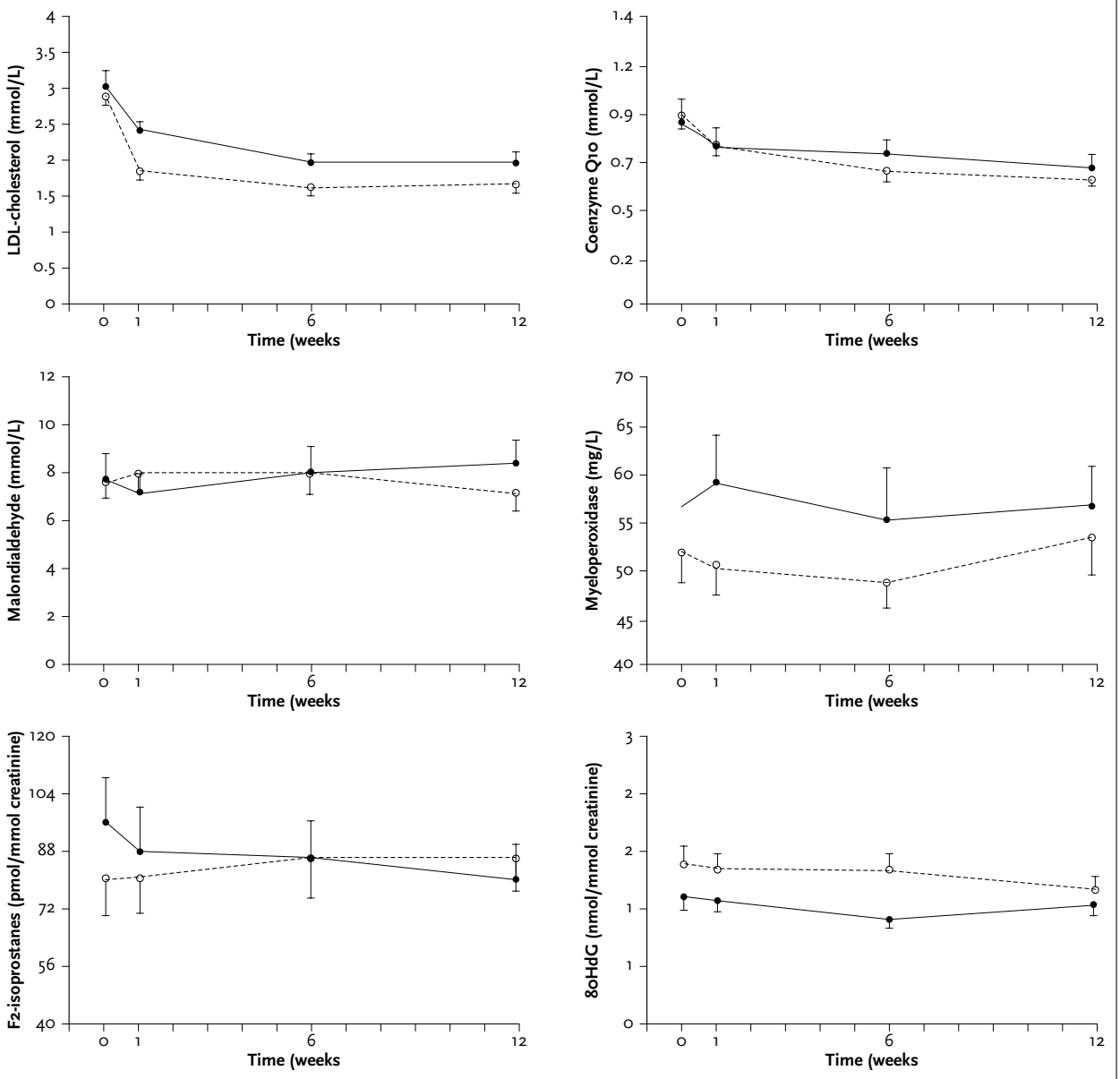
In the present study it was observed that after 12 weeks of treatment with atorvastatin and simvastatin resulted in an equimolar LDL cholesterol reduction, with simvastatin having an initial faster effect in the first week. In addition, a decrease in plasma coenzyme Q10 concentration was observed in both treatment arms. Neither atorvastatin nor simvastatin affected levels of MPO, MDA and 8-OHdG. Although F2-isoprostanes decreased significantly in the atorvastatin group, no statistical differences were observed between the two statin groups.

Effects of atorvastatin and simvastatin on coenzyme Q10 and oxidative stress markers

In addition to the cholesterol-lowering effects of statins, several studies have indicated that the beneficial effects of statins may be due to pleiotropic effects. Mevalonic acid, the product of (HMG-CoA) reductase reaction, is the precursor not only of cholesterol but also of nonsteroidal isoprenoid compounds. It is thought that many of the pleiotropic effects are mediated by inhibition of isoprenoids, such as GTP-binding proteins, which may serve as lipid attachments for intracellular signalling molecules.[23] As a result of the common biosynthetic pathway, the concentration of isoprenoid-containing plasma coenzyme Q10 may also be affected by statin treatment. Indeed, after 12 weeks of intervention, we observed a 25% and 34% reduction in plasma coenzyme Q10 levels in the atorvastatin and simvastatin group, respectively.

The principal and most studied product of polyunsaturated fatty acid peroxidation is MDA. The effect of both statins on plasma MDA concentration has been investigated in several studies. As reported by one research group, atorvastatin compared with simvastatin significantly reduced MDA levels in patients with coronary heart disease[7] as well as in patients with type 2 diabetes mellitus.[8] During statin treatment, MDA levels were also decreased in women with polycystic ovary syndrome, but no differences were observed between the atorvastatin and simvastatin group.[9] Although MDA is widely used as a proxy of oxidative damage, the validity of it has been criticised by lack of specificity and problems with post-sampling formation.[24] Furthermore, the most common MDA methods are insufficiently sensitive and are confounded by interferences.[12] To increase the specificity of the MDA assay we used HPLC with fluorescence detecting.[20] Using this technique, no significant differences in MDA concentration were seen in the two statin groups during follow-up.

Figure 2. *Changes in mean LDL cholesterol, coenzyme Q10, malondialdehyde, myeloperoxidase in plasma, and F2-isoprostanes and 8-hydroxy-2-deoxyguanosine (8-OHdG) in urine measured at baseline and after 1, 6, and 12 weeks using simvastatin (open circles) and atorvastatin (closed circles). Error bars indicate SE.*



Myeloperoxidase (MPO) is linked to oxidative stress by its role in catalysing the formation of oxidising agents.[25] Atorvastatin significantly reduced serum MPO in patients with acute coronary syndrome.[26] In contrast, Meuwese *et al.* found significantly increased MPO levels in heparin plasma after two-year treatment with atorvastatin 80 mg or simvastatin 40 mg.[27] This discrepancy can probably be explained by the type of collection tube (presence and type of anticoagulant) used to sample blood. EDTA plasma is the preferred specimen for measurement of MPO concentration as it appears unaffected by *ex vivo* release of MPO from leukocytes.[19] In the present study we did not observe differences in MPO measured in EDTA-plasma between groups or separately in either group.

The effect of statin treatment on 8-OHdG has been investigated before. In a cross-sectional evaluation of haemodialysis patients no differences in 8-OHdG levels were seen between subjects with or without statin treatment.[28] Notably, the results of this study were not stratified for the type of statin; i.e. simvastatin, atorvastatin, fluvastatin, or pravastatin. In contrast to atorvastatin, the effect of simvastatin on 8-OHdG has

not yet been studied in humans. It has been shown that atorvastatin did not have a significant effect on urinary 8-OHdG in hypercholesterolaemic patients.[29] In contrast, 8-OHdG concentrations in urine decreased significantly in patients with type 2 diabetes mellitus after one month of atorvastatin administration, but changed little at two and three months.[30] These studies all have in common that 8-OHdG was analysed by ELISA, which may not be the preferred procedure for its measurement. Commercial ELISAs seem to overestimate 8-OHdG in urine due to cross reaction of urea,[31] and therefore interpretation is adversely affected by methodological inaccuracies.[32] LC-MS/MS is reliable, sensitive and highly selective compared with other techniques and for these reasons we used this method in the present study. Using LC-MS/MS we did not observe effects on 8-OHdG urine levels upon treatment with either simvastatin or atorvastatin.

Previous studies examining the impact of statin treatment on F2-isoprostanes in urine showed inconclusive outcomes. Significantly decreased levels were found in some studies,[33-36] while other papers reported no effect on the concentration of F2-isoprostanens following statin treatment.[37-40] Although different statins were evaluated in those studies, the type of statin could not explain this disagreement. Apart from one study,[37] urinary F2-isoprostanes were determined by immuno-assays. Importantly, several studies compared immuno-assays with mass spectrometry for the measurement of F2-isoprostanes concentrations,[41-43] and without exception a poor correlation was found due to overestimating by the immuno-assay procedure. As a result, it has been concluded that an immuno-assay based method is not a valid substitute for techniques based on mass spectrometry, because both techniques are not equivalent and may not even measure the same compound. Therefore we used LC-MS/MS in the present study. After 12 weeks a slight but significant reduction was observed in the atorvastatin group, which was absent in the simvastatin group. However, the changes in F2-isoprostanes between the two treatment allocations was not significant.

Strengths and limitations of the study

There are some limitations to this study. In each group a relatively small number of subjects were examined, but clinically important effects of statins on oxidative stress would have undoubtedly been observed. In addition, the study population was rather heterogeneous, but all subjects join an increased CVD risk and benefit statin treatment. The currently most specific markers of oxidative stress, F2-isoprostanes and 8-OHdG, were measured in urine, but not in plasma. We preferred urine because both markers are less stable during handling and storage of plasma. Moreover, urine concentrations may better reflect systemic oxidative stress. Major strengths of our study were the measurement of oxidative stress markers at four different time points using sensitive and specific techniques.

In summary, simvastatin caused a faster initial LDL lowering than atorvastatin, but the overall LDL cholesterol reduction was comparable. With the exception of a slight reduction in urinary F2-isoprostanes in the atorvastatin group, no oxidative stress lowering effects of the two statins were seen during the follow-up of 12 weeks using specific markers of oxidative stress measured by selective techniques. Lowering of oxidative stress in patients at high risk for CVD may not be an important aspect in the protective pleiotropic effects of atorvastatin and simvastatin.

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DISCLOSURES

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CASE REPORT

Why you should ask your patients about their fishing hobbies

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ABSTRACT

Patients who use immunosuppressive agents, in particular medication that blocks tumour necrosis factor-α, are at risk for mycobacterial infections. Besides the typical *Mycobacterium tuberculosis* infection, also atypical mycobacterial disease may occur. Here we demonstrate two patients with such atypical mycobacterial infection due to swimming and fishing water contact. We propose that patients, before starting with immunosuppressive therapy, are counselled about risk factors for mycobacterial disease.

KEYWORDS

TNF-α, ulcer, Mycobacteria

What was known about this topic?
Tumour necrosis factor alpha (TNF-α) antagonists are important in the treatment of various autoimmune and inflammatory diseases. Mycobacterial infection can complicate treatment and demand cessation of therapy.

What does this add?
Cutaneous mycobacterial infections in patients using TNF-α antagonists may present atypically. Asking about fishing hobbies and aquariums can be an important link to diagnosis. Despite adequate antibiotic regimens, reintroduction of the TNF-α antagonist can give rise to a relapse of infection.

INTRODUCTION

Tumour necrosis factor alpha (TNF-α) antagonists, such as adalimumab, play a pivotal role in the treatment of various autoimmune and inflammatory diseases. The drawback is the increased susceptibility for mycobacterial infections. Patients are routinely screened for *Mycobacterium tuberculosis* before starting therapy. The increased risk for atypical (cutaneous) mycobacterial infections such as *Mycobacterium marinum,* however, should also be taken into consideration.[1,2] We report two cases of *M. marinum* infection with an atypical presentation in patients on adalimumab.

CASE REPORTS

Case one, a 69-year-old male, treated for rheumatoid arthritis with methotrexate, prednisolone, and since one year also adalimumab, was referred with erythematous-livid (pustulo)papules and a few partly necrotic nodules on his right lower back, hand, and both legs (*figure 1*). He had a history of fishing in Dutch open freshwater and regularly cleaned his granddaughter's freshwater aquarium. Histology of a nodule showed a granulomatous infiltrate with acid-fast bacilli in the Ziehl-Neelsen and Wade-Fite stain; polymerase chain reaction (PCR) and culture were positive for *M. marinum* or *ulcerans*. *M. marinum* was suspected and adalimumab was interrupted, while ethambutol and clarithromycin were initiated. Four months later, when the patient was in clinical remission of the mycobacterial infection, etanercept was introduced because of rheumatoid arthritis disease activity, provoking a relapse. Etanercept was withdrawn and rifampicin was added.

The second case, a 55-year-old male, treated with adalimumab during the last year for psoriatic arthritis, presented with multiple small ulcers on his left calf.

Figure 1. *Mycobacterium marinum infection. Clinical presentation of case 1. Multiple lenticular to nummular erythematous-livid papules and nodules (size 5-30mm) with central crusts, and few miliary to lenticular pustules (2-5mm) on the right upper and lower leg, left lower leg, right abdomen and right hand*

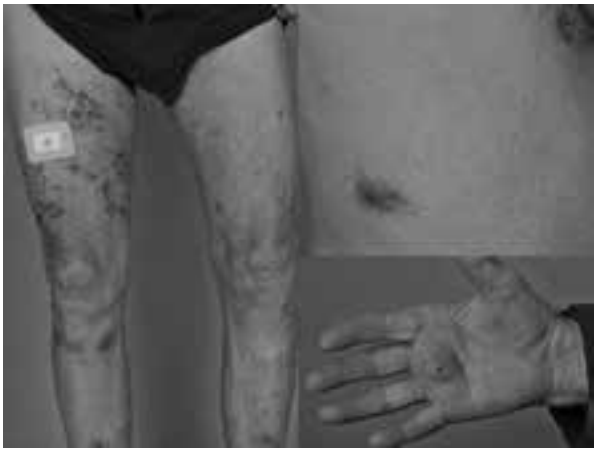
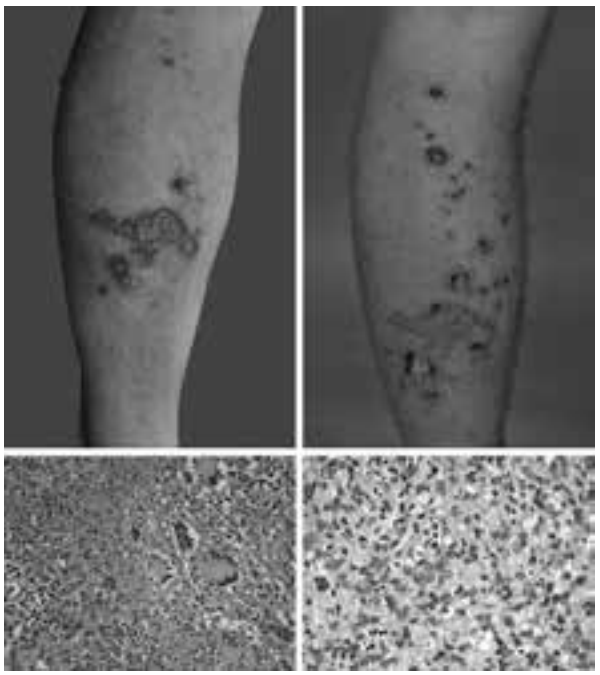


Figure 2. *Mycobacterium marinum infection. Clinical and histological presentation of case 2. Multiple disseminated ulcers (size 5-30 mm) with sharply defined violaceous borders on the left calf, and improvement of lesions during antibiotic treatment for M. marinum. Skin biopsy shows a granulomatous infiltrate with focal necrosis (HE stain, original magnification x 40), while the Wade-Fite stain reveals numerous acid-fast bacilli (original magnification x 400)*



Because of positive bacterial swabs he was consecutively treated with three regimens of different antibiotics, not resulting in wound healing. Further history, specifically on contact with water prior to the appearance of the ulcers, revealed that he went bathing in the Dead Sea in Israel after a minor trauma on his left calf and, after returning to the Netherlands, had frequent contact with open freshwater while fishing. Histopathology (border ulcer) showed granulomatous infiltrates with acid-fast bacilli in the Ziehl-Neelsen and Wade-Fite stain (*figure 2*). PCR and culture of an ulcer indicated *M. marinum* or *ulcerans*; given the history, *M. marinum* was suspected. Following cessation of adalimumab and initiation of ethambutol and clarithromycin clinical improvement was achieved (*figure 2*). When adalimumab was reintroduced four months later, reactivation ensued. Adalimumab was therefore withdrawn indefinitely.

DISCUSSION

Cutaneous mycobacterial infections are usually self-limiting, showing one or a few nodules in a sporotrichoid pattern on the dorsal surfaces of hands or feet. In contrast, patients using TNF-α antagonists demonstrate a progressive course of disseminating ulcers and nodules, due to impaired granuloma formation.[3] Various types of TNF-α antagonists have different pharmacological features and different risks of infections. Recently *M. marinum* infections were reported after the use of various TNF-inhibiting agents, but up to now adalimumab has only once been implicated.[4] Reintroduction or switch to an alternative TNF-α antagonist under continued antibiotic regimen is not always successful as both our cases confirm.[5] Despite antibiotic treatment as in immunocompromised patients, both cases developed a relapse after reintroduction of TNF-α blockade. Withdrawal of adalimumab had unfavourable effects on the patients' medical condition and quality of life: both experienced a disabling relapse of arthritis.

CONCLUSION

Our cases underline that patients' hobbies may carry a risk of infection with *M. marinum*. We propose that, before starting immunosuppressive therapy, patients are counselled about this risk, as mycobacterial infections not only require long-term antibiotic treatment, but also withdrawal of otherwise highly effective medication.[6] The risk of relapse after reintroduction of TNF-α blockade emphasises the value of prevention.

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Conflicts of interest:
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PHOTO QUIZ

A patient with a tumour in the breast and extensive haematomas

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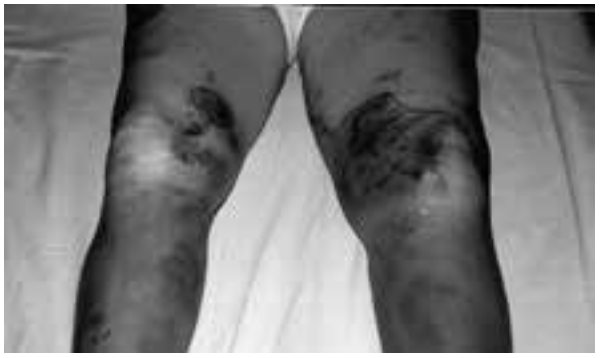
CASE REPORT

A 74-year-old woman was admitted to hospital due to extensive haematoma on both legs and swelling of the knees. The painless haematomas had arisen spontaneously. There was no history of trauma, haematemesis or gum bleeding. On admission the patient complained of a dry mouth, burning sensation of the eyes and fatigue for the last two months. Her social history revealed that she lived alone and prepared her own food. She ate mainly white bread without toppings three times a day and porridge. As a supplement for the lack of fruit and vegetables, she had started vitamin B supplements. She did not use any other medications or alcohol. A year before admission a general physician had diagnosed a lump in the right breast. However, due to strong religious considerations, she had refused further medical advice. On physical examination a tired looking woman was seen. Blood pressure was 140/90 mmHg, the pulse was regular at 120 beats/min. A 4 cm large tumour was palpated in the right mamma. The abdomen and both legs showed large skin haemorrhages, up to 15 cm in diameter, enclosed by not sharply defined erythema (*figure 1*). Routine laboratory investigations showed a haemoglobin level of 6.6 mmol/l, otherwise normal electrolytes, kidney function and serum albumin (36 g/l). The prothrombin time (PT), partial thromboplastin time (aPTT) and thrombocyte count were within normal ranges.

Figure 1. *Skin haemorrhages of both legs*



WHAT IS YOUR DIAGNOSIS?

See page 373 for the answer to this photo quiz.

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A patient with pure red cell aplasia after allogenic stem-cell transplantation

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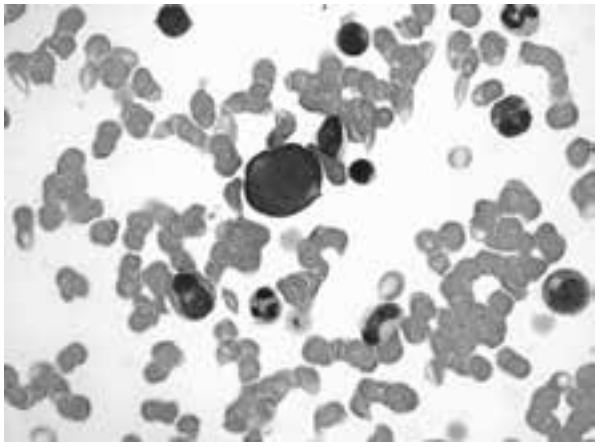
CASE REPORT

A 58-year-old male patient was seen on our outpatient clinic with persistent anaemia after partial T-cell depleted allogenic stem-cell transplantation because of hypoplastic myelodysplastic syndrome (MDS). To keep his haemoglobin level above 5.0 mmol/l he received two units of blood transfusion every two weeks. There were no signs of occult blood loss, infectious diseases or graft-versus-host-disease. The patient was on antiviral medication and trimethoprim-sulfamethoxazole (TMP-SMZ) as *Pneumocystis jiroveci* pneumonia (PJP) prophylaxis. Immunosuppressant drugs were slowly tapered and finally stopped two months after transplantation. His haemoglobin level did not improve after discontinuation of TMP-SMZ.

Physical examination showed no abnormalities. Laboratory investigation revealed: haemoglobin 4.8 (8.4-10.8 mmol/l), leucocytes 4.3 (4.0-11.0 x10^9/l), thrombocytes 283 (150-400 x10^9/l), and reticulocytes 3.8 (25-125 x10^9/l). Vitamin B_{12} and folic acid levels were normal. Ferritin level was high because of repeated blood cell transfusions. Chimerism analysis showed 100% donor chimerism. A crista biopsy and aspiration was performed nine months after transplantation to look for the cause of anaemia.

Figure 1. *Giemsa stained bone marrow smear*



WHAT IS YOUR DIAGNOSIS?

See page 374 for the answer to this photo quiz.

Netherlands
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PHOTO QUIZ

An accidental finding of multiple abdominal and pelvic tumours

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CASE REPORT

A 44-year-old man with a history of a splenectomy in childhood due to a traumatic splenic rupture presented at the emergency department with fever, abdominal discomfort, and pain in the left buttock. He received pneumococcal vaccinations every five years. Upon physical examination painful flexion of the left hip was observed, while abdominal examination was normal. A contrast enhanced computed tomography (CT) of the abdomen and pelvis showed fluid around the left hip joint, which was aspirated, cultured and diagnosed as a septic arthritis with *Streptococcus agalactiae*. In addition, the CT revealed three tumours with homogenous contrast enhancement in the left upper abdomen with diameters of 2.5 to 3.5 cm, and a tumour in the pelvis of 7.6 x 4.3 cm (*figure 1*).

WHAT IS YOUR DIAGNOSIS?

See page 375 for the answer to this photo quiz.

Figure 1. *Contrast enhanced CT images showing tumours with homogeneous contrast uptake in the pelvis (left) and in the left upper abdomen (right). The tumours are indicated by the white arrows*

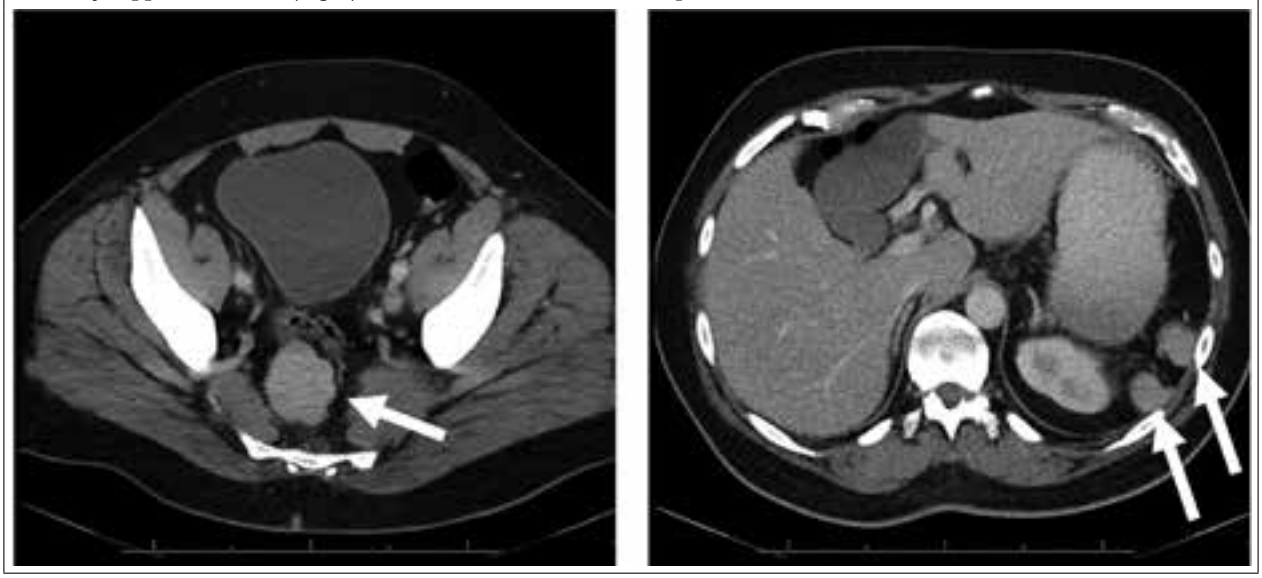

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PHOTO QUIZ

Multiple spots on bone: diagnostic challenge or spot diagnosis?

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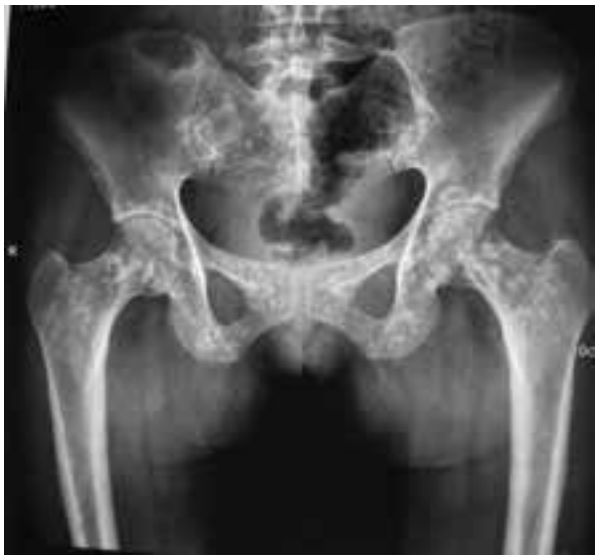
CASE REPORT

A 25-year-old female presented to us with complaints of pain over her right hip for one day following a fall from standing height. On examination there was no swelling/effusion/tenderness over the hip joint. Range of motion was full and painless. Radiographs showed no evidence of fracture or joint effusion, but multiple scattered lesions of variable size were seen over the whole pelvis and proximal femur *(figure 1)*.

WHAT IS YOUR DIAGNOSIS?

See page 376 for the answer to this photo quiz.

Figure 1. *Anteroposterior radiographs of the pelvis showing multiple sclerotic foci of variable sizes in the ileum, acetabulam, femoral head and proximal femur without narrowing of the joint spaces*



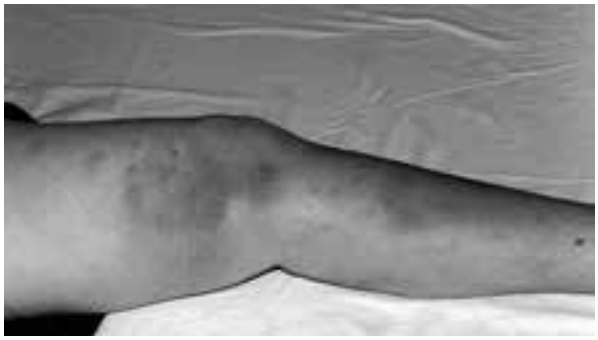
ANSWER TO PHOTO QUIZ (PAGE 369)

A PATIENT WITH A TUMOUR IN THE BREAST AND EXTENSIVE HAEMATOMAS

DIAGNOSIS

Additional determinations of the vitamin status revealed vitamin C and D deficiencies. The vitamin C level was <5 μmol/l (10-15 μmol/l), confirming the clinical diagnosis of scurvy. It took some effort to convince the patient of the necessity for a balanced diet, but a normal intake could be achieved. Following ten days of supplemental vitamin C prescription, an almost full recovery from the haemorrhages was seen (*figure 2*).

Figure 2. *Recovery of the skin following vitamin C suppletion*



DISCUSSION

The skin is an accessible organ and may provide the internist with major clues to a clinical diagnosis. Haemorrhages, or extravasation of blood in the skin, are often described as purpura. This term simply refers to purple or colouring of the skin. Haemorrhages can be divided on the one hand into small lesions, which do not bland with pressure, known as petechiae. These pin point lesions, up to 3 mm, are well-known features of capillary and platelet disorders. Larger lesions on the other hand, called ecchymosis, are frequently seen in trauma and clotting factor deficiencies. However, many other diseases may present with (non)-palpable purpura, for instance thromboembolic, immune complex disease and small vessel vasculitis.[1,2] Vitamin C or ascorbic acid is a necessary cofactor in the hydroxylation of pro-collagen. Reduced collagen formation is associated with capillary fragility, bleeding risk and poor wound healing. Bleeding of the gums and gingivitis are well-known symptoms in this classic disease. Small haemorrhages can merge into a vast haematoma, usually localised at pressure points, such as the buttocks and legs ('saddle' phenomenon). In accordance with a citation of Sydenham centuries ago,[3] 'where the scurvy ends, then the dropsy begins', we think that the patient's vitamin B supplements prevented her from developing neuropathy. A more detailed history in our patient revealed that she had chronic diarrhoea too. The patient believed that this complaint was due to various food components, fruits in particular, and had further limited her daily intake. The lump in her breast eventually turned out to be breast cancer.

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A PATIENT WITH PURE RED CELL APLASIA AFTER ALLOGENIC STEM-CELL TRANSPLANTATION

DIAGNOSIS

The diagnosis is pure red cell aplasia due to Parvo B19 infection.

Giemsa stained bone marrow smear showed a red cell aplasia. Numerous giant proerythroblasts were present in the aspirate, with a high nuclear/cytoplasmic ratio. In combination with pure red cell aplasia, these changes in erythroid precursors are highly suggestive of infection with human parvovirus B19. Immunohistochemistry for parvovirus showed nuclear expression of infected cells. DNA PCR for parvovirus was performed on blood and was positive.

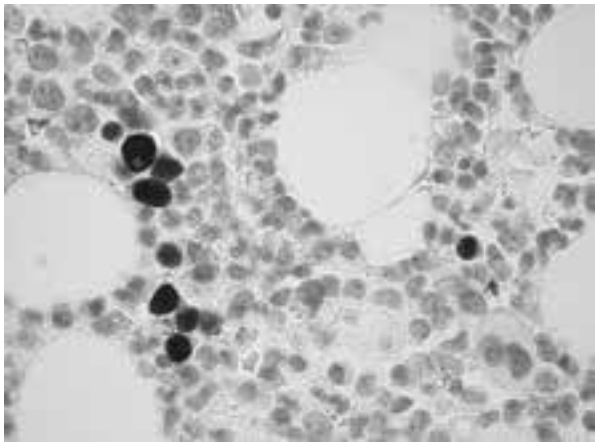
Most cases of parvovirus B19 infection are asymptomatic. The most common presentation of infection is erythema infectiosum, known as the fifth disease, a childhood exanthema characterised by a 'slapped cheek' rash.[1]

In the absence of antiviral immunity, as in our immunocompromised patient, red-cell aplasia can be a manifestation of persistent Parvovirus B19 infection. Parvovirus B19 infects erythroid progenitor cells by binding to the receptor known as the P antigen. Subsequent viral replication in erythroid progenitor cells leads to cellular lysis, which is characteristically manifested as pure red cell aplasia on bone marrow examination.[2]

The anaemia is severe and requires transfusions. A persistent B19 infection often responds to a five- or ten-day course of immune globulin at a dose of 0.4 g per kilogram of body weight.[3,4]

After initiation of immune globulin substitution the haemoglobin level in our patient improved to 6.7 mmol/l within two weeks.

Figure 2. *Immunohistochemical staining for Parvovirus highlights the viral inclusions*



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ANSWER TO PHOTO QUIZ (PAGE 371)

AN ACCIDENTAL FINDING OF MULTIPLE ABDOMINAL AND PELVIC TUMOURS

DIAGNOSIS

In our patient, who was admitted with a septic arthritis, we made an accidental finding on CT of multiple tumours in the abdomen and pelvis. The tumours had a homogenous contrast enhancement, with a Houndsfield unit value of 86 after contrast administration. The subsequent ultrasound examination corroborated with the homogenous aspect of the tumours on CT imaging. We established the diagnosis of splenosis based on the fact that the patient had a history of a traumatic splenic rupture and splenectomy, the masses had a similar attenuation on CT to the expected appearance of otherwise normal splenic tissue, and the tumours had a homogenous aspect on CT as well as ultrasound imaging. Based on the combination of these findings malignant disease is unlikely.

Splenosis is a rare benign condition of heterotopic autotransplantation of splenic tissue in another anatomic compartment that can occur after splenic rupture or splenectomy.[1] The cause of splenosis most likely pertains to direct seeding or haematogenous spread of splenic tissue. It occurs most frequently in the abdominal and pelvic cavities, but can also be found in other locations, such as in the liver, kidney, pancreas, thorax, cerebrum or subcutaneous tissue[1,2] Splenosis differs from accessory spleens, which are congenital and supplied by the splenic artery, and usually found near the splenopancreatic or gastrosplenic ligament. Splenosis is often found incidentally, but can also present symptomatically, for example with gastrointestinal bleeding, haemoptysis, chest pain, bowel obstruction or hydronephrosis.[1] It can be challenging to differentiate splenosis from malignant disease based on CT and ultrasound imaging studies.[3] Therefore, the medical history is pivotal in guiding the diagnostic process. If the diagnosis remains unsure, nuclear scintigraphy using heat damaged red blood cells tagged with technetium-99 can be performed to establish a definite diagnosis, as splenic tissue has a high uptake of damaged erythrocytes.[4]

Our patient developed septic arthritis, which raises the question whether splenic tissue in splenosis is functional or not? Connell *et al.* recently reviewed the literature on this topic and discussed that autotransplanted splenic tissue has a different microanatomy, with less white pulp, and does not have the phagocytic capacity of normal splenic tissue.[5] Multiple case series have reported fatal pneumococcal and meningococcal septicaemia despite the presence of splenosis.[5] Therefore, splenosis should not be considered protective against infection. Post-splenectomy patients with signs of infection should be treated aggressively, regardless of the presence of splenosis.

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DIAGNOSIS

Osteopoikilosis was first described in 1915 by Albers-Schönberg as a sclerosing bone dysplasia of unknown cause.[1] It is also referred to as spotted bones or osteopathia condensans disseminata. A diagnosis of exclusion, cases may be under-reported. Prevalence in the general population is unknown, but an older retrospective review reported an estimated prevalence of 1 in 50,000.[2] The lesions have been described in all age groups, and although prevalence studies have shown a higher frequency among men, the apparently unequal sex distribution may be a result of referral bias in the literature (men are more likely than women to present to hospital with traumatic injuries requiring radiological investigation).[2] Osteopoikilosis exists in hereditary (autosomal dominant transmission) and sporadic forms and is characterised by defective endochondral bone formation. It is associated with a heterozygous mutation in LEMD3 that encodes an inner nuclear membrane protein; the precise function of this protein remains to be elucidated.[3]

The condition is usually asymptomatic, but in 15-20% patients there may be joint pain and joint effusions.[4] In our patient, the pain was relieved by oral analgesics (diclofenac sodium) for one week. Most reported cases of osteopoikilosis are identified during the investigation of unrelated problems in which there is no clinical history suggestive of either malignant or systemic disease. In such situations, no further workup is necessary. The characteristic radiological feature is multiple, punctate, sclerotic, round or oval foci symmetrically distributed in a predominantly periarticular fashion within the epiphyseal and metaphyseal regions. The lesions are noted in a fairly symmetric distribution, especially around the knee and shoulder, along with the pelvis, carpal and tarsal bones. The lesions are less common in the skull, ribs, vertebral bodies and mandible. Although further investigation is unnecessary in typical osteopoikilosis, when radionuclide bone scans are performed, their results are negative. The microscopic features of the lesion are identical to those encountered in bone islands. In clinical and radiological follow-up of osteopoikilosis, the lesions remain stable.

Osteopoikilosis is typically an asymptomatic incidental finding, but it can be associated with other diseases. The major differential diagnoses are osteoblastic metastases, and tuberous sclerosis. The symmetric distribution, the propensity for epiphyseal and metaphyseal involvement, and the uniform size of the foci are features that suggest osteopoikilosis, a diagnosis that is supported by a normally appearing bone scan. Skeletal metastasis and tuberous sclerosis are characterised by asymmetric distribution, common involvement of axial skeleton, including spine, osseous destruction, variation in size, and positive scintigraphic findings. In these situations bone scintigraphy plays an important role in distinguishing them from osteopoikilosis.

In conclusion, although benign, osteopoikilosis may sometimes be difficult to diagnose. The characteristic imaging features and bone scintigraphy may help in reaching the diagnosis and excluding other differential diagnosis.

ACKNOWLEDGEMENT

We thank the patient for allowing us to publish this report and image.

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SPECIAL REPORT

Treatment of hepatitis C monoinfection in adults – Dutch national guidelines

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ABSTRACT

In this new Dutch guideline for hepatitis C virus infection we provide recommendations for the management of hepatitis C infection. Until 2012 the standard for treatment consisted of pegylated interferon alpha (peg-IFNα) and ribavirin. The advent of first-generation direct antiviral agents such as boceprevir and telaprevir has changed the concept of treatment of adult chronic hepatitis C genotype 1 infected patients.

There are three benefits of boceprevir and telaprevir. They increase the likelihood of cure in 1) naive genotype 1 patients and 2) in patients who did not respond to earlier treatment with peg-IFNα and ribavirin, while 3) allowing shortening of treatment duration from 48 weeks to 24 or 28 weeks, which is possible in 40-60% of non-cirrhotic naive (boceprevir and telaprevir) and relapsing patients (telaprevir).

The use of boceprevir and telaprevir is associated with multiple side effects and awareness of these side effects is needed to guide the patient through the treatment process. This guideline, formulated on behalf of The Netherlands Association of Hepato-gastroenterologists, The Netherlands Association of Internal Medicine, and The Dutch Association for the Study of Liver Disease, serves as a manual for physicians for the management and treatment of acute and chronic hepatitis C virus monoinfection in adults.

KEYWORDS

Boceprevir, hepatitis C, guidelines, pegylated interferon, protease inhibitor, ribavirin, telaprevir

INTRODUCTION

Hepatitis C virus (HCV) infection resulting in chronic liver disease is highly prevalent in Europe.[1] With the introduction of interferon therapy, later combined with ribavirin, eradication of HCV infection became a reality. The last innovation in this field came a decade ago with the introduction of pegylated interferon alpha (peg-IFNα). Further advances in the therapy of HCV infection were in the most part restricted to refinements of the existing dual therapy with peg-IFNα and ribavirin (combination abbreviated to PR).

The watershed in the field came with the clinical introduction of two direct-acting antiviral agents (DAAs) boceprevir (Victrelis®) and telaprevir (Incivo®). From 2012 these two DAAs were allowed on the market in the Netherlands and are reimbursed by the health insurance companies for the treatment of chronic HCV genotype 1 infection in adults with compensated liver disease (including cirrhosis). Phase 3 studies, including more than 2700 patients, have documented the high antiviral potency of these agents against HCV genotype 1.[2-6]Accordingly,

the treatment of chronic HCV genotype 1 infected patients has changed and led to the introduction of new national guidelines in several countries, and an update of the EASL and AASLD guidelines.[7-9] The last Dutch guideline on the treatment of HCV infection stems from 2008.[10] In order to guide the clinician through the changed therapeutic environment we provide the reader with a completely revised guideline with concise recommendations for the management and treatment of HCV monoinfection in adults. For the complete guideline we refer to www.mdl.nl.

Table 1. *Treatment responses*

Category	Characteristics
Rapid viral response (RVR)	HCV RNA undetectable at week 4
Extended rapid viral response (eRVR)	HCV RNA undetectable at week 4 and week 12
Early viral response (EVR)	HCV RNA undetectable at week 12 or a decrease by >2 log
Delayed viral response (DVR)	HCV RNA >2 log decrease but detectable at week 12, undetectable at week 24
End of treatment response (ETR)	HCV RNA undetectable at end of treatment
Sustained viral response (SVR)	HCV RNA undetectable after 24 weeks of follow-up

BACKGROUND

The clinical progression of chronic HCV infection varies among patients. Some have only minimal structural hepatic changes even after prolonged infection, while others rapidly develop complications such as cirrhosis and hepatocellular carcinoma (HCC).[11,12] The progression of histological deterioration is independent of HCV genotype and the concentration of HCV RNA in plasma (viral load), but is related to host factors such as gender, obesity, presence of concomitant liver disease, lifestyle aspects (e.g. alcohol use), and the existence of an untreated co-infection with hepatitis B virus (HBV) or human immunodeficiency virus (HIV).[13-15] The overall mortality is increased due to cirrhosis and HCC, but also due to an increased risk of extrahepatic manifestations such as cardiovascular and renal diseases.[16,17] In contrast, curing HCV infection with antiviral therapy diminishes the risk of cirrhosis and HCC and consequently improves survival compared with patients with persistent viraemia.[18,19]

There are at least six distinct HCV genotypes. In the Netherlands, ~50% of chronic hepatitis C is caused by genotype 1a and 1b, ~30% by genotype 3, whereas genotype 2 and 4 both account for ~10% of chronic HCV infected patients. Genotype 5 and 6 are uncommon in the Netherlands.[20,21]

The primary goal of therapy is to eliminate HCV infection which is defined as undetectable plasma HCV RNA 24 weeks after termination of treatment defined as sustained virological response (SVR) (see *table 1* for abbreviations). With PR given for 24 or 48 weeks, SVR can be achieved in 40-60% of HCV genotype 1 or 4 infected patients and in 70-80% of patients infected with HCV genotype 2 or 3.[9,22,23]

NATURAL HISTORY

In Europe, the incidence of acute HCV infection is around 1 per 100,000 persons per year. This probably underestimates the true incidence because acute HCV infection is asymptomatic in approximately 80% of cases.[9] After infection, formation of HCV antibodies can take months, which implies that plasma HCV RNA analysis should be used to diagnose acute HCV infection.[24]

Spontaneous clearance of HCV infection occurs in 20-30%. Spontaneous clearance is unlikely to happen 12 weeks after infection and treatment should subsequently be initiated to prevent development of chronic HCV infection.[25,26]

Persistence of plasma HCV RNA for more than six months constitutes a chronic HCV infection. It is thought that chronic hepatitis C affects ~3% of the world population, i.e. 170 million individuals.[27] The prevalence in the Netherlands varies between 0.1-0.4%.[28,29] European prevalence rates are higher (0.4-4%).[30] Chronic hepatitis C progresses slowly, over a time frame of 15-50 years. Cohort studies suggest that 10-20% of all infected patients will eventually develop end-stage liver disease, typically after two to three decades.[12,31] In cirrhotic patients, the annual rate of HCC is 1-4% and chronic hepatitis C induced HCC accounts for one-third of all HCCs.[11]

INITIAL EVALUATION

As of 2012 treatment of hepatitis C in the Netherlands is preferably restricted to one of the 40 certified and specialised viral hepatitis treatment centres.[32]

The initial evaluation of a chronic hepatitis C patient consists of a detailed medical history evaluation, which includes assessment of the source of the HCV infection, presence of current or past alcohol abuse, and use of concomitant medication. Evaluation includes physical examination with special attention to signs of chronic liver disease, cirrhosis and liver failure (e.g. spider nevi, palmar erythema, gynaecomastia, ascites). Laboratory tests should include a full blood count, liver enzymes and function, thyroid and kidney function, and plasma HCV RNA and genotype.[10] Current guidelines recommend vaccination against hepatitis A and hepatitis B for those who are seronegative.[9,33]

Pretreatment assessment of liver fibrosis or cirrhosis can be important as this may influence indication, strategy and success of treatment.[9,11,34] Abdominal ultrasound, liver biopsy or elastography are therefore part of the work-up. Liver biopsy remains the golden standard for fibrosis assessment. Non-invasive tests such as transient elastography (FibroScan®) or the use of biomarkers may be useful to identify or exclude cirrhosis. However, the ability of FibroScan® to discriminate between fibrosis stage F1 and F3 is limited.[35,36]

Positive predictors of SVR with PR therapy can be classified as pretreatment or on-treatment factors. In general, the most important positive pretreatment predictors for SVR are: response to previous PR-based treatment, e.g. naive patients and patients who relapsed to previous therapy respond better than partial and null responders (see *table 2* for classification of patient categories), interleukin (IL) 28B CC polymorphism (exclusively HCV genotype 1), and low stage of fibrosis. Other predictors are low baseline viral load (<600,000 IU/ml), genotype non-1, non-HIV co-infection, age under 40 years, and non-black race.[37-39] The most important on-treatment positive predictive factor for achieving SVR is attaining a rapid viral response (RVR) (see *table 1*).[40,41] Other known on-treatment factors are decline in haemoglobin concentrations during PR therapy in hepatitis C genotype 1, ribavirin plasma concentrations and treatment adherence.[42-44] With the use of DAAs, the predictive value of IL28B polymorphism is limited.[45] In addition, DAAs are more effective in genotype 1b than in genotype 1a patients.[3,4,46] On-treatment laboratory testing should occur regularly and should include HCV RNA (at the selected time points), haemoglobin, total leucocytes, neutrophils, thrombocytes, and liver enzymes.

Table 2. *Treatment categories according to the host response during previous treatment*

Category	Characteristics
Naive patients	No previous treatment
Relapsers	HCV undetectable at end of treatment, but detectable after 24 weeks of follow-up
Partial responders	>2 log HCV RNA decline at week 12, but detectable HCV RNA at week 24
Null responders	<2 log HCV RNA decline at week 12
Non-responders	Null response or partial response
Viral breakthrough	Detectable HCV RNA at any time during treatment after previous undetectable HCV RNA during antiviral therapy

INDICATIONS AND CONTRAINDICATIONS FOR ANTIVIRAL THERAPY

Treatment should be considered in all patients who do not have contraindications, especially in those with METAVIR F3 and F4 and should be strongly considered in patients with METAVIR F2 fibrosis. In patients with METAVIR ≥F2 alternatively, therapy can be postponed until more DAAs have become available, allowing interferon-free regimens. There are subgroups with limited benefits from chronic hepatitis C treatment. First, elderly patients (age >70 years) or patients with (longstanding) asymptomatic disease and a low stage of fibrosis (METAVIR ≤F2).[47] Second, absolute contraindications (such as decompensated cirrhosis or uncontrolled depression, psychosis, epilepsy, pregnancy or planning to become pregnant, and other severe medical diseases) and relative contraindications (such as thrombocytopenia <90 x 10^9/l, neutrophil count <1.5 x 10^9/l, anaemia (haemoglobin <8 mmol/l), renal insufficiency (GFR <30 ml/min), or ongoing alcohol or drug abuse) may preclude therapy. In patients with relative contraindications benefits of treatment should be balanced carefully against the increased risk of side effects.[9,48] Patients with concomitant HIV or HBV infection or other liver diseases and those with contra-indications listed above, were excluded from the phase 3 studies with boceprevir or telaprevir. As a consequence, treatment strategies formulated below cannot be applied to these patients. Finally, patients with virological failure on boceprevir or telaprevir therapy create a cohort of non-responders. Given the extensive cross-resistance that can develop in patients failing either boceprevir or telaprevir, retreatment with the other drug is not advisable. If treatment is postponed, patients should be monitored yearly. Cirrhotic patients should be subjected to abdominal ultrasound for HCC screening once or twice a year.[49]

ANTIVIRAL THERAPY

Acute hepatitis C

Patients with acute HCV monoinfection should be treated if HCV RNA is still positive three months after exposure, because spontaneous clearance is unlikely to happen at this stage.[26,50] Therapy consists of peg-IFNα monotherapy (peg-IFNα-2a: 180 μg/week, peg-IFNα-2b: 1.5 μg/kg/week) for the duration of 24 weeks. With peg-IFNα monotherapy, SVR rates are more than 90%. The addition of ribavirin has no proven benefit.[26,51]

Acute HCV infection is frequently reported in HIV co-infected male homosexual patients and for management the reader is referred to appropriate guidelines.[52,53]

Chronic hepatitis C

Patients with HCV genotype 1

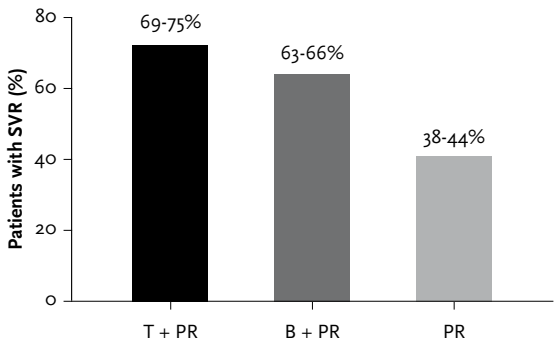
Both boceprevir and telaprevir can only be used in combination with PR for treatment of adult chronic HCV genotype 1 infected patients with compensated liver

disease. Peg-IFNα and ribavirin dosage instructions are either peg-IFNα-2a 180 µg/week in combination with ribavirin 1000 mg (<75 kg) or 1200 mg (≥75 kg) per day or peg-IFNα-2b 1.5 µg/kg/week in combination with ribavirin 800-1400 mg (<65 kg: 800 mg, 65-80 kg: 1000 mg, 81-105 kg: 1200 mg, and >105 kg: 1400 mg). Both peg-IFNα 2a or 2b, can be prescribed with either boceprevir or telaprevir.[54,55] Boceprevir should be taken orally three times a day with eight hour intervals. Telaprevir can be taken two (1125 mg) or three (750 mg) times a day, with 12 and 8 hours intervals, respectively. Telaprevir should be taken with food (preferably containing at least 20 gram of fat) and boceprevir with a small meal to increase bioavailability.[56,57] There are no head-to-head studies that compare boceprevir and telaprevir, which makes it difficult to compare their relative efficacy.[58,59] SVR rates are assumed to be comparable for both DAAs. The main differences are related to the side-effect profiles, the use of a four-week lead-in period with boceprevir, and the duration of DAA treatment.

With the new DAAs SVR rates have increased to 65-75% in treatment naive patients.[2-4,60] Some 70-90% of patients who relapsed after PR treatment achieved SVR with boceprevir or telaprevir triple therapy compared with 25-30% in PR control arms. Partial responders obtained SVR in 40-60% with triple therapy compared with 7-15% with PR alone. Null responders achieved SVR in about 30% with telaprevir therapy in combination with PR, compared with 5% treated with PR alone (*figure 1* and *2*).[5,6]

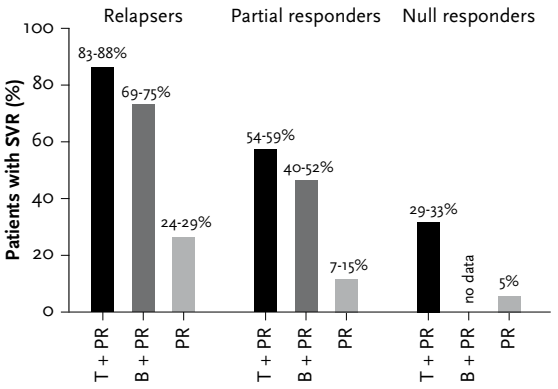
A significant proportion of naive patients (44-65%) in phase 3 studies with boceprevir or telaprevir in combination with PR met the criteria for response-guided therapy (RGT) and can be treated for a shorter period (see 'Treatment strategies'). Success rates are very high in these patients (>90%).[2,4] The main advantages of RGT are that it allows shortening of treatment and prevents unnecessary exposure to side effects.[61]

Figure 1. *SVR rates in treatment naive patients with HCV genotype 1*



PR = peg-IFNα and ribavirin; T = telaprevir; B = boceprevir; SVR = sustained viral response.

Figure 2. *SVR rates in treatment experienced patients with HCV genotype 1*

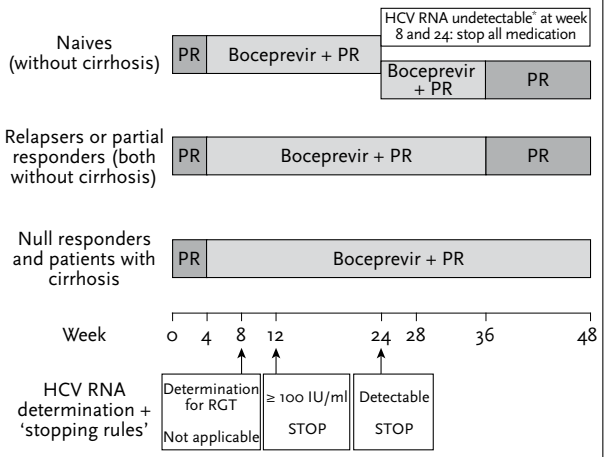


PR = peg-IFNα and ribavirin; T = telaprevir; B = boceprevir; SVR = sustained viral response.

Treatment strategies

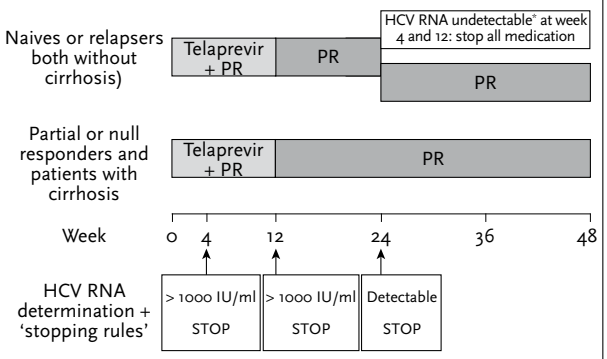
Depending on the host response during previous treatment and the presence of cirrhosis, the optimal treatment strategy for both DAAs follows from *figure 3* and *4*. Important considerations about the implementation of these strategies are described here. First, regarding the rules for discontinuation, alternative time points and tolerated levels of viral load are used in DAA regimens. Second, the concept of RGT is dissimilar with respect to its duration and eligibility of patients. RGT can be applied for non-cirrhotic treatment naive patients (boceprevir and telaprevir) and previous relapsers (telaprevir).[2,4,62] In these cases, duration of treatment can be limited to 24 weeks (telaprevir) or 28 weeks (boceprevir) (*figure 3* and *4*). Accurate quantitative and qualitative plasma HCV RNA measurement is crucial for choosing the right treatment strategy as this is the indicator for treatment success.[2-6] There are several test characteristics that need to be fulfilled: a lower limit of quantification of 25 IU/ml and a lower limit of detection of 10-15 IU/ml are mandatory in the DAA era. In this respect, RGT can only be applied when HCV RNA is undetectable at selected time points.[56,57] It is important that a 'detectable but below the limit of quantification' HCV RNA result does not equal an 'undetectable' HCV RNA result.[63] A small proportion of naive chronic HCV genotype 1 patients with an RVR and favourable prognostic factors (low viral load <600,000 IU/ml, ≥F2 fibrosis, IL28B CC genotype) do not have added benefit from DAAs and can be treated with PR protecting them from DAA side effects.[64] If RVR is not achieved, introduction of boceprevir at week 4 is recommended.[2] On the other hand, retreatment with DAAs in cirrhotic null responders should carefully be discussed considering the low SVR rates (~14%), the lack of alternatives, and likelihood of adverse events.[65]

Figure 3. *Boceprevir treatment strategies*



***with a lower limit of detection of 10-15 IU/ml.**

Figure 4. *Telaprevir treatment strategies*



***with a lower limit of detection of 10-15 IU/ml.**

Patients with HCV genotype 2 and 3

Boceprevir and telaprevir are not registered for the treatment of chronic HCV genotype 2 and 3 infected patients.[66] Current treatment is 24 weeks of peg-IFNα-2a 180 μg/week or peg-IFNα-2b 1.5 μg/kg/week with ribavirin 800 mg a day. If there are baseline factors associated with a poor response, ribavirin should be dosed based weight.[9] SVR rates are around 70-80% in these patients.[9,67]

In case of intolerability for peg-IFNα dosage can be adjusted (peg-IFNα-2a 135 μg/week or peg-IFNα-2b 1.0 μg/kg/week) without compromising SVR rates. Sixteen weeks of treatment with peg-IFNα and weight-based ribavirin can be applied to patients with an RVR who cannot complete 24 weeks of treatment because of severe side effects. This strategy is only applicable for patients with favourable baseline factors. However, with shortened therapy there is a slightly increased risk of viral relapse in genotype 3 patients.[64,68,69]

In patients with chronic HCV genotype 2 and 3 infection without an RVR and concomitant advanced liver fibrosis or cirrhosis or failure on previous treatment, a 48-week treatment strategy may be followed.[34,67]

Patients with HCV genotype 4, 5 and 6

For genotype 4, 5 and 6 current PR consists of 48 weeks of peg-IFNα with weight-based ribavirin (see section 'antiviral therapy of HCV genotype 1 infection' for peg-IFNα and ribavirin dosage). SVR rates range from 43-70%.[70] Naive genotype 4 patients with positive prognostic factors (≥F2 fibrosis, low baseline viral load and an RVR) are eligible for shortened therapy of 24 weeks.[71,72]

VIRAL RESISTANCE

Both boceprevir and telaprevir are highly specific inhibitors of the viral NS3/4A serine protease. The nucleoside sequence of the NS3/4A protease varies among HCV genotypes. As a result, the antiviral activity of the protease inhibitors differs between the HCV genotypes. Both DAAs were specifically designed for HCV genotype 1 and have limited activity against other genotypes.[66,73,74]

The high mutation rate results in a large diversity in the viral population, which may lead to the selection of protease inhibitor cross-resistant variants, resulting in treatment failure. Therefore, neither of these DAAs can be used as monotherapy and can only be prescribed in combination with PR to prevent the emergence of viral resistant strains.[75-77]

DRUG-DRUG INTERACTIONS

Boceprevir and telaprevir are substrates for CYP3A and P-glycoprotein (PgP).[56,57] Compared with boceprevir, telaprevir is a stronger inhibitor of CYP3A and PgP. Drug interactions can be expected when one of both DAAs is used in combination with other drugs which are also CYP3A or PgP inhibitors or inductors enhancing the risk of drug toxicity or a decreased efficacy of the involved drugs. Because of the somewhat different profiles, interactions may vary between the two agents. Therefore, information and advice cannot be implemented equally for both boceprevir and telaprevir. Before starting treatment with DAA-combination therapy, we recommend to check for all possible interactions on http://www.hep-druginteractions.org/, the Dutch handbook for drug interactions with anti-HCV infection agents, and/or consult a pharmacist.[78,79]

Some practical examples: the use of boceprevir and telaprevir leads to impaired efficacy of oral oestrogen containing contraceptives, due to low oestrogen concentrations. Therefore, the use of two nonhormonal containing contraceptives is recommended during and at least two months after cessation of boceprevir or

telaprevir.[80,81] Also, the use of both DAAs with simvastatin should be avoided as concomitant use results in increased drug levels of simvastatin, putting the patient at risk for rhabdomyolysis.[82,83] Furthermore, drug levels of escitalopram, a frequently used selective serotonin reuptake inhibitor (SSRI), are lowered during boceprevir and telaprevir usage.[83]
Supplementary file 1 summarises the most important interactions that should be avoided or interactions that require caution. If information on possible interactions is lacking, consider temporary discontinuation of the drug.

SIDE EFFECTS

PR treatment is frequently accompanied by side effects, such as flu-like symptoms, anaemia, neutropenia, thrombocytopenia, and depression. These side effects influence quality of life and may result in dosage reduction or premature treatment discontinuation. This can be prevented by close monitoring and management of side effects.[42,84]
With the addition of boceprevir and telaprevir to PR new side effects have emerged while other side effects may be aggravated.[85] For example, rash and (anal) pruritus affects ~50% of patients taking telaprevir while dysgeusia occurs in 40% of patients treated with boceprevir.[2-6] The most important side effects and their management strategies are discussed below.

Anaemia

Phase 3 trials have clearly shown that PR with boceprevir, but especially with telaprevir, results in a higher frequency of anaemia than PR alone.[2-6] Ribavirin dose reduction in patients treated with boceprevir or telaprevir does not compromise efficacy and is the first step of choice.[86,87] Ribavirin should be reduced by 200 mg per step. During treatment ribavirin can be up-titrated again when haemoglobin levels are acceptable (≥7.0 mmol/l). Dose reduction of ribavirin as opposed to dose maintenance supported by erythropoietin in patients with triple therapy is equally effective in terms of achieving SVR.[88] If used, erythropoietin agents should be discontinued when haemoglobin reaches the threshold of 7.5 mmol/l.[89] Blood transfusion should be saved for exceptional cases. For patients treated with PR (e.g. non genotype 1 patients), dose reduction should be postponed as long as possible as this negatively influences the chance of SVR.[42] When interference is necessary, ribavirin or peg-IFNα dose reduction, use of erythropoietin agents or blood transfusions can be considered. No recommendation can be given for the preferred strategy.

Neutropenia

The incidence of neutropenia is higher in patients treated with PR in combination with a DAA. Although there is little evidence that neutropenia puts the patient at risk for an infection, current recommendations stipulate peg-IFNα reduction when the neutrophil count falls below 0.75 x 10^9/l. Furthermore, (temporary) discontinuation of peg-IFNα should be performed when the neutrophil count drops further (<0.5 x 10^9/l).[9,90] There is no room for granulocyte colony-stimulating factor because of unclear benefit and high costs.[91]

Thrombocytopenia

Thrombocytopenia <90 x 10^9/l is a relative contraindication for treatment of chronic HCV infection.[9,92] Peg-IFNα reduction is recommended when the platelet count drops below 50 x 10^9/l and should be discontinued when the platelet count falls below 25 x 10^9/l. When the platelet count increases again, peg-IFNα can be restarted at a reduced dose.[9]

Rash management

Rash is a common side effect of PR and occurs even more frequently with telaprevir. Moreover, 4-7% of patients in phase 3 trials assigned to telaprevir had to discontinue all antiviral therapy due to dermatological side effects.[3,4,6] It develops typically on the trunk, extremities and friction sites, it is generally mild by nature and can be treated with local cooling ointment (unguentum emolliens) or with local corticosteroid therapy (class 3) and antihistamines. Patients with rash grade 2 to 4 need to be referred to a dermatologist without delay.[93] Severe rash (grade 3) is defined as involvement of more than 50% of body surface or if systemic symptoms occur (fever, lymphadenopathy, arthralgia, or a rise in creatinine or ALAT). In this case, telaprevir has to be discontinued and if there is no improvement within one week, PR also needs to be discontinued.[94] Generally, the rash will disappear within a couple of weeks after stopping telaprevir. Rare events with telaprevir are the drug reaction with eosinophilia and systemic symptoms (DRESS), Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN). All treatment should be stopped immediately, a dermatologist should be consulted immediately, and glucocorticoids should be considered.[94]

Psychiatric side effects

Psychiatric side effects such as depression, agitation, irritability, insomnia, lack of concentration and emotional instability put the patient at risk for PR dose reduction, lower treatment adherence and premature treatment cessation resulting in lower SVR rates.[42,95] Prophylactic treatment with an SSRI should be considered in all patients with a history of depression or signs of depression at baseline.[96] Apart from pretreatment evaluation of feasibility of treatment and possible drug interactions, consider consulting a psychiatrist and/or a specialist in addiction medicine to ensure safety and drug compliance.

FOLLOW-UP AFTER ANTIVIRAL THERAPY

HCV RNA should be tested 24 weeks after the end of treatment. If HCV RNA is negative, SVR is achieved and the patient can be considered to be cured from chronic HCV infection with only a minimal risk of viral recurrence.[97] Recent data suggest that negative HCV RNA 12 weeks post-treatment is probably sufficient to confirm SVR, although this needs further evaluation.[98]

Hypothyroidism can arise during but also after termination of treatment. Consequently, thyroid function should also be assessed during the first two years after treatment.[84] Cirrhotic patients should be followed-up, preferably in a specialised Dutch viral hepatitis centre, because they still remain at risk for cirrhosis-related complications. As per the guidelines, abdominal ultrasound has been advised in the follow-up of these patients to screen for HCC and endoscopic assessment for oesophageal varices.[49,99]

THE FUTURE

With the introduction of boceprevir and telaprevir the development of novel DAAs and immune modulatory therapy with less side effects than peg-IFNα does not stop. There is intense interest for novel agents that avoid the use of peg-IFNα. Indeed, several HCV polymerase inhibitors are in advanced stages of clinical development. Without doubt therapeutic options will expand to other genotypes. In addition, efforts to design better options for difficult to treat patients [for example with HBV or HIV coinfections) will be necessary.

Furthermore, a new group of DAA non-responders will emerge. How and when these patients will be eligible for anti-HCV infection therapy is uncertain. Consequently, these patients will probably be excluded from upcoming trials with second-generation DAAs, which means that at this time, treatment options for this group are limited.

CONFLICTS OF INTEREST

Drs. M.H. Lamers: none

Drs. M.M.T.J. Broekman: none

Prof. Dr. D.M. Burger: received research grants, honoraria for advisory boards and speakers fees from Merck and Tibotec/Janssen

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Prof. Dr. B. van Hoek: member of the advisory board for Janssen, Merck, Roche, and Novartis

Prof. Dr. C.A. Boucher: His employer the Erasmus Medical Center, Erasmus University has received research grants from Merck and Roche, consultancy fees from Merck, and travel support from Janssen and Gilead

Dr. J.T. Brouwer: member of the advisory board from Merck

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SPECIAL REPORT

Vitamin B_{12} deficiency and the lack of its consequences in type 2 diabetes patients using metformin

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ABSTRACT

Objectives: To study vitamin B_{12} concentrations in patients with type 2 diabetes with and without metformin use and to identify risk factors and consequences of low vitamin B_{12} concentrations.

Research design and methods: This study had a cross-sectional design. During eight weeks all patients with type 2 diabetes visiting the diabetic outpatient clinic of the Isala Clinics in Zwolle were approached for participation. Participation included measurement of haemoglobin, mean corpuscular volume and vitamin B_{12} levels. Data on neuropathy were retrospectively searched for in the patient records. Vitamin B_{12} deficiency was defined as serum B_{12} concentrations <150 pmol/l.

Results: In the total cohort (n=298), the overall prevalence of vitamin B_{12} concentrations <150 pml/l was 9.7% (95% CI 6.6-13.7%). In type 2 diabetes patients not taking metformin (n=134), the prevalence was 4.4% (95% CI 1.6-9.4%) compared with 14.1% in metformin users (n=164) (95% CI 9.2-20.4%; p=0.006). Each 100 mg step in metformin dose increased (OR=1.081, p=0.014), whereas PPI use lowered (OR=0.322, p=0.037) the odds of having a vitamin B_{12} deficiency in logistic regression. Nevertheless, metformin use did not predict the chance on having anaemia or neuropathy.

Conclusion: Among patients with type 2 diabetes using metformin, the prevalence of vitamin B_{12} deficiency is higher than compared with patients not using metformin. However, metformin use did not predict the chance of having anaemia or neuropathy.

KEYWORDS

Anaemia, metformin, neuropathy, type 2 diabetes mellitus, vitamin B_{12} deficiency

INTRODUCTION

Metformin is the drug of choice in the treatment of type 2 diabetes.[1] One of the possible side effects of metformin that has gained attention over the last few years is a decreased concentration of vitamin B_{12} due to decreased absorption.[2] It is estimated that this problem occurs in approximately 10-30% of patients using metformin[2] and is associated with a 4-24% decrease of vitamin B_{12} (B_{12}) concentrations.[3,4] Although it may take up to five years before a deficiency manifests itself,[5] it potentially has serious consequences, with an increased risk of macrocytic anaemia and neurological disturbances. However, when acknowledged and treated in time, these consequences can be prevented.[6]

In one of the earliest studies on metformin and B_{12} deficiency, published in 1972, a prevalence of B_{12} deficiency of 5.6% among type 2 diabetes patients using metformin was found.[2] Subsequent studies showed a prevalence of 5.8-22.6%.[7,8] In a recent randomised, placebo-controlled trial in type 2 diabetes patients using insulin, 196 patients were given metformin for 4.3 years while 194 patients received a placebo. The mean duration of diabetes was 14 (SD=9) years in the metformin group and 12 (SD=8) years in the placebo group. At baseline, 1.6% of patients using metformin and 2.2% patients on placebo had a B_{12} deficiency. The average daily metformin dose in the metformin group was 2050 mg. Of the participants treated with metformin, 9.9% had a vitamin B_{12} deficiency (defined as a concentration <150 pmol/l) at the end of the trial compared with 2.7% in placebo-treated patients (p=0.004). Incidences of anaemia or neuropathy were not reported.[4]

Since these studies do not describe what the actual prevalence of B_{12} deficiency is among metformin-treated type 2 diabetes patients, the aim of this study was to

determine its prevalence in a secondary care setting and to compare it with the prevalence of B_{12} deficiency in non-metformin treated type 2 diabetes patients, treated in the same setting. In addition, the relationship between metformin use and presence or absence of anaemia and neuropathy was investigated.

PATIENTS AND METHODS

Study design, participants and procedures

This study had a cross-sectional design to describe the prevalence of vitamin B_{12} deficiency among a sample of individuals with type 2 diabetes at the outpatient clinic of the Isala Clinics, Zwolle, the Netherlands.

All patients aged 18 years or older who were being treated for type 2 diabetes at the outpatient clinic were eligible for inclusion, regardless of metformin use. Participants with diabetes after necrotic pancreatitis, late-onset autoimmune diabetes of adults (LADA) or use of cobalamin injections or tablets were excluded.

Prior to and during their regular scheduled visit to their treating internist, patients were informed about this study. Subsequently, patients could consult one of the investigators to receive more information and sign informed consent when they had agreed to participate. During this visit, information about metformin use and dosage, supplemental vitamin use, proton pump inhibitor (PPI) use, height and weight was gathered. Electronic patient records were consulted for additional patient characteristics and diabetes complications. Finally, blood samples were collected directly following this visit.

Measurements

Haemoglobin (Hb) and mean corpuscular volume (MCV) were measured with the SE9000 Haematology Analyser (Sysmex); total vitamin B_{12} was measured with the Modular Analyser (Roche Diagnostics). Anaemia was defined as Hb <8.5 mmol/l in men and <7.5 mmol/l in women. Macrocytosis was defined as MCV >100 fl. Vitamin B_{12} levels <150 pmol/l were considered to indicate deficiency. Vitamin B_{12} levels were grouped as follows: deficiency (<150 pmol/l), low-normal (150-220 pmol/l) and normal (>220 pmol/l). Patient record was searched to determine the presence of neuropathy. Neuropathy was considered present when the treating internist recorded this in the patient records.

Statistical analysis

Assuming a 10% prevalence of B_{12} deficiency in metformin-using patients in the study by De Jager *et al.*,[4] power analysis showed that 158 patients were needed to estimate the prevalence of B_{12} deficiency in type 2 diabetes patients using metformin with a 95% confidence interval width of 10%. Investigated categorical variables included sex, insulin use, use of other DM medication, proton pump inhibitor use, use of vitamin B containing supplements, nephropathy, retinopathy, neuropathy and macrovascular complications. Continuous variables included age, body mass index, duration of type 2 diabetes, duration of metformin use, duration of insulin use, Hb and MCV levels. Associations between B_{12} deficiency and categorical variables were determined with Fisher's exact test. Associations between continuous variables and vitamin B_{12} deficiency were determined with Student's *t* test or the non-parametric Mann-Whitney U test, depending on the distribution of the continuous variables. A multivariate analysis, using binary logistic regression, was used to estimate the influence of factors associated with a B_{12} deficiency. A multiple linear regression analysis was performed to estimate the impact of factors influencing B_{12} levels. The same procedures were performed to estimate the impact of variables influencing Hb levels and predicting anaemia or neuropathy. A (two-sided) p-value of less than 0.05 was considered statistically significant. All analysis were performed using SPSS version 18.0, Inc, Chicago, Il, USA.

The study was performed in accordance with the Declaration of Helsinki. Informed consent was obtained from all patients, and the protocol was approved by the medical ethics committee of the Isala Clinics in Zwolle.

RESULTS

Patient characteristics

Patients were included between 1 April and 27 July 2012. A total number of 426 patients were eligible for participation in the study of whom 114 (27%) patients declined participation. In addition, eight patients were excluded because they used intramuscular cobalamin injections and six patients gave informed consent but did not have their blood drawn. Subsequently 298 (70%) patients were included in the present study. Of these patients, 164 (55%) had used metformin for at least two weeks, with a maximum of 31.9 years. For two patients these records were not available. For 38 patients using metformin, there were no data on the exact duration of metformin use.

Patient characteristics of all patients classified by metformin use are shown in *table 1*. Patients using metformin were younger, had a shorter median diabetes duration, showed neuropathy less frequently and less often used insulin.

Vitamin B_{12} deficiency

Serum vitamin B_{12} levels ranged from 72-873 pmol/l among all patients. There were 29 (9.7%, 95% confidence interval

Table 1. *Characteristics of type 2 diabetes patients*

	All patients (n=298)	Metformin use (n=164)	No metformin use (n=134)	p-value
Age (years) (mean, SD)[a]	64.8 (11.6)	62.6 (11,9)	67.2 (10.8)	0.001*
Sex (male) (n, %)[c]	158 (52.8)	90 (55.2)	67 (49.6)	0.353
Body mass index (kg/m²) (median (P25-P75)[b]	31.1 (27.6-35.7)	31.6 (27.6-35.9)	30.8 (27.5-35.4)	0.400
Type 2 diabetes (years) (median (P25-P75)[b]	14.9 (8.9-20.9)	12.1 (7.6-17.9)	17.9 (12.1-23.9)	<0.001*
Metformin use (years) (median (P25, P75, min, max)[b]	-	4.9 (1.7-8.1-0.04-31.9)	-	-
Insulin use (n, %)[c]	256 (86.2)	128 (79.0)	128 (94.8)	<0.001*
Duration of insulin use (median (P25-P75)[b]	8.3 (4.9-13.9)	7.1 (3.9-10.7)	10.5 (6.8-15.9)	<0.001*
Use of other DM medication[d] (n, %)[c]	64 (21.7)	50 (31.3)	14 (10.4)	<0.001*
Proton pump inhibitor use (n, %)[c]	130 (43.6)	74 (45.4)	56 (41.5)	0.558
Use of vitamin B containing supplements (n, %)[c]	25 (9.4)	15 (9.2)	10 (7.4)	0.677
Microvascular complications (n, %)[c]	148 (50.0)	72 (45.0)	75 (55.6)	0.080
Nephropathy (n, %)[c]	60 (20.0)	30 (18.6)	29 (21.5)	0.562
Retinopathy (n, %)[c]	70 (23.6)	41 (25.5)	29 (21.5)	0.493
Neuropathy (n, %)[c]	66 (22.3)	28 (17.4)	38 (28.1)	0.035*
Macrovascular complications (n, %)[c]	105 (35.7)	51 (32.1)	54 (40.0)	0.180

[a]Student's *t* test; [b]Mann Whitney U test; [c] Fisher's exact test; [d]diabetes medication other than metformin or insulin; *difference between metformin users and non-metformin users.

(CI) 6.6-13.7%) subjects with B_{12} deficiency (<150 pmol/l), 65 (21.8%, 95% CI 17.3-26.9%)) patients with low-normal B_{12} levels (150-220 pmol/l) and 204 (68.5%, 95% CI 62.8-73.7%) patients with normal B_{12} levels (>220 pmol/l). In metformin users, B_{12} deficiency was present in 14.1% (95% CI 9.2-20.4) and in non-metformin users 4.4% (95% CI 1.6-9.4%). The absolute difference in prevalence was 9.7% (95% CI 3.2-6.1) (p =0.003). Furthermore, as depicted in *table 2*, metformin users more often had low-normal vitamin B_{12} levels (p<0.001). Only five patients (1.7%, 95% CI 0.5-3.9%) had B_{12} levels below 100 pmol/l, all using metformin.

Prediction of vitamin B_{12} deficiency

In linear regression, metformin dosage was the only variable able to predict vitamin B_{12} levels significantly. When correlating dose increases of metformin with B_{12} concentrations, each incremental increase of 100 mg in metformin dose was associated with an incremental decrease in vitamin B_{12} levels of 3.77 pmol/l (95% CI -6.79 to -0.81). In logistic regression, each dose increase of 100 mg metformin increased odds for B_{12} deficiency by 8% (OR 1.08; 95% CI 1.02-0.15). PPI use reduced odds for B_{12} deficiency by 68% (OR 0.32; 95% CI 0.11-0.94). In this study, duration of metformin use did not have a significant effect in this model. Results of this regression analysis are shown in *table 3*.

Consequences of vitamin B_{12} deficiency

In the metformin group 23.3% showed anaemia, compared with 22.3% in the group not using metformin (95% CI of the difference -1.1 to 8.6; p=0.82). Sex was the only factor influencing odds for anaemia (OR 2.26; 95% CI 1.04-4.93 when male).

Of all individuals with type 2 diabetes, 68 (22.8%) were anaemic of whom 14 (4.7%) also had a vitamin B_{12} deficiency. Of the 29 patients with B_{12} deficiency 14 (48%) were anaemic. Among 269 patients without B_{12} deficiency 44 (20%) were anaemic (p=0.002). In the metformin group, patients with B_{12} deficiency were also more likely to have anaemia (43.5%), compared with metformin-using patients without B_{12} deficiency (20.0%) (p=0.03). There was only one patient with a macrocytic anaemia in both the metformin and the non-metformin group. In both the metformin and non-metformin group three patients had a microcytic anaemia. Mean MCV in the metformin group was 89.1 fl compared with 90.2 in the non-metformin group (p=0.08).

Table 2. *Metformin use and vitamin B_{12} levels*

		Vitamin B_{12} levels			
		Deficient(<150 pmol/l)	Low-normal(150-220 pmol/l)	Normal(>220 pmol/l)	Total
Patients not on metformin	n (%)	6 (4.4)	18 (13.3)	111 (82.2)	135 (45.3)
Patients on metformin	n (%)	23 (14.1)	47 (28.8)	93 (57.1)	163 (54.7)
Patients (total)	n (%)	29 (9.7%)	65 (21.8%)	204 (68.5%)	298 (100.0%)

Table 3. *Logistic regression analysis for possible predicting factors for B_{12} deficiency*

	Odds ratio (95% CI)	p-value
Sex (if male)	0.745 (0.277, 2.006)	0.560
Age	0.998(0.955, 1.043)	0.931
BMI	0.971 (0.892, 1.056)	0.490
Type 2 diabetes duration	1.005 (0.902, 1.119)	0.930
Metformin 100 mg	1.081 (1.016, 1.150)	0.014*
Metformin duration	0.983 (0.869, 1.111)	0.780
Insulin use	0.581 (0.106, 3.178)	0.531
Insulin duration	1.018 (0.898, 1.153)	0.781
PPI	0.322 (0.111, 0.936)	0.037*
Vitamin B-containing supplements	0.993 (0.187, 5.276)	0.993
Other medication[a]	0.562 (0.121, 2.608)	0.462
Constant	0.389	0.654

[a]**Diabetes medication other than metformin or insulin.**

Anaemic patients had mean vitamin B_{12} levels of 294.3 pmol/l compared with 323.1 pmol/l in patients without anaemia (p= 0.44). Patients with metformin use less often had a neuropathy (17.4%, 95% CI 11.9-24.1%) compared with patients without metformin use (28.1%, 95% CI 20.8-36.5%) (p=0.04). In logistic regression, only duration of diabetes was found to predict chances of neuropathy (OR 1.078; 95% CI 1.043-1.114).

DISCUSSION

The prevalence of B_{12} deficiency among secondary care treated type 2 diabetes patients was 14.1% in metformin users (median metformin use 4.9 years) and 4.4% in non-metformin users. In multivariate models, metformin was a positive, and PPI use a negative predictor of this deficiency. Of all patients (regardless of metformin use), 22.8% were anaemic and 22.3% had neuropathy. Metformin use of 4.9 years was not related to anaemia. It is possible that the chronic disease type 2 diabetes in itself is sufficient to explain the anaemia in this population. Also, 4.9 years of metformin use was related to a lower prevalence of neuropathy than when no metformin was used.

Although the higher prevalence of vitamin B_{12} deficiency among metformin users is in line with previous studies, the magnitude is slightly different. In 1970 in the first described cohort in type 2 diabetes patients using metformin for less than five years, a prevalence of 5.6% among metformin users was found.[2] Pflipsen *et al.* found a prevalence of 22.6% among patients with type 2 diabetes using metformin, in a primary care setting.[8] In this study, vitamin B_{12} concentrations below 100 pg/l (74 pmol/l) or between 100-350 pg/l (74-258 pmol/l) combined with elevated methylmalonic acid or homocysteine levels were defined as deficiency, therefore this study may have well overestimated the prevalence. In a recent study by De Jager *et al.* the same definition of vitamin B_{12} deficiency was used as in the current study. They found a 9.9% prevalence of B_{12} deficiency in patients treated with metformin for 4.3 years.[4] Reinstatler *et al.* defined B_{12} deficiency as B_{12} ≤148 pmol/l and found a prevalence of B_{12} deficiency of 5.8% in a cohort of patients followed for six years.[6]

Ting *et al.* studied risk factors of B_{12} deficiency in patients receiving metformin. The dose of metformin was the strongest independent predictor of vitamin B_{12} deficiency and a longer duration of treatment with metformin was associated with a higher prevalence.[9] In our study we also saw an association between the decreasing B_{12} levels and increasing metformin dose. In accordance with the present study, Reinstatler *et al.* found no clear increase in the prevalence of deficiency as the duration of metformin use increased.[7]

In the current study the decreased occurrence of neuropathy should be interpreted with caution as no objective measures were undertaken to observe the presence of neuropathy. The occurrence of anaemia, however, with actual Hb measurements in every patient, could not be predicted by metformin use after a mean duration of use of 4.9 years. This points toward an important caveat: there is a discrepancy between clinical and biochemical signs of vitamin B_{12} deficiency. Although limited research is available on the question whether biochemical deficiency progresses into clinical deficiency, this does not seem likely.[6]

To identify B_{12} deficiency in metformin-treated type 2 diabetes patients, some authors recommend regular screening of metformin users for B_{12} deficiency or even standard suppletion with, for instance, calcium to reverse the disturbed vitamin B_{12} uptake.[2,4,10,11] There are some important remarks to make in this context. First, it is important to realise that 150 pmol/l as cut-off point for B_{12} deficiency is arbitrarily chosen. Especially with the elderly, B_{12} values below 150 pmol/l are commonly found, and most of them are clinically irrelevant.[5,12] Second, not much is known about consequences of B_{12} deficiency in metformin-using patients. Suppletion is of course not desirable when this does not give any health gain.

The present study adds to this discussion by again defining a prevalence, confirms the influence of metformin on a vitamin B_{12} deficiency and shows that, although metformin increases B_{12} deficiency rates, it does not increase odds for anaemia or neuropathy after 4.9 years treatment with metformin. This last finding argues against standard screening and/or suppletion of vitamin B_{12} in metformin-treated type 2 diabetes patients. We would therefore like to plead for more research focusing on the consequences of a metformin-induced B_{12} deficiency to determine whether screening and suppletion are necessary.

CONCLUSIONS

The prevalence of B_{12} deficiency in secondary care type 2 diabetes patients using metformin was estimated at 14.1%. The prevalence is significantly higher in patients treated with metformin compared with non-metformin users. Metformin use, however, does not predict the odds for anaemia or neuropathy after 4.9 years treatment with metformin.

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LETTER TO THE EDITOR

Successful treatment after short course of telaprevir-based therapy in chronic hepatitis C infected patient

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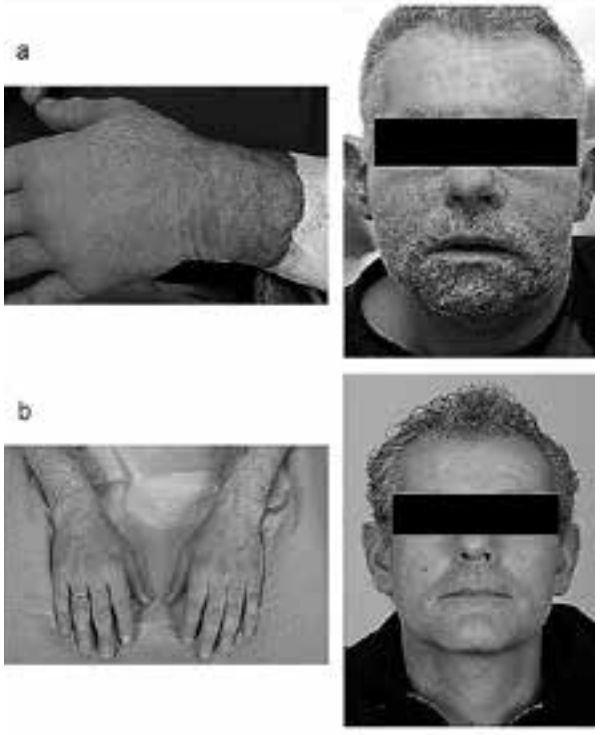
To the editor,

Since 2012, triple therapy with a combination of pegylated interferon-alpha 2a/2b, ribavirin and a novel NS3/4A serine-protease inhibitor (telaprevir or boceprevir) is the standard of care for chronic hepatitis C genotype 1a/b (HCV).[1,2] Together with a substantial increase in sustained viral response (SVR) rates and shortened duration of therapy, additional side effects of the direct acting antiviral agents (DAA) are increasingly being recognised.[3] We describe a patient who stopped telaprevir-based triple therapy after only six weeks due to severe toxic skin reaction caused by the telaprevir, and achieved an SVR despite several unfavourable characteristics.

A 48-year-old man, HCV-infected during a period of intravenous drug use in the 1990s, started triple therapy consisting of peginterferon alpha-2a, ribavirin and telaprevir. HCV was genotype 1a, with a baseline HCV-RNA concentration of 2.0 x 10^6 IU/ml, an ALAT level of 180 U/l and no severe fibrosis or cirrhosis by fibroscan. His IL28B polymorphism was C/T (rs1279860). The first four weeks of triple therapy passed uneventfully while his HCV-RNA viral load dropped to <15 IU/ml (Cobas Taqman V2.0, Roche). Over the next two weeks he gradually developed a toxic skin reaction on the arms, legs, rump and face for which topical corticosteroid ointment and oral antihistamines failed. The rash worsened, the patient's condition deteriorated and he developed a secondary impetigo in the face (*figure 1*). He was admitted to hospital and the triple therapy for HCV was discontinued. The consulted dermatologist performed a skin biopsy showing lymphohistiocytic infiltrates and increased numbers of eosinophilic granulocytes in the dermis confirming the clinical diagnosis of telaprevir-induced toxic skin reaction (grade 3). He recovered completely. He had an undetectable HCV-RNA level 12 and 24 weeks after treatment discontinuation and was therefore successfully treated for HCV.

Figure 1. *Severe toxic skin reaction caused by telaprevir*



A) Grade 3 skin rash (defined as a generalised skin eruption involving >50% of the body surface area plus epidermal detachment) developed after 4-6 weeks of telaprevir-based triple therapy; B) Normalisation of all skin lesions after treatment discontinuation and achievement of SVR

Successful very short courses of therapy have rarely been described,[4] while this is the first patient with telaprevir-

based triple therapy. Of note, there were a number of unfavourable pretreatment characteristics such as HCV genotype 1a infection, C/T IL28B polymorphism and high baseline viral load that would lower his chances of SVR in the first place. On the other hand, the patient exhibited very favourable viral kinetics in the first weeks of therapy, which has been shown to be the most important predictor of treatment success.[5] Furthermore, it could be hypothesised that this patient's severe side effects were indicative of a strong immune response, which could have contributed to a rapid forced viral clearance of HCV.

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