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EDITORIAL

Nontraditional and traditional factors in renal atherosclerosis

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In this issue of the Netherlands Journal of Medicine, the reader will find two interesting views on traditional and nontraditional risk factors for the progression of renal disease and cardiovascular disease on the one hand, and a new view of a traditional renin-angiotensin system (RAAS) with respect to peritoneal dialysis treatment on the other.

The review by Nanayakkara touches on the toughest problem we currently face in nephrology: patients with renal failure die of cardiovascular disease and we do not understand the mechanism. This is illustrated on the one hand by the 'inverse epidemiology', a horrible term indicating that we do not find the same relationships between traditional risk factors and cardiovascular disease in patients with end-stage renal disease as in people without renal disease. Another factor must have taken control; the authors indicate that the pathophysiological process in end-stage renal disease is probably dominated by an increase in oxidative stress driving inflammation, endothelial dysfunction and anaemia.[1] On top of all this is a disturbance in calcium/phosphate metabolism and a defect in the vascular repair by endothelial progenitor cells.[1] On the other hand, the 'nontraditional' risk factors are unable to explain the increased morbidity and mortality. There is a lack of tools to accurately measure these factors, in particular oxidative stress, in humans, as well as a lack to strongly inhibit these factors. Finally, as is the case for cholesterol and for haemoglobin, these factors fail to fulfil Koch's postulates; this stresses so much the necessity to perform careful clinical testing of hypothetical constructs. All in all, a definitive proof that these factors are of eminent importance is lacking. Hidden in this review are some extremely challenging issues. One is the clear separation of initiating factors for cardiovascular disease (i.e. risk factors), factors that form a reflection of the actual disease process, biomarkers, and factors that are involved in maintenance and repair (such as endothelial progenitor cells). There is currently confusing nomenclature, and the reason that nontraditional risk factors may not assist strongly in predicting cardiovascular disease may well be related to the notion that these factors are indicators of the disease process initiated by the traditional risk factors (e.g. CRP). Second is that, although the model that is presented by the authors is attractive and strongly supported by experimental data, it illustrates the strong need to better understand the clinical pathophysiology of atherosclerosis in renal disease. It clearly is not the traditional cardiovascular disease we have been associating with increased lipids levels and diabetes, but another disease. Finally, the review illustrates the necessity for *in vivo* assessment of the mechanisms and the need for more potent tools to manipulate the mechanisms that favour progression of cardiovascular disease in humans with renal disease.

The second view by Kolesnyk[2] discusses a very traditional risk factor for renal and cardiovascular disease, the RAAS. It sums up the evidence that inhibition of the RAAS is beneficial for the progression renal disease and CVD. Somehow intriguing is that the RAAS is not considered a traditional risk factor, while altogether complying very nicely with Koch's postulates. The authors extend their views beyond the conventional patterns: they consider whether ACEi or ARB administration is beneficial for the membrane function of the peritoneal membrane in peritoneal dialysis patients. The evidence is not extremely strong, but supports a role for angiotensin II in the fibrosis of the peritoneal membrane. These authors take a stand for a traditional factor in the process that limits the application of peritoneal dialysis, fibrosis of the peritoneal membrane. Implications of local RAAS activity have substantially accumulated in the last decade and a role of the RAAS in the pathophysiology of peritoneal membrane pathology is likely. They also emphasise that the involvement of the RAAS in the progression of renal disease in transplant patients is unresolved; this in the face of all the strong evidence supporting a role for the RAAS in chronic kidney disease. It so much emphasises that the RAAS will never be really traditional.

It is very nice to have these two views in one issue of the Journal, the one emphasising a new mechanism, the other emphasising a new role for an old mechanism. The authors are to be complimented for their summaries and views of where things stand in these two areas. We are now at the stage where these issues will need to be translated from theory to practice; one more call for translational studies with joint efforts of basic researchers and clinicians. One of the tools we have at hand is applying the knowledge about the RAAS as much as we can, and we should. The other is to perform genuine translational research, using patient materials, bringing this to the lab and then back again to the clinic. It would not be a surprise if these studies would again place angiotensin II very central in the pathogenesis of renal disease.

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REVIEW

Vascular disease and chronic renal failure: new insights

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ABSTRACT

Premature cardiovascular disease (CVD) is a frequent complication in patients with chronic kidney disease (CKD). The traditional (Framingham) risk factors only partly explain the high prevalence of CVD in these patients and nontraditional risk factors/markers such as oxidative stress, persistent inflammation, cardiovascular ossification, endothelial dysfunction and anaemia are prevalent and seem to play an important role in the pathogenesis of CVD in CKD patients. In addition, the so-called reverse epidemiology phenomenon, which occurs in advanced kidney disease, complicates the search for causative mechanisms. Here we review a few recently developed concepts regarding the high incidence of CVD in CKD patients.

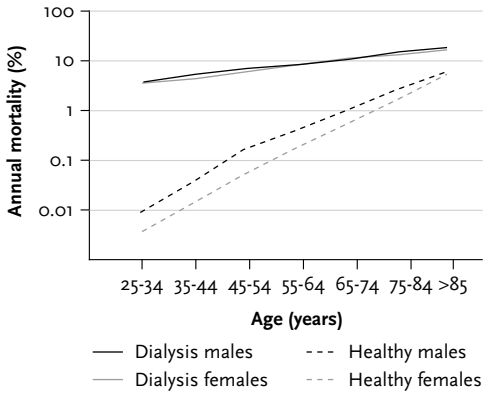
KEYWORDS

Chronic renal disease, cardiovascular risk, anaemia, sleep disturbances, reactive oxygen species, inflammation

INTRODUCTION

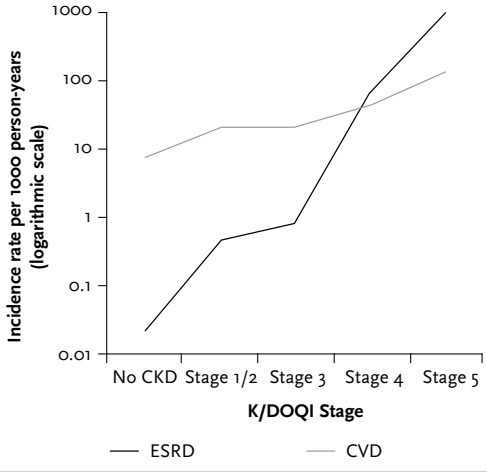
As in the general population, cardiovascular disease is the major cause of death in patients with end-stage renal disease (ESRD), accounting for about 40% of total mortality.[1,2] Life expectancy is severely reduced in ESRD patients compared with the general population, suggesting that the incidence and case fatality of cardiovascular disease is increased in ESRD patients. Indeed, it has been shown that total and cardiovascular mortality is increased 20- to 30-fold in ESRD patients (*figures 1* and *2*).[1,2] The risk of nonfatal cardiovascular disease is also 10-30

Figure 1. *Epidemiology of cardiovascular disease in haemodialysis patients[2]*



Adapted from Foley et al.

Figure 2. *Incidence of end-stage renal disease (ESRD) and cardiovascular disease (CVD) events according to baseline chronic kidney disease (CKD) stage. Data derived from the PREVEND study[101]*



times higher in patients with ESRD compared with the general population.[3] ESRD patients are therefore prone to the development and/or progression of cardiovascular disease.[4]

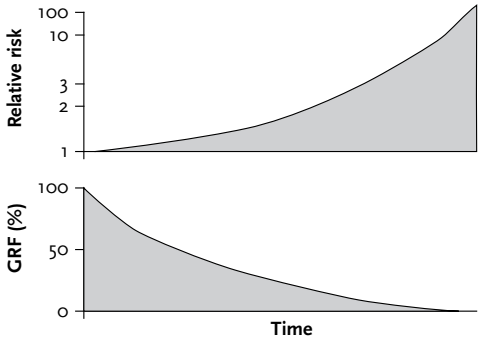
Cardiovascular disease (CVD) comprises a group of conditions, which can be divided into ischaemic and nonischaemic conditions. Examples of nonischaemic disease include valvular heart disease and arrhythmia. Ischaemic cardiovascular disease includes coronary artery disease, ischaemic cardiomyopathy, stroke, peripheral vascular disease, ischaemic nephropathy and renovascular disease. Sudden death (cardiac arrest) is also frequently the result of ischaemic vascular disease. Ischaemic vascular disease is generally the result of atherothrombosis. Lipoprotein retention and inflammation play a role in the early phase of this disease leading to arterial narrowing, whereas later on inflammation is implicated in plaque rupture and thrombosis.[5] In United States Renal Data System (USRDS) about 60% of all cardiac deaths in dialysis patients are attributed to cardiac arrest/cause unknown or arrhythmia.[6] Coronary artery disease coupled with diminished tolerance to myocardial ischaemia by left ventricular hypertrophy and ischaemic myocardial fibrosis, rapid electrolyte shifts in haemodialysis patients and derangements in autonomic function may all contribute to this increased risk of sudden cardiac death.[6]

Despite the marked increased risk of cardiovascular disease, several large randomised trials in ESRD patients have consistently failed to show survival benefit from multiple new treatment strategies, which were aimed to reduce cardiovascular disease, such as increased dialysis dose,[7,8] homocysteine-lowering therapy,[9] intensified nutrition,[10] lipid-lowering with statins,[11] treatment with angiotensin-converting enzyme inhibitors,[12] and normalisation of haemoglobin with erythropoietin.[13,14] While some of these interventions do have significant beneficial effects on the incidence of cardiovascular disease in the general population, the reason for the lack of benefit of these interventions in ESRD patients is unclear. One can postulate that the risk factors involved in the atherogenesis in patients with ESRD markedly differ from those of the general population or that the stage of atherosclerosis in these patients is so advanced that it has become resistant to the therapies that have been used.

CARDIOVASCULAR RISK IN MILD TO MODERATE KIDNEY DISEASE

Estimates suggest that prevalence of mild to moderate chronic kidney disease is high in the Netherlands.[15,16] Earlier stages of chronic kidney disease (CKD) have also been associated with increased cardiovascular morbidity and mortality (*figures 1* and *2*).[1,17-21] A recent review of 85 publications, involving a total of 552,258 subjects, concluded that an undeniable link exists between the deterioration of renal function and the development of cardiovascular disease.[22] The risk of cardiovascular disease is already increased in very early stages of chronic kidney disease (at an estimated glomerular filtration rate (GFR) of approximately 75 ml/min) and increases continuously with decrease in renal function (*figure 3*).[22] In addition, recent studies have shown that patients with moderate chronic kidney disease are at a high risk of developing congestive heart failure[23] and that the majority of these patients have coronary heart disease.[24,25] A Norwegian study with 65,604 stage 2 to 3 CKD patients demonstrated that these subjects had a higher risk for development of premature cardiovascular death than progression to ESRD[26] and Keith *et al.* confirmed these findings in patients with stage 2, 3, and 4 CKD.[27]

Figure 3. *The relationship between estimated glomerular filtration rate and the risk of cardiovascular death*



Bron: Ronco C, Cruz DN (eds). Hemodialysis – From Basic Research to Clinical Trials. Contrib Nephrol. Basel, Karger, 2008, vol 161, pp 63-7.

TRADITIONAL AND NONTRADITIONAL RISK FACTORS

The clear association between reduced kidney function and cardiovascular risk may, at least partly, be the result of a relationship between total atherosclerotic burden and decreased renal function, because intrarenal atherosclerosis (ischaemic renal disease) is a common cause of reduced renal function in patients with atherosclerosis.[28] However, even patients with a primary nonatherosclerotic renal disease such as polycystic kidney disease have an elevated risk of cardiovascular disease.[29,30] Traditional atherosclerotic risk factors such as age, dyslipidaemia, hypertension, diabetes mellitus, smoking and sedentary lifestyle play an important role in the occurrence of cardiovascular mortality in patients with chronic kidney disease.[31] However, these factors only partially explain the

cardiovascular morbidity and mortality in patients with mild to advanced chronic kidney disease, suggesting a pathophysiological role for additional risk factors.[32-35]

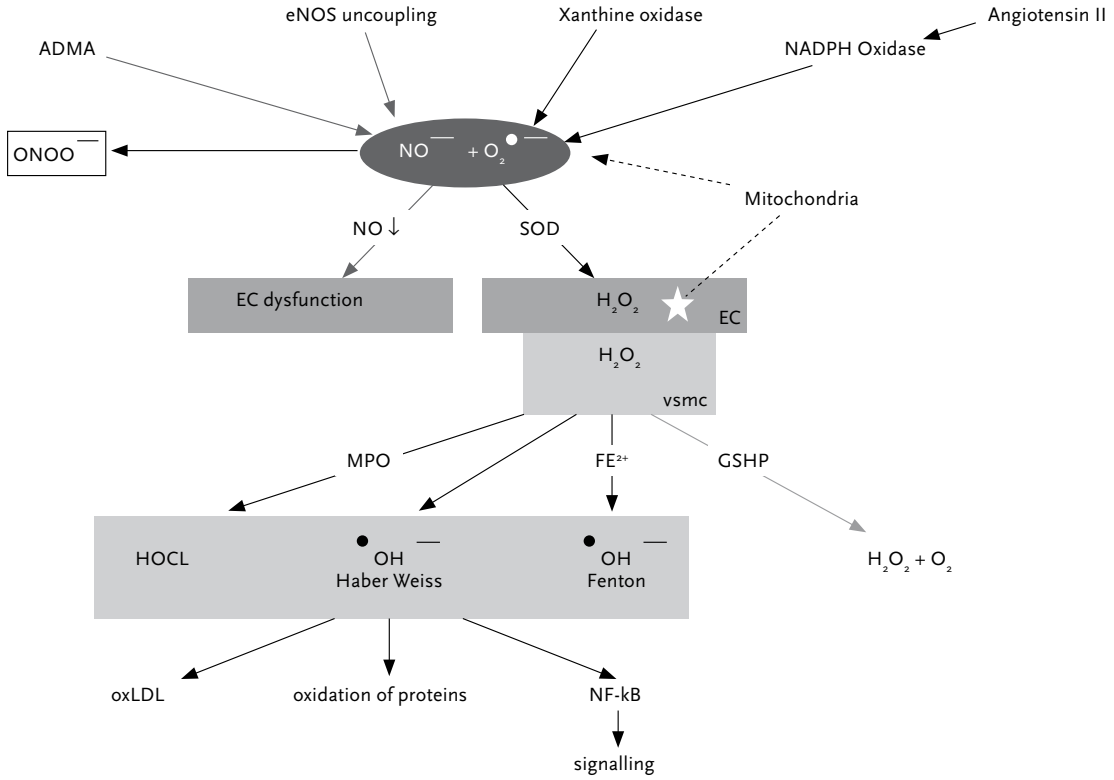
The so-called novel risk factors are markers of putative mechanisms involved in cardiovascular risk such as oxidative stress, endothelial dysfunction, cardiovascular ossification (calcification), inflammation, anaemia and disturbances in the sleep pattern. These novel markers are thought to play a role in the development of atherosclerosis in patients with CKD.[33,36,37] However, it should be emphasised that these traditional and nontraditional risk factors do overlap and do not operate in separate rigid compartments. While numerous studies have demonstrated that the traditional (Framingham) risk factors only partially explain the excess CVD in advanced CKD patients,[32-35] in a population of elderly CKD patients Shilpak *et al.* demonstrated that traditional CV risk factors had a stronger association with CV mortality than nontraditional risk factors.[31] The association between traditional and nontraditional risk factors and CVD is also complicated by the reverse epidemiology phenomenon seen in patients with CKD.[38] Reverse epidemiology refers to alterations in the normal relation between risk factors and clinical outcome. In specific populations, such as patients with ESRD, this abnormal relation can be so strong that more or less reversal of the usual association between risk factor and clinical outcome occurs. For example, in dialysis patients high body mass index (BMI) and higher serum cholesterol are correlated with decreased cardiovascular morbidity and mortality. Also the association between blood pressure, serum homocysteine, serum parathyroid hormone, serum creatinine and cardiovascular morbidity and mortality is reversed in dialysis patients compared with the general population.[38] The common occurrence of persistent inflammation and protein energy wasting in advanced CKD seems to a large extent to account for this paradoxical association between traditional risk factors and CV outcomes in this patient population.[39] Preliminary studies suggest that the reverse epidemiology phenomenon is also present in patients with CKD not yet on dialysis.[40] This phenomenon of reverse epidemiology sometimes makes it difficult to target traditional risk factors in an effective manner because determination of an optimal target for risk factors such a blood pressure and LDL cholesterol is uncertain, especially in patients with advanced CKD. In this review, we aim to discuss some new concepts on this complex association between CKD and CVD.

Oxidative stress

Imbalance between production of reactive oxygen species (ROS) and antioxidant defence results in oxidative stress (*figure 4*), which may arise either from deficiencies of antioxidants (such as glutathione, ascorbate or α-tocopherol) or increased formation of ROS such as peroxynitrite ($ONOO^-$), hypochlorous acid (HOCL) or superoxide anions.[41] High levels of oxidative stress markers such as F_2-isoprostanes, advanced glycosylation end products, malonyldialdehyde and oxidised LDL have been demonstrated in patients with mild to moderate CKD and in ESRD patients.[42] Oxidative modification of low-density lipoproteins (LDL) is thought to be a key step in the initiation of atherosclerosis.[43] Therefore, one may consider the oxidative stress hypothesis as a unifying concept of increased CVD risk in CKD patients.[36] Numerous studies have demonstrated an inverse association between different markers of oxidative stress and eGFR and in addition graded increase in oxidative stress has been demonstrated with longer duration of dialysis therapy.[44,45] This increased oxidative stress is probably due to increased production of ROS as increased NAD(P)H oxidase activity has been reported in patients with even mild CKD.[46] Whether there is also a deficiency in the antioxidant defence system is still a matter of debate.[42] While some studies demonstrate a reduction in intracellular or plasma antioxidant factors such a superoxide dismutase, catalase or glutathione peroxidase other studies showed no reduction in total antioxidant capacity.[47,48]

Even so, the causal relation between oxidative stress and CVD in CKD patients has not yet been established. Only a small number of epidemiological studies that evaluate the association between surrogate markers of oxidative stress and CVD have been performed and results have been inconclusive.[49] Even fewer data are available on intervention trials aimed at reducing the oxidative stress. Most of the intervention trials aimed at reducing cardiovascular disease in ESRD patients yielded disappointing results, but the SPACE study, in which 196 patients with ESRD were treated with 800 IU vitamin E, demonstrated encouraging results on composite CV endpoints and myocardial infarction.[50] In the Antioxidant Therapy in Chronic Renal Insufficiency (ATIC) study 18 months of a stepwise treatment strategy with pravastatin, vitamin E and homocysteine lowering therapy on top of well-controlled blood pressure lowering led to a significant increase in endothelial function and a significant reduction in carotid intima-media thickness.[51] However, whether the reduction in oxidative stress was primarily responsible for these favourable effects was unclear. In another small study, N-acetylcysteine was shown to reduce cardiovascular events in ESRD patients.[52] However, these studies were too small and too short and the effects on oxidative stress biomarkers were not well documented. Therefore, although existing data suggest a probable role for oxidative stress in increased atherogenecity in CKD patients, further studies are needed to examine the pathophysiological role of oxidative stress and the effects of oxidative stress reducing treatment strategies in CKD.

Figure 4. *Schematic simplification of the generation, disposal and effects of reactive oxygen species in renal disease*



This figure shows a simplified representation of superoxide (O_2-) and hydrogen peroxide (H_2O_2) generation and effects. In renal dysfunction, the availability of NO is already jeopardised by increased levels of ADMA. Angiotensin II (Ang II) stimulates intracellular formation of ROS such as the superoxide anion and hydrogen peroxide. Ang II activates several subunits of the membrane-bound multicomponent NAD(P)H oxidase and also increases ROS formation in the mitochondria. The increased O_2-, which are formed by NADPH oxidase and xanthine oxidase, will further decrease the available NO levels, inducing EC and vascular VSMC dysfunction. Also superoxide reacts with NO to form peroxynitrite ONOO- which is damaging to tissues and induces mitochondrial dysfunction.
SOD converts superoxide is into H_2O_2 which can freely enter the cell. The less reactive oxidant hydrogen peroxide (H_2O_2) is then reduced to water and oxygen by catalase or glutathione peroxidase The glutathione system is paramount for protection against oxidative threats. Alternatively, H_2O_2 may be converted into hydroxyl (OH--) radicals, the most reactive and toxic of the ROS, through the Haber–Weiss or the Fenton reactions. These radicals are highly unstable and will interact with proteins, lipids or nucleic acids. In the presence of myeloperoxidase (MPO, from neutrophils), H_2O_2 forms additional oxidants.
Finally, ROS-dependent signalling events, mostly related to NF-kB, will have detrimental effects on the cell's function and the possibility of (irreversible) oxidation of proteins and other molecules in the cells (e.g. AGEs).
ADMA = asymmetric dimethylarginine; ROS = reactive oxygen species; SOD = superoxide dismutase; EC = endothelial cell; VSMC = vascular smooth muscle cell; GSHP = glutathioneperoxidase; MPO = myeloperoxidase; NF-kB = nuclear factor kB; AGE = advanced glycosylation end products.

Inflammation

Numerous studies have reported an association between chronic renal failure and different markers of inflammation such as C-reactive protein (CRP), IL-6, TNF-α, fibrinogen suggesting that CKD is a low-grade inflammatory process with leucocytes being the key mediators in this process.[42,53] Moreover, inflammatory markers are predictors of kidney function deterioration implying that persistent inflammation may also be a risk factor for further deterioration of kidney function.[54] CRP formed locally in the kidney reduces nitric oxide production and induces monocyte recruitment and foam cell formation.[55] In addition, it has been demonstrated that elevated CRP, IL-6 and fibrinogen are independent predictors of CV outcomes in patients with CKD.[56] Therefore, one could postulate that inflammation promotes both deterioration in renal function and CVD. Although precise mechanisms that contribute to the high prevalence of inflammation in CKD are unknown, ROS have been proposed as a potential contributor. Oxidative stress is able to activate transcriptor factors such as NF-κB which regulates inflammatory mediator gene expression.[57] Presence of antioxidants has been shown to prevent NF-κB activation by ROS and depletion of reduced glutathione facilitates activation of NF-κB.[58]

Both dialysis-related and dialysis-unrelated factors may contribute to the high prevalence of inflammation in CKD patients. Intercurrent clinical events, chronic infections with *Chlamydia pneumoniae*, peridontitis, biofilm formation in haemodialysis patients, truncal obesity, and volume over load have all been implicated as culprits in this process. In addition, there is a significant interindividual variation in the prevalence of inflammation which may point to genetically determined variations in the inflammatory response.[56]

Endothelial dysfunction

Endothelial dysfunction reflects decreased bioavalability of nitric oxide (NO) and precedes structural changes and clinical manifestations of atherosclerosis.[59] Levels of asymmetric dimethylarginine (ADMA), a methylated product of L-arginine, are elevated in CKD. ADMA is known to inhibit endothelium-dependant NO bioavailability and high ADMA concentrations are associated with higher carotid intima media thickness (IMT) and CV events in patients with CKD.[60] In addition, Ravani *et al.* demonstrated that ADMA represents a strong and an independent risk marker for progression of CKD.[61] Pharmacological interventions with statins, vitamin E, and homocysteine-lowering therapy aimed at reducing plasma ADMA have shown inconsistent results.[62] However, improved endothelial function after administration of both soy protein and α-lipoic acid in CKD patients has been linked to decreased ADMA levels.[49]

The pathophysiological mechanisms leading to albuminuria in renal disease are complex and manifold.[63] However, albuminuria is also thought to be a reflection of generalised increase in endothelial permeability or endothelial dysfunction.[64] The association between albuminuria and CV risk is independent of an association between impaired kidney function and CV risk.[65] In addition, albuminuria is a much stronger predictor of decline in kidney function than mild kidney failure, and other cardiovascular risk factors such as inflammation, abnormalities in fibrinolysis, and dyslipidaemia are strongly associated with both albuminuria and endothelial dysfunction.[49]

Thus, in early CKD both the estimated glomerular filtration rate (eGFR) and low-grade albuminuria seem to play a pathophysiological role in increased CVD. Results from the HUNT II study, performed in 9000 Norwegians, demonstrated that mild changes in both eGFR and albuminuria were independently associated with increased CVD.[66] Stam *et al.* demonstrated that for each decrease of 5 ml/min/1.73 m^2 of GFR, the relative risk of CV death increased by 22% (relative risk 1.22; 95% CI 1.09 to 1.36) and much of this risk was accounted for by albuminuria (adjusted models relative risk 1.17; 95% CI 1.04 to 1.31). Rodondi *et al.* also demonstrated that albuminuria had a stronger association with atherosclerosis than eGFR.[67] Although in early stages of CKD, an increase in albumin excretion seems to be more important than a small decrease of eGFR, it seems unlikely that albuminuria accounts for the exponential increase in CVD risk once the eGFR decreases to under 60 ml/min/1.73 m^2. However this is still a matter of debate.

Interruption of the renin-angiotensin axis with ACE inhibitors or angiotensin II receptor blockers (ARBs) seems to be pivotal in reducing albuminuria. In the PREVEND intervention trial, patients with albuminuria and without hypertension were randomised to one of two arms, an ACE inhibitor *vs* placebo or a statin *vs* placebo.[68] Use of an ACE inhibitor reduced albuminuria and CV mortality, although this improvement could have been related to reduction of blood pressure.

In response to endothelial injury, endothelial progenitor cells (EPC) are mobilised from the bone marrow to act as repair cells. In CKD there is impaired migratory activity and a decreased number of EPC in the circulation, which may play a role in the progression of atherosclerosis.[69] In fact, low levels of EPC predicted the occurrence of CV disease and death in patients with coronary artery disease.[70]

However, a causal association between endothelial dysfunction and CVD in CKD patients remains to be established. Therefore endothelial dysfunction should be considered a cardiovascular marker until such time that more studies illuminating this issue become available.

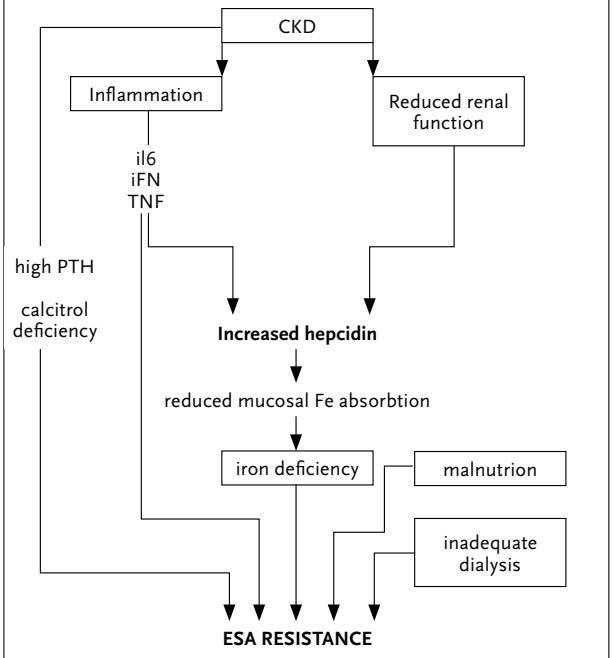
Anaemia

Anaemia frequently occurs in CKD and becomes more prevalent as renal function deteriorates. The prevalence of anaemia reaches about 50% in stage 4 CKD whereas in stage 5 anaemia is almost universal.[71] In patients with CKD, anaemia is a common contributor to poor quality of life (QOL), hospitalisation and mortality. There are several physiological explanations for this finding: anaemia is associated with a poor delivery and utilisation of oxygen, a decreased immune response and impaired cognition. Moreover anaemia in CKD is linked to left ventricular hypertrophy (LVH), angina and congestive heart failure (CHF).[71,72]

The severity of anaemia depends both on the cause of the CKD and the degree of GFR loss. In CKD the primary cause of anaemia is insufficient erythropoietin (EPO) production in combination with insufficient response to EPO caused by the mechanism usually described as 'anaemia of chronic disease'. Secondary causes that contribute to the development of anaemia include absolute iron deficiency, hyperparathyroidism and a shorter lifespan of red blood cells caused by uraemia.[71-73]

Anaemia of chronic disease develops as the result of a chronic inflammatory disorder.[74] In CKD the overall inflammatory response is enhanced and inflammatory cytokines such as hepcidin and Il-6 are increased.[75] Enhancement of erythropoiesis by EPO requires intact EPO signalling (EPO receptor, downstream JAK/STAT signalling and transcriptional response) and effective mobilisation of iron stores.[73] Hepcidin inhibits the efflux of iron into plasma transferrin by downregulating ferroportin, the efflux channel for iron in macrophages and in enterocytes (*figure 5*).[76] Enhanced synthesis of hepcidin thus leads to inhibition of iron absorption in the small intestine and sequestering of iron in macrophages, resulting in limited iron availability for erythropoiesis. In addition to its effect on iron metabolism, hepcidin may contribute to EPO

Figure 5. *Mechanisms leading to EPO resistance in patients with various degrees of renal dysfunction*



Inflammation and hepcidin, which links inflammation and iron metabolism, are pivotal factors involved in EPO resistance. Both inflammation and reduced renal function increase plasma hepcidin levels. Hepcidin reduces iron availability in among other ways through a reduction in mucosal iron uptake. Furthermore, inflammation per se as well as disturbed phosphate and vitamin D metabolism directly reduce bone marrow response to EPO. When renal function further deteriorates additional factors that induce EPO resistance become important such as inadequate renal replacement therapy and protein energy wasting. ESA = erythropoiesis stimulating agents.

resistance through a direct inhibitory effect on erythroid progenitor proliferation and survival.[77] In patients with CKD and in haemodialysis patients, it has been shown that hepcidin levels are higher than in healthy controls.[78-80]

From a biological point of view, it is plausible that anaemia has an impact on cardiac structure, function and outcomes. Following the large amount of observational data suggesting a 'cause and effect' relationship between anaemia and outcomes, a series of erythropoietin treatment trials was undertaken. Starting in the late 1990s, different hypotheses were investigated in clinical trials: early *vs* late anaemia correction and higher *vs* lower haemoglobin targets in both dialysis and predialysis CKD patients.[81] It is important to note that these studies compared actively treated groups with different target haemoglobin levels and that no placebo-controlled trials were performed. The main overall result is that these studies failed to demonstrate that normalisation of haemoglobin levels using EPO is beneficial and, importantly, that normalisation of haemoglobin levels may be associated with worse outcomes.[82] As an example three major clinical trials investigating the effect of different target haemoglobins on anaemic CKD patients (the Anaemia CORrection in Diabetes (ACORD),[83] Correction of Hemoglobin and Outcomes in Renal Insufficiency (CHOIR)[84] and Cardiovascular Risk Reduction by Early Anaemia Treatment with Epoetin Beta (CREATE)[85] are shown in *table 1*.

The mechanisms underlying the higher mortality rate are unclear. Increased tendency for developing thrombosis or an elevated blood pressure may contribute to an increase in lethal CV events. Other mechanisms such as deregulation of production/responsiveness of vasoactive factors may also play a role. In addition, it is still unclear whether the higher mortality is related to the higher haemoglobin target itself or to the means by which the haemoglobin level is achieved. A secondary analysis of the CHOIR study points to the dose of EPO used rather than the achieved haemoglobin level.[86]

The Trial to Reduce Cardiovascular Events with Aranesp Therapy (TREAT) is the first randomised, placebo-controlled study to investigate whether raising haemoglobin in CKD patients with erythropoietin is beneficial.

Table 1. *Major clinical trials investigating the effect of different target levels of haemoglobin on anaemic CKD patients*

Study	Primary endpoint(s)	Secondary endpoints
ACORD	Change in LVMI from baseline	LV volumes; LV ejection fraction (LVEF); fractional shortening
CHOIR	Time to death; MI; hospitalisation for CHF and stroke	Time to renal replacement therapy; hospitalisation following CV and any cause; QOL
CREATE	Time to first CV event (incl. sudden death, MI, acute HF, stroke, transient ischaemic attack, angina pectoris) resulting in hospitalisation for ≥24 h or prolonged hospitalisation; complications of peripheral vascular disease (necrosis, amputation)	Death from CV and any cause; CHF; the need for CV intervention; hospitalisation following CV and any cause; changes in LVMI and LV volumes; time to initiation of renal replacement therapy; changes in BMI; serum albumin level; C-reactive protein level; changes in QOL, Hb level and weekly epoetin dose; the need for dialysis and transfusion and decreases in eGFR

LVMI = left ventricular mass index; MI = myocardial infarction; CV = cardiovascular; (C)HF = (congestive) heart failure; LVEF = left ventricular ejection fraction; QOL = quality of life; Hb = haemoglobin; eGFR = an estimated glomerular filtration rate.

In summary, although it is clear that anaemia and EPO resistance is associated with increased morbidity and mortality, the beneficial effect of treatment of anaemia using EPO on mortality in CKD remains to be established.

CARDIOVASCULAR OSSIFICATION

In the search for factors that might contribute to the enhanced cardiovascular risk in recent years, the role of renal disease-induced abnormalities in calcium-phosphorus metabolism has become apparent. A strong association has been described between hyperphosphataemia, hyperparathyroidism and CV disease.[87] This increased CV disease is probably caused by increased vascular calcification and evidence is emerging that optimising treatment of calcium and phosphate alterations may decrease CV risk in CKD patients.[88] Likewise in patients with CKD, phosphorus levels are also associated with cardiovascular outcome. Several factors including parathyroid hormone (PTH) and vitamin D play a critical role in maintaining plasma phosphate levels.[89] Fibroblast growth factor 23 (FGF23) has recently been identified as an important regulator of systemic phosphate balance.[90] FGF23 is a 32-kDa protein that is predominantly expressed in osteocytes in the bone and in the endothelial cells that line the venous sinusoids of the bone marrow.[91] Under physiological conditions FGF23 promotes phosphaturia and suppresses the 1 α-hydroxylase activity, thus leading to a reduction in 1,25-dihydroxyvitamin D levels. Transgenic mice over-expressing FGF23 have reduced plasma phosphate concentration, phosphaturia and reduced renal phosphate sodium co-transporter.[92] The phosphate balance is altered in CKD and in these patients very high levels of FGF23 have been demonstrated.[93] As the number of viable nephrons decreases in CKD, in spite of the high FGF23, the net phosphate excretion does not increase sufficiently. This high phosphate level in combination with the reduction in 1,25-dihydroxyvitamin D levels leads to secondary hyperparathyroidism. However, the exact role of FGF23 in renal osteodystrophy has not been established. Fliser *et al.* demonstrated a correlation between increased FGF23 concentration and progression of chronic renal failure suggesting that FGF23 may play a role in progression of renal failure.[94] Other related factors such as calcium phosphate product and PTH also correlated with progression of renal failure in these subjects. Therefore, further studies are needed to better understand the role of FGF23 in patients with CKD.

Disturbances in circadian rhythm

An upcoming field of interest is the effect of disturbances in circadian rhythms in CKD. Circadian rhythms are fluctuations in nearly all bodily functions with a period of about 24 hours. In renal patients some of these circadian processes are disrupted. For example, sleep disturbances are much more prevalent in ESRD patients than in the general population.[95] In dialysis patients several studies on the impact and importance of sleep problems on quality of life revealed that sleep disturbances have a major effect on vitality and general health of these patients.[96] These sleep disturbances can have multiple causes, e.g. sleep apnoea and/or restless legs/periodic limb movement disorder, dialysis treatment or pathology of renal disease.

A key mechanism for the circadian sleep-wake rhythm disorders in CKD might be the disturbance of the circadian rhythm of the pineal hormone melatonin. The nocturnal melatonin rise above a certain threshold (Dim Light Melatonin Onset) declines with decreasing kidney function and is absent in many daytime haemodialysis (HD) patients. Exogenous melatonin intake in HD patients led to significant improvement of sleep parameters and melatonin rhythm.[97] In addition to the modulation of the circadian sleep-wake rhythm, several functions of melatonin have been put forward, such as influences of melatonin on the cardiovascular system. Firstly, melatonin shows antioxidative properties.[98] Secondly, the absence of a dipping blood pressure profile was associated with the absence of a nocturnal melatonin rise and administration of melatonin was associated with lowering of both systolic and diastolic blood pressure.[99,100] Further research on the effect of melatonin on blood pressure regulation and cardiovascular risk profile is warranted.

Future directions

Although in CKD many both traditional and nontraditional risk factors are associated with increased morbidity and mortality, data substantiating that intervention in these risk factors improves outcome is limited. In contrast, recent studies have shown that, for instance, cholesterol reduction and haemoglobin normalisation fails to improve prognosis. Therefore randomised controlled trials are indispensable to ascertain the optimal levels and optimal interventions for traditional and nontraditional risk factors in CKD and ESRD patients. There are clear indications that impaired renal function is an independent risk factor for developing cardiovascular disease in the general population. However, in spite of numerous studies in patients with CKD the pathophysiological mechanisms leading to this increased risk are unclear. Future research should in our opinion focus on studying mechanistic pathways rather than cross-sectional associations between the individual risk factors and CV mortality and morbidity in CKD patients.

Ideally, determinants of the excess cardiovascular risk in patients with CKD will be compared with patients without CKD, in a prospective population-based study with the exclusion of patients with CV disease at the baseline. Furthermore, experimental mechanistic studies in patients with mild kidney failure may also help to

identify the impact of traditional risk factors such as hypertension, obesity and diabetes and their interaction with nontraditional risk factors in CKD patients. Finally, randomised controlled trials are necessary to ascertain the optimal levels and optimal intervention for traditional risk factors in CKD and ESRD patients.

In conclusion, factors such as endothelial dysfunction, oxidative stress, vascular ossification, inflammation and anaemia are strongly interrelated and thought to play an important pathophysiological role in the initiation and progression of CVD in patients with CKD. However a causal relation between these factors and increased CVD in CKD patients remains to be further elucidated. Intervention studies designed to test whether the above-mentioned factors are not only markers but also aetiological risk factors may provide further information that may lead to novel therapeutic options.

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ERRATUM

Unfortunately, in the article 'Reintroduction of Riva-Rocci measurements to determine systolic blood pressure?' from the authors Verrij E, van Montfrans G, Bos J-W, which was published in Neth J Med. 2008 Dec;66(11):480-2, the initials of one of the authors were incorrect. Bos J-W should have been Bos WJ.
We apologise for any inconvenience.

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REVIEW

Effects of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers in patients with chronic kidney disease

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ABSTRACT

Since about three decades, inhibitors of the renin-angiotensin system have been available in clinical practice. Although angiotensin-converting enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARB) were primarily aimed at treatment of hypertension and heart failure, more of their positive effects were discovered later on. Patients with chronic kidney disease were recognised to profit the most from treatment with these agents; however some blind spots are still present. Patients with advanced renal failure are almost always excluded from the trials; patients with end-stage renal disease form the least studied population of all and outcomes of treatment with ACEi/ARB are still uncertain in these cohorts. The aim of this review is to summarise and update the evidence about effects of AII inhibitors in patients with chronic kidney disease with the specific emphasis on patients treated with dialysis. Lately a novel indication for ACEi/ARB administration, especially for peritoneal dialysis patients, has been proposed. It is based on the capacity of these drugs to inhibit the local tissue renin-angiotensin system, which results in less development of peritoneal fibrosis and a longer life for the peritoneal membrane. The most recent available data are presented in this review.

KEYWORDS

ACE inhibitors, angiotensin II receptor blockers, chronic kidney disease, dialysis

CONTROLLING HYPERTENSION

Hypertension is the major risk factor in developing and progression of nondiabetic and diabetic chronic kidney disease (CKD). Currently the prevalence of hypertension in the general population is about 1 billion people worldwide and a further rise is predicted for the near future.[1] Development of hypertension is highly associated with older age (over 60 years), non-Hispanic black race and body mass index ≥30.[2] In order to prevent end-organ damage and development of major cardiovascular events, blood pressure (BP) should be well-controlled. However the current situation is far from optimal worldwide, especially in CKD patients.[2,3] In patients with existing nephropathy, the goal of hypertension management involves not only cardiovascular protection by lowering BP to the appropriate level, but also slowing the progression of kidney disease. The latter often includes management of proteinuria, which is itself associated with both the risk of cardiovascular disease and progression to end-stage renal disease.[4] Therefore, it is of great importance to choose an appropriate antihypertensive agent for patients with CKD.

An increase in the renin-angiotensin-aldosterone activity is one of the major factors involved in the hypertension seen in patients with CKD. Angiotensin II (AII) is known to mediate systemic haemodynamic changes as well as changes in intrarenal circulations.[5] Moreover, this hormone has been recognised to play a key role in sustaining proteinuria and progression of kidney disease.[5,6] Therefore, inhibiting effects of AII and lowering blood pressure with drugs that block the renin-angiotensin system (RAS) is a major component of CKD treatment.[7] Can ACEi and ARB achieve the optimal blood pressure target? This usually depends on how aggressive BP management should be. According to the different guidelines, the majority of CKD patients would benefit from a BP level lower than 130/80 mmHg. However, one should be aware about serious side effects of aggressively lowering BP in patients with

advanced kidney disease and end-organ damage. Besides, there is currently no evidence whether diabetic patients and patients with nondiabetic nephropathy with proteinuria >1 g/d would definitely benefit from the low BP target.[8] In patients without diabetes and a level of proteinuria between 0.3 and 1g/dl strong consideration is given to achieving a BP level lower than 130/80 mm/Hg, unless a specific trial were to show otherwise.[8] However, as stated above, one should be aware of the difficulty to reach such a BP target, especially in diabetic patients. In four randomised controlled trials (RCTs) in diabetic nephropathy, the usual number of antihypertensive drugs needed to achieve a diastolic BP of <85 mmHg was three, which indicates that such a task requires multiple drug therapy.[9-11] However, in patients with CKD, AII inhibitors should be considered a first-line therapy because of their effects beyond BP control alone and additional benefit for high-risk patients.

AII INHIBITORS AND CARDIOVASCULAR PROTECTION

Primary ACEi were aimed to treat hypertension and management of heart failure. Knowing AII to be involved in vasoconstriction, hypertrophy of cardiovascular cells as well as in the fibrotic process in the heart and vessels, cardiovascular protection can be expected from ACEi/ARB treatment.[12,13] The classical SAVE and SOLVD trials showed a significantly lower mortality risk in patients with heart failure receiving the ACEi captopril and enalapril.[14,15] Later the HOPE study confirmed these findings by showing a reduction in the risk of myocardial infarction, stroke and risk of death due to a CV event by 20 to 30% in patients with or without heart failure treated with ramipril.[16] Afterwards two trials with contradicting results were published: one showed that perindopril reduced CV mortality, nonfatal MI and cardiac arrest in patients with stable angina pectoris,[17] the other could not confirm such results by using trandolapril.[18]

Cardiovascular disease (CVD) is a leading cause of death among CKD patients.[19] Retrospective analyses of the SAVE and HOPE trials came to the conclusion that treatment with ACEi was associated with an equal or even a greater risk reduction of all-cause mortality in the group of patients with renal insufficiency compared with the ones with a normal glomerular filtration rate (GFR).[20,21] A substudy of HOPE showed that adding ramipril to the antihypertensive regimen in patients at high risk of cardiovascular events decreased cardiovascular events by 25%.[22] Medications that inhibit the RAS are known to reduce CVD complications in patients with diabetic nephropathy.[9,11,23] In diabetic nephropathy two studies reported CVD outcome as a secondary endpoint. One showed that congestive heart failure was less frequent in the losartan-treated group compared with placebo or the group treated with amlodipine.[11] However, in this trial no difference was shown with regard to CV morbidity, such as the occurrence of MI, stroke, or unstable angina. Another trial also reported less admissions for heart failure and a trend towards less nonfatal MI for patients receiving losartan.[9] However, neither of these trials were aimed to study cardiovascular morbidity and mortality in the first place.[24] Recently, new data have become available: results of a big multinational RCT in which primary outcomes were cardiovascular events in high-risk individuals with various vascular disease, treated either with ARB alone or in combination with ACEi.[25] In the ONTARGET trial both ramipril and telmisartan appeared to be equally effective to prevent a major cardiovascular event in a wide range of high-risk patients, including ones with CKD.

Overall there is not enough evidence on effects of ACEi/ARB treatment of patients with CKD and CVD to reduce cardiovascular complications. Patients with advanced kidney disease are very often excluded from the big RCTs and therefore a clinical trial powered specifically for such outcomes in high-risk CKD patients is required.

EFFECTS ON PROTEINURIA AND PROGRESSION OF KIDNEY DISEASE

Proteinuria is very often present in CKD and its magnitude directly influences the rate of renal function deterioration.[26] For more than a decade ACEi/ARB are known to have pronounced antiproteinuric and renoprotective properties, independently from their primary antihypertensive effect. This was first shown in patients with type 1 diabetic nephropathy in a CAPTOPRIL trial in 1993.[23] The study showed that compared with placebo, in patients receiving captopril there was a 30% reduction in proteinuria, 43% reduction in the risk of doubling of serum creatinine and a 50% reduction in the combined endpoint of death, need for dialysis or transplantation. These changes were observed independently of the BP levels.

In the last ten years a number of studies have been performed investigating the ability of ACEi/ARB to decrease the rate of progression of proteinuria and diabetic nephropathy.[27-29] The main findings of the biggest trials performed with AII inhibitors in patients with CKD I-IV were primary focused on renal outcomes and are summarised in *table 1*.

In patients with nondiabetic kidney disease several large studies confirmed the pronounced antiproteinuric and renoprotective effects of ACEi: ramipril was associated with a major reduction of proteinuria, slower GFR decline and risk of doubling serum creatinine or progression to end-stage renal disease (ESRD).[30,31] Two studies comparing benazepril with placebo on top of other antihypertensive

Table 1. *Randomised controlled trials on effects of ACEi/ARB with primary renal endpoints in patients with diabetic nephropathy, mild to moderate renal insufficiency and proteinuria*

Study	Number of patients	Regimen compared	Mean follow-up	Effect on reduction of proteinuria	Effect on renal function preservation	Other effects
CAPTOPRIL, 1993	409	Captopril *vs* placebo	3 years	+		Reduction in combined endpoint of death and need for dialysis
RENAAL, 2001	1513	Losartan *vs* placebo	3.4 years	+	+	Reduction in combined endpoint of death, progression to ESRD
IDTN, 2001	1715	Irbesartan *vs* amlodipine *vs* placebo	2.6 years	+	+	Reduction in combined endpoint of death, progression to ESRD
BENEDICT, 2004	1200	Trandolapril *vs* verapamil *vs* both *vs* placebo	48 months	+	+	ACEi slowed progression to microalbuminuria
REIN-2, 2005	338	Ramipril *vs* ramipril +felodipine; normal *vs* low BP target	19 months	-	-	No differences in renal outcomes

BP = blood pressure; ESRD = end-stage renal disease; ACEi = angiotensin-converting enzyme inhibitors.

regimens confirmed the above effects of ACEi.[32,33] It is worth mentioning that one of them, an AIPRI study, was focused on renoprotective properties of benazepril in patients with CKD of various aetiologies, but patients with glomerular disease were found to have the greatest profit from such treatment compared with the ones with polycystic kidney disease, nephrosclerosis or interstitial nephritis.[33] The data on major trials in patients with nondiabetic CKD are given in *table 2*.

The classic CAPTOPRIL study provided evidence that the stage of CKD and the amount of proteinuria are the main factors that determine the benefit from the use of an AII inhibitor. Patients with serum creatinine of >180 mmol/l had the greatest effect from using ACEi when compared with those with minor renal insufficiency (<90 mmol/l). A couple of other studies together with a meta-analysis showed ACEi/ARB to have their best renoprotective effect in patients with the largest amounts of proteinuria[31,33] and an estimated GFR of <60ml/min.[34] Therefore, ACEi/ARB have renoprotective qualities, which are the most pronounced in patients with proteinuria and advanced kidney disease.

ACEi '*vs*' or 'and' ARB?

Generalising all the information available today, it appears that both ACEi and ARB can provide sufficient renal and cardiovascular protection.[8,24] However, more evidence is needed to prove these medications to be equivalent in patients with similar clinical conditions. A few trials already contributed to this. One compared telmisartan

Table 2. *Randomised controlled trials on the effects of ACEi/ARB on primary renal endpoints in patients with nondiabetic nephropathy, moderate renal insufficiency and proteinuria*

Study	Number of patients	Regimen compared	Mean follow-up	Effect on reduction of proteinuria	Effect on renal function preservation
AIPRI, 1996	583	Benazepril *vs* placebo	3 years	+	Reduction of risk of doubling of serum creatinine or progress to ESRD
REIN, 1997	166	Ramipril *vs* placebo	16 months	+	Lower risk of GFR decrease, doubling of serum creatinine or progression to ESRD
AASK, 2001	1094	Ramipril *vs* metoprolol *vs* amlodipine; normal *vs* low BP goal	3-4 years	Patients with proteinuria >1g/d in the group with low BP goal had slower GFR decline	Lower risk of combined end-point of death, 50% decrease of GFR or reaching ESRD
Hou *et al.*, 2006	224	Benazepril *vs* placebo	3-4 years	+	Decreased risk of doubling of serum creatinine, ESRD or death

BP = blood pressure; ESRD = end-stage renal disease; GFR = glomerular filtration rate.

and enalapril with regard to their effects on the change of GFR, proteinuria, serum creatinine, BP level, rates of ESRD and cardiovascular events and all-cause mortality in patients with type 2 diabetes.[35] The study's conclusion was that these two agents are similar in providing long-term cardioprotection and renoprotection. One of the main objectives of the recent ONTARGET trial was to compare long-term cardiovascular effects of telmisartan and ramipril in high-risk patients with different vascular illnesses. The investigators found ACEi and ARB to be equal from that prospective. With regard to renal outcomes, although this was not the primary aim of the study, it appeared that telmisartan's effects on major renal outcomes were similar to ramipril in patients with a high vascular risk.[36] However the same trial confirmed the earlier observation, that ARB in general are better tolerated then ACEi which have a higher incidence of hyperkalaemia, cough and may induce angioedema.[37] On the other hand more evidence is available for the effectiveness of ACEi in clinical practice. Together with the higher cost of ARB this may influence the clinician's choice. With regard to the combination of ACEi and ARB there is a still ongoing discussion. In theory such a combination could provide better blockade of the RAS and therefore be more effective in reaching the goal to protect renal function. However, the up-to-date findings are controversial. On one hand such a combination was shown to be effective in terms of treatment of proteinuria regardless of BP changes.[8,38,39] On the other hand, the recent ONTARGET trial did not show any advantage over monotherapy with regard to the decline of GFR and the need for chronic dialysis,[4,36] as well as the rate of cardiovascular events. Additionally, monotherapy has been proven to be well tolerated while combination therapy showed a higher risk for developing hypotension and hyperkalaemia.

To summarise all of the above, it should be noted that for patients with chronic kidney disease both ACEi and ARB can provide appropriate control of blood pressure and proteinuria as well as similar renal and cardiovascular protection. Today there is still more evidence for efficacy of ACEi, but already many good-quality studies have shown ARB to be equivalent. Regarding the combined use of these two RAS blocking agents, more evidence is needed to answer specific questions for the treatment of patients with different types and severity of CKD.

Use of ACEi/ARB in patients treated with dialysis

After reaching the end stage of chronic kidney disease, the majority of patients will start renal replacement therapy with one of the two types of dialysis. It has been stated that in dialysis patients the risk of cardiovascular mortality is 10 to 20-fold higher than in age- and sex-matched general population without kidney damage.[40] Hypertension is one of the most important risk factors of cardiovascular complications in patients treated with dialysis.[41] About 80% of patients requiring dialysis treatment are hypertensive.[42] Controlling hypertension in patients with ESRD is a well-recognised problem which often requires administration of multiple medications. Antihypertensive agents of different groups are applicable for blood pressure control; however there is a lack of evidence about their efficacy and about BP targets for patients on dialysis. A couple of recent systematic reviews and meta-analyses collected evidence from randomised trials and concluded that hypertension should be treated in patients on dialysis; however, no superiority of any antihypertensive medications was proven.[43,44]

Although β-blockers, calcium-channel blockers and AII inhibitors have been shown to be suitable for BP control in patients on dialysis,[44,45] the last mentioned may provide an additional benefit in this high-risk patient population. Activation of the RAS is recognised to be essential for hypertension and the increased risk of cardiovascular events in dialysis patients. It has been shown that in such patients a chronic overactivity of RAS is often present, together with increased activity of plasma renin.[46] These factors together with expansion of the extracellular volume and interdialytic weight gain create a vicious circle in which management of hypertension in haemodialysis (HD) patients remains difficult. However, there is enough evidence to state that HD patients, especially those with increased plasma renin activity (PRA), would benefit from adding drugs that inhibit AII into their antihypertensive regimen. A number of studies have been done, which showed significantly reduced mortality risk for ESRD patients with cardiovascular disease treated with ACEi.[47,48] Two studies showed a survival benefit for HD patients receiving ACEi;[49,50] however, data suggest that only 30 to 50% patients on dialysis are prescribed these medications.[45,46,51-53]

Apart of their direct effect on BP, ACEi/ARB have also shown the ability to reduce an increased sympathetic nerve discharge in patients with chronic kidney disease and high renin levels.[54] Patients on HD often have overactivity of the sympathetic nervous system, which is another reason for the development of hypertension.[55] Such symptoms as xerostomia and thirst were found to be highly associated with higher interdialytic weight gain and chronic fluid overload.[56] The latter direct impact on hypertension in HD patients and makes it more treatment resistant. AII has also been claimed to be a dipsogenic agent and couple of studies have shown previously that ACEi could reduce thirst in patients undergoing HD.[57-59] In the first double-blind, placebo-controlled trial with a crossover design in 25 HD patients, the use of enalapril was associated with a reduction in thirst, oral fluid intake and, consequently, in weight gain between dialysis sessions.[58] However, the other studies could not confirm such an effect of ACEi and ARB.[60,61] One recent study investigated the antidipsogenic

effect of dual blockade of RAS with ACEi and ARB, and also failed to confirm the hypothesis.[62] The possible explanations for such discrepancy could be the small size of the referenced studies (usually less than 30 patients), as well as differences in the studied population; however antidipsogenic properties of AII inhibitors need more investigation.

ACEi/ARB use in patients on peritoneal dialysis

Until recently AII inhibitors were generally used in patients undergoing peritoneal dialysis (PD) because of their effects on the cardiovascular system. In the last ten years, a number of studies have been done to investigate the ability of these medications to suppress local RAS and attenuate peritoneal fibrosis development, and therefore to prolong the 'effective life' of the peritoneal membrane. Experimental and clinical studies which were focused on specific effects of AII inhibitors in long-term peritoneal dialysis patients are presented in the last part of this review.

AII inhibitors as antifibrotic agents

PD has a survival advantage over haemodialysis in the first couple of years of renal replacement therapy (RRT).[63] However, after long-term PD (>2 years) the technique and patient survival deteriorates.[63-65] This could partially be explained by loss of the residual renal function (RRF) and changes in the peritoneal membrane.[66] During long-term treatment with peritoneal dialysis the peritoneal membrane is affected by solutions with high concentrations of glucose and glucose degradation products (GDPs).[67] Besides, uraemic toxins as well as inflammatory cytokines induced by acute and chronic inflammation may also contribute to the damaging process.[66] Morphological changes in the peritoneal membrane associated with long-term peritoneal dialysis treatment include interstitial fibrosis, loss of the mesothelial cell layer, neoangiogenesis and vasculopathy.[66,68,69] These are associated with the main functional disturbances – high solute transport and ultrafiltration failure – which lead to inadequate PD treatment.[70,71] The changes in the peritoneal membrane are mediated by several growth factors. The most relevant ones are vascular endothelial growth factor (VEGF)[72-75] and transforming growth factor β1 (TGF-β1).[76-79] The latter appears to be related to the AII, which is produced by the local RAS, and is present in human peritoneal mesothelial cells (HPMC).[80] Locally produced AII regulates cell growth and synthesis of extracellular matrix and therefore has all the properties of a growth factor.[81,82] In HPMC, AII acts as a profibrotic agent, inducing production of a fibronectin and glucose-induced TGF-β1.[80,83] It has been shown that their expression can be significantly reduced by the ACEi and ARB.[80,84] Production of VEGF, the growth factor essential for the development of ultrafiltration failure, was also shown to be attenuated by ACEi/ARB in a recent *in vitro* study.[85]

Animal studies

A number of studies have been done in experimental animal models, which confirmed the findings of the above cell culture studies. The use of ACEi enalapril and lisinopril in rats showed decreased fibrosis and angiogenesis.[86-88] Also lisinopril and valsartan (an ARB) have been found to reduce levels of TGF-β1 and VEGF in rats' PD effluent.[89] The ARBs irbesartan and olmesartan were also shown to protect against peritoneal fibrosis caused by bacterial peritonitis and PD fluid with an acidic pH.[90,91] ACE inhibition was also beneficial in a murine model of chlorhexidine/ethanol induced encapsulating peritoneal sclerosis (EPS); in this model oral administration of quinapril for up to 56 days markedly reduced peritoneal thickening.[92]

Studies in humans

Relatively little is known about specific effects of ACEi/ARB in PD patients. The most relevant of these include their impact on peritoneal membrane function, residual renal function, PD technique and patient survival.

Effects on peritoneal transport

Studies focused on effects of these medications on peritoneal membrane transport can be divided into short- and long-term. In the first short-term study a decrease in peritoneal protein loss was observed in 12 continuous ambulatory peritoneal dialysis (CAPD) patients treated with the ACEi captopril.[93] After a few years the same group found a similar effect for the ARB, irbesartan.[94] In contrast, the study by Favazza *et al*. comparing effects of clonidine, enalapril and nifidipine, showed higher peritoneal clearances of creatinine and β2-microglobulin with enalapril.[95] Other authors were not able to show any effect of enalapril or losartan on peritoneal transport in CAPD patients in short term.[96,97] Given the discrepancy of these results, more studies are needed to provide clarity. Knowing that long-term peritoneal membrane changes do not occur before two to three years on PD, studies with sufficiently long follow-up could give an answer whether the long-term use of AII inhibitors can influence peritoneal transport. A first single-centre study focused on effects of ACEi/ARB on peritoneal membrane transport in long-term PD patients was performed by our group.[98] Our major finding was a different time course of small solute transport during the first three to four years of PD treatment. Patients treated with ACEi/ARB showed a slight decrease in the mass transfer area coefficient (MTAC) of creatinine and urea. This was different from the controls in which an increase in time of treatment was found. It suggested inhibition of peritoneal angiogenesis which

is in agreement with results from experimental studies. In another study we were able to confirm the above results on 217 incident CAPD patients participating in the Netherlands Cooperative Study on Adequacy of Dialysis (NECOSAD) treated with PD for at least two years.[52] Once again, patients treated with ACEi/ARB showed a slight decrease of their 24-hour dialysate/plasma-creatinine ratio during the follow-up while an increase was observed in controls.

Effects on PD technique and patient survival

Given all of the above findings, it was also hypothesised that membranoprotective properties of ACEi/ARB could positively influence the technique survival of PD. Our study showed a tendency for patients treated with ACEi/ARB for at least 75% of their time on PD to have a better technique survival although such an assumption could not be statistically confirmed.[52] A possible explanation for this could be the fact that in the NECOSAD database only a very small number of patients are documented as being switched to HD due to problems with peritoneal transport, and therefore the real magnitude is hard to detect.[65]

With regard to survival of PD patients, the effects of ACEi/ARB were found to be controversial. Recently, Fang *et al* showed a significantly lower mortality risk in those receiving ACEi/ARB *vs* untreated patients.[51] Use of these medications was associated with reduced all-cause mortality. Factors, associated with mortality were age, low serum albumin and congestive heart failure. In contrast, a study done by our group did not find a survival benefit with regard to ACEi/ARB treatment.[52] A possible explanation for the discrepancy of these results is the difference between the studied cohorts. Besides, in observational studies it is hard to prove a link between treatment and outcome as confounding by indication can never be avoided.[99]

Effects on residual renal function

A number of clinical trials provided evidence for a survival benefit for PD patients with preserved residual renal function (RRF).[100-103] This can be explained by the fact that, unlike dialysis, native kidneys not only remove small solutes, but also protein-bound substances by active secretion in the proximal tubules. Better preserved RRF is also associated with less comorbidity,[103-105] better fluid and nutritional status.[106,107] Although there is plenty of evidence for the renoprotective effects of AII inhibitors in patients with chronic kidney disease stage I-IV,[9,11,108] the presence of such effect in PD patients is a subject of controversy. A large observational study in more then 1000 PD patients showed that development of anuria was delayed in those receiving ACE inhibitors.[53] However, these results were not confirmed by a smaller single-centre study.[109] Two RCTs also suggested renoprotective properties of ACEi/ARB in PD: they both showed a different time course of residual glomerular filtration rate (rGFR) as well as a longer duration of anuria development for treated *vs* untreated patients.[110,111] However, the findings of these two RCTs are somewhat contradictory: one showed a temporary decrease of rGFR after the start of treatment with lisinopril, while the other reported a major increase after starting losartan.

The difference in the RCTs could be partially explained by confounding by indication, also known as selection by prognosis. The distinct difference between RCTs and observational studies, such as cohort studies, is that an RCT can provide evidence for a causal relationship because it has the potential to avoid confounding by indication.[99,112] The patients most often prescribed ACEi/ARB use these drugs because of hypertension, heart failure and diabetes mellitus. However, these conditions themselves are associated with a more rapid decline in residual renal function.[53,113]

Use of ACEi/ARB in patients after kidney transplantation

After receiving a kidney transplant CKD patients form another special cohort in which possible effects of other immunosuppressive medications have been barely studied. Not much evidence exists with regard to a potential positive influence of ACEi/ARB on cardioprotection, patient and graft survival. Available data from observational and randomised controlled trials provide rather controversial results. The most recent observational studies reported better outcomes in patients treated with ACEi/ARB compared with untreated patients, which included improved patient and graft survival.[114-116] On the other hand, recently published systematic review and meta-analysis of randomised controlled trials on the use of antihypertensives in kidney transplant recipients concluded that the use of ACEi/ARB led to clinically important reductions in GFR, and therefore may have detrimental effects on clinical outcomes.[117] However, it should be mentioned that such a conclusion was made on the basis of a few studies with a rather small patient cohort, which did not report highly relevant endpoints, such as graft loss, cardiovascular events and patient death. The controversy of existing results together with a general lack of evidence creates great diversity in ACEi/ARB use in kidney transplant recipients. This was confirmed by investigators of the ongoing Long-Term Deterioration of Kidney Allograft Function (DeKAF) study, who also showed that many patients taking these medications at the time of transplantation have them discontinued, due to a fear of suboptimal allograft function postoperatively, and possible contribution to significant anaemia after transplantation.[118]

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CONCLUSION

Drugs that inhibit the RAS are proven to be effective in the treatment of hypertension and heart failure. In patients with chronic kidney disease these medications appeared to bring benefit beyond their direct effects on the cardiovascular system, resulting in preservation of renal and peritoneal function and improved patient survival. There is some evidence that patients with ESRD and after receiving kidney transplant may also profit from these main properties of ACEi/ARB, but more research is needed for clarity. It has been shown that ACEi/ARB are usually prescribed in less than a half of patients on dialysis, which means that these drugs are being underused. The novel effects of these drugs discovered makes the target population for their administration much wider, especially in patients on renal replacement therapy.

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REVIEW

New developments in the treatment of patients with multiple myeloma

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ABSTRACT

Much progress has been made in the treatment of patients with multiple myeloma (MM). The introduction of new drugs such as thalidomide, bortezomib and lenalidomide has created more possibilities for patients than many years before. In addition, autologous peripheral blood stem cell transplantation after high-dose melphalan has become the standard of care for younger patients. Allogeneic stem cell transplantation is an experimental option for those younger patients with a human leucocyte antigen identical donor. Because of these rapid developments and many treatment options we need good quality clinical studies that can guide us in what to do in everyday practice. This review will focus on those studies that have changed the treatment guidelines for patients with MM.

KEYWORDS

Multiple myeloma, bortezomib, thalidomide, lenalinomide, stem cell transplantation

INTRODUCTION

Multiple myeloma (MM) is a malignant plasma cell disorder which is diagnosed in nearly 700 patients in the Netherlands each year. The disease is characterised by the clonal proliferation of plasma cells in the bone marrow, which produce a monoclonal immunoglobulin (paraprotein or M-protein). This patient-specific M-protein can be detected in the serum or in the urine as free light chains. Typical clinical and laboratory features in patients with MM include bone pain, due to lytic lesions, osteoporosis, anaemia, renal insufficiency, hypercalcaemia and increased susceptibility to infections.

The treatment of MM has been very cumbersome for a long time because the disease is relatively resistant to conventional chemotherapeutic therapy. Since the majority of plasma cells do not divide, cell cycle dependent cytotoxic agents are of limited effectiveness. Alkylating agents such as melphalan and cyclophosphamide and corticosteroids are the most effective *conventional* agents for the treatment of this disease.

In addition, the interaction of myeloma cells with extracellular matrix proteins and bone marrow stromal cells, as well as osteoblasts and osteoclasts, play a crucial role in the drug resistance of the disease, the so-called 'cell adhesion-mediated drug resistance'. Several antiapoptotic factors are secreted by the bone marrow microenvironment, such as interleukin 6 (IL-6) which induces resistance to drug-induced apoptosis. Myeloma cells also secrete several cytokines which further stimulate IL-6 production, neoangiogenesis, osteoclast proliferation and osteoblast inhibition. Additionally, the transcription factor nuclear factor kappa B (NF-κB) is constitutively expressed in MM cells also leading to drug resistance.

Internationally and within the Haemato Oncology Foundation for Adults in the Netherlands (HOVON) clinical studies are performed that should be translated into daily patient care and hopefully will lead to better survival of patients with MM. Indeed, a recent SEER (Surveillance, Epidemiology, and End Results) programme analysis demonstrated a clear survival benefit of three years in younger patients with MM diagnosed in the years 2002-2004 compared with previous calendar periods.[1]

TREATMENT OF YOUNGER PATIENTS (≤65 YEARS)

A way to overcome the above-described drug resistance in MM is to further increase the dosing schedule of a drug. Since melphalan has moderate nonhaematological toxicity, high doses can be used in younger patients.

However, this treatment induces severe and prolonged myelosuppression. This can be overcome by the infusion of autologous haematopoietic stem cells that are collected before the administration of high-dose therapy. This autologous peripheral stem cell transplantation (PBSCT) has no antitumour effect of its own, but is a form of rescue treatment after high-dose therapy. The presence of malignant plasma cells in the infused autologous stem cell product has no influence on the relapse risk after PBSCT, and because CD34+ stem cell selection increases the infection risk post-transplant, unselected stem cell products are used for this procedure.[2]

Autologous peripheral blood stem cell transplantation

Several prospective, randomised studies have been performed comparing conventional chemotherapy with high-dose therapy combined with PBSCT.[3-7] The HOVON 24 study compared single nonmyeloablative intensive treatment with double, intensive treatment consisting of intermediate dose melphalan with additional high-dose cyclophosphamide, total body irradiation (TBI) and autologous PBSCT in previously untreated patients. A significantly higher proportion of patients achieved a complete response (CR) on protocol treatment with the high-dose therapy (32 *vs* 13%, p<0.001) and outcome was also better in terms of progression-free survival (PFS) but not overall survival (OS).

As a result of this and other studies which demonstrated improved response rates (RR), PFS rates and in some studies also improved OS rates, autologous PBSCT after high-dose melphalan (HDM) has become the standard of care for younger patients (≤65 years) and eligible patients should always be referred to hospitals with transplantation facilities for this part of their treatment.

Another important conclusion from these studies was that achievement of a CR (negative immunofixation of serum) and very good partial response (VGPR, reduction of M-protein >90%) are significantly correlated with PFS and OS.

If this relation between remission rate and survival exists, several additional treatment strategies can be developed that increase remission rates, which should lead to better outcome in the first-line treatment of younger MM patients. One strategy is to increase the remission rate before autologous PBSCT and the other is to increase the remission rate after first autologous PBSCT.

Novel drugs and autologous PBSCT

The introduction of thalidomide (Softenon) in 1999, bortezomib (Velcade) in 2005 and lenalidomide (Revlimid) in 2007 for the treatment of relapsed MM created the possibility to investigate these intensification strategies in newly diagnosed patients.

Prospective randomised studies comparing thalidomide-based induction regimens with conventional regimens such as VAD (vincristine, adriamycin and dexamethasone) followed by intensive therapy with HDM and autologous PBSCT have been performed, including the HOVON 50 study. In this study higher responses were achieved with the addition of thalidomide. At least PR was achieved in significantly more patients with thalidomide combined with adriamycin and dexamethasone (TAD) compared with VAD, 71 *vs* 57% respectively, p=0.001.[8] Response after total protocol treatment including HDM and autologous PBSCT and maintenance treatment was also better in the TAD arm compared with VAD, 88 *vs* 79% (p=0.005) with also better VGPR + CR rates, 66 *vs* 54% (p=0.005).

These better RR translated into a significantly better PFS of 34 months compared with 25 months (p<0.001) after a median follow-up of 52 months (*table 1*). However, OS was not significantly different with a median of 60 months in the control arm and 73 months in the thalidomide arm (p=0.77). This is possibly explained by the difference in

Table 1. *Phase III trials comparing different induction regimens and autologous PBSCT ± maintenance therapy*

Author	Conditioning	ORR after PBSCT	Median EFS/PFS (months)	Median OS (months)
Barlogie et al. 2006[6]	VAD + 3 cycles cyclo/ etoposide/ cisplatin/dex ± T 400 mg	Double PBSCT CR rate only 43 *vs* 62%	Est. 50 *vs* 60	Est. 86 *vs* not reached (ns)
Lokhorst et al. 2009[8]	VAD *vs* TAD T 200 mg	79 *vs* 88% CR+VGPR 54 *vs* 66%	25 *vs* 34	60 *vs* 73 (ns)
Sonneveld et al. 2008, abstract[10]	VAD *vs* PAD	84 *vs* 94% CR+VGPR 60 *vs* 73%		
Harousseau et al. 2008, abstract[11]	VAD *vs* Bor/Dex 4 cycles	CR+VGPR 42 *vs* 62%		

ORR=overall response rate; PBSCT=peripheral blood stem cell transplantation; EFS=event-free survival, PFS=progression-free survival; OS=overall survival; CR=complete response; VGPR=very good partial response; est=estimated; ns=not significant; VAD=vincristine-adriamycin-dexamethasone; cyclo=cyclophospahmide; T=thalidomide; TAD=thalidomide-adriamycin-dexamethasone; PAD=bortezomib-adriamycin-dexamethasone; Bor/Dex=bortezomib/dexamethasone.

survival after relapse. Relapsed patients who had received thalidomide had a median OS after relapse of 20 months *vs* 31 months (p=0.009) for the patients in the VAD arm.
Patients in the TAD arm had more neurological toxicity, mainly peripheral neuropathy, 31 *vs* 21% (p=0.008). With the use of low-molecular-weight heparin prophylaxis in patients receiving TAD, the incidence of venous thromboembolism was almost similar in both study arms, 8 *vs* 4%.
Comparable with the HOVON 50 study, also Barlogie *et al*. did not find any survival benefit in patients receiving additional thalidomide at induction, double autologous PBSCT, consolidation and maintenance therapy, despite increased RR and event-free survival (EFS) rates.[9] This was also due to the shorter median survival after relapse of 1.1 years in the thalidomide arm compared with 2.7 years in the control group. Since this Total Therapy II schedule is very different from the treatment regimens applied in the Netherlands, these findings cannot be easily translated into our daily practice. However, the notion that long duration of thalidomide use in the induction regimen and also in maintenance treatment can be detrimental when treating a subsequent relapse in these patients, as was also found in the HOVON 50 study, is important.
In the HOVON 65 phase III study, bortezomib was studied in induction therapy combined with adriamycin and dexamethasone (PAD) compared with VAD induction and followed by autologous PBSCT and maintenance therapy. This seems to be an effective regimen with very high response rates (≥PR) at interim analysis of the first 300 patients after HDM and autologous PBSCT of 92% in the PAD arm and 77% in the VAD arm (p=0.01) and CR rates of 15 *vs* 4% (p=0.05).[10] These results confirm the results of the IFM 2005/01 trial which also demonstrated superiority of bortezomib in the induction regimen compared with VAD for response rates.[11] Data on PFS and survival are therefore eagerly awaited.
Because information on trials using lenalidomide in the induction regimen before autologous PBSCT is scarce, its use will be discussed in the section of patients >65 years.
In conclusion, the introduction on thalidomide into the induction regimen before autologous PBSCT has proven benefit in response rates *and* PFS in randomised controlled trials. Therefore, thalidomide combined with (adriamycin and) dexamethasone is recommended as first-line therapy in the induction regimen of younger patients eligible for autologous PBSCT.

Treatment after autologous PBSCT

Strategies exploring ways to increase remission rates after the first HDM and autologous PBSCT are: second HDM with autologous PBSCT within a few months after the first procedure, allogeneic SCT (see section on allogeneic SCT) or maintenance/consolidation therapy. Randomised trials have shown that double autologous PBSCT may increase PFS, but only in patients not having at least a VGPR after the first stem cell infusion.[12,13] However, these trials were performed with the 'older' induction regimens, questioning its significance nowadays and there is no consensus regarding this strategy.
The goal of maintenance or consolidation therapy after HDM and autologous PBSCT is to improve the quality and duration of response, both of which should lead to a better survival. Interferon-α was used as maintenance therapy but meta-analysis of randomised trials showed a minimal benefit in survival and considering the costs and side effects of interferon treatment this approach has been abandoned.[14]
In 2006, the French IFM group published the 99-02 trial demonstrating that maintenance treatment with thalidomide 400 mg daily and pamidronate started two months after a double autologous PBSCT until disease progression improved overall survival compared with no maintenance or pamidronate therapy only.[15] After relapse, the one-year probability of survival was similar. In a sub-analysis of the study only patients who did not achieve a VGPR or CR benefited from the thalidomide maintenance suggesting an action of mostly tumour reduction, such as consolidation therapy, rather than a maintenance effect. In an Australian study thalidomide consolidation therapy of 200 mg daily for 12 months combined with prednisolone 50 mg on alternate days until disease progression compared with prednisolone therapy alone after single autologous PBSCT improved the PFS and OS rate at three years in the thalidomide arm.[16] Also this effect was more pronounced in the no CR/VGPR group in a sub-analysis. Another study on thalidomide maintenance was recently retracted.[17]
In the HOVON 50 study there was no separate randomisation for maintenance treatment and patients who had induction treatment with VAD continued with interferon-α and patients with TAD induction with thalidomide 50 mg daily for two years. Therefore, a separate conclusion on the value of this maintenance treatment cannot be made.
In conclusion, maintenance therapy after autologous PBSCT may have benefit; however, the optimal duration and the effect once maximal response is achieved is currently unknown. Therefore, thalidomide maintenance therapy should not be given until relapse but possibly for a fixed period.

TREATMENT OF ELDERLY PATIENTS AND PATIENTS NOT ELIGIBLE FOR HIGH-DOSE MELPHALAN

The majority of patients diagnosed with MM will be older than 65 years and therefore ineligible for HDM. Until recently, the mainstay of treatment for these patients

was melphalan combined with prednisone (MP) or dexamethasone-based regimens. In the past few years, several trials showed important improvements in patient outcome by addition of novel agents to these regimens.

Melphalan-prednisone based studies

The IFM and the GIMEMA group published the first phase III studies that showed the superiority of adding thalidomide to MP (*table 2*). The GIMEMA trial compared six 4-weekly cycles of MP with MP-thalidomide (MPT) in patients between 60 and 85 years, using thalidomide continuously (100 mg/day) in the MPT regimen, followed by maintenance thalidomide of 100 mg until relapse.[18] Median follow-up was 38 months. The RR was significantly higher in patients treated with MPT compared with MP (69 *vs* 48%, p<0.001). In addition, CR and VGPR were significantly higher after MPT (29 *vs* 11%, p<0.001). Although after MPT the PFS improved (median 22 *vs* 15 months, p=0.004), no difference was seen in OS (median 48 *vs* 45 months, p=0.79).

The IFM 99-06 trial did show improvement of survival using MPT *vs* MP.[19] In patients aged 65 to 75 years, twelve 6-week cycles of MPT were tested with dosages up to 400 mg thalidomide, *vs* the same MP regimen without thalidomide. There was no maintenance therapy in either arm. Median follow-up was 51.5 months. The RR was superior using MPT (76 *vs* 35%, p<0.0001) and even more importantly, the median OS improved from 33 months to 52 months (p=0.001). A trial also performed by IFM in an even older patient group, aged 75 to 89 years, confirmed the benefit of adding thalidomide in both RR and OS.[20] The Nordic study group and the HOVON 49 study also tested MP *vs* MPT. MP(T) therapy was continued until plateau and thalidomide maintenance was given in the MPT group in both studies. The Nordic group showed improvement in PFS, but not in OS. The HOVON 49 study, however, showed improvement in RR (66 *vs* 45%, p<0.001), median PFS (15 *vs* 11 months, p=0.002) as well as median OS (40 months *vs* 31 months, p=0.05).[21]

In conclusion, addition of thalidomide to the MP regimen leads to clear improvement in responses and PFS. The beneficial effect on OS was demonstrated in three studies. As expected, the MPT regimen is more toxic than MP alone. An increased risk of thromboembolic events, which mainly occurs within the first four months of therapy, necessitates prophylactic anticoagulation with aspirin or low-molecular-weight heparin.[22] Furthermore, over 50% of patients develop peripheral neuropathy after prolonged use of thalidomide, although grade 3/4 neuropathy only arises in 2 to 9%.

Lenalidomide, an analogue of thalidomide, has demonstrated significant activity in relapsed and refractory MM patients in combination with dexamethasone[23,24] (see section on relapsed MM for further details). A phase II study from Italy combined lenalidomide with MP as first-line treatment in elderly patients (median age 71 years, range 57-77). Lenalidomide dosage was 5 or 10 mg per day, yielding a PR or better in 81% of patients, including 24% CR. The EFS and OS were 92 and 100% at one year, respectively.[25] The results of a recently closed phase III trial testing MP *vs* MP with lenalidomide are awaited.

Lenalidomide is not registered for use as first-line treatment in the Netherlands and can only be given to newly diagnosed patients in the context of clinical trials.

After promising results in a phase II study,[26] the Spanish PETHEMA group tested addition of bortezomib to MP (MPV) *vs* MP alone, in previously untreated elderly patients. As with thalidomide, also the addition of bortezomib resulted in a better RR of 71 *vs* 35% (p<0.001), and very good CR rates of 30 *vs* 4% (p<0.001).[27] More importantly, MPV-treated patients had a better OS after a median follow-up of 16.3 months, hazard ratio 0.61 (p=0.008), but there was a higher rate of gastrointestinal symptoms, peripheral neuropathy and herpes zoster infections. It is recommended to use valacyclovir prophylaxis to prevent these herpes zoster reactivations with bortezomib use. This VISTA trial led to registration of MPV in first-line therapy for elderly patients not eligible for high-dose therapy and who cannot be treated with thalidomide due to comorbidity or side effects.

Table 2. *Phase III trials comparing MP vs MPT or MPV ± maintenance*

Author	Treatment	ORR	Median PFS/EFS (months)	Median OS (months)
Palumbo *et al.* 2008[18]	MP *vs* MPT	48 *vs* 69%	15 *vs* 22	45 *vs* 48 (ns)
Facon *et al.* 2007[19]	MP *vs* MPT	35 *vs* 76%	17.8 *vs* 27.5	33 *vs* 52
Hulin *et al.* 2009[20]	MP *vs* MPT	31 *vs* 62%	18.5 *vs* 24.1	29.1 *vs* 44
Wijermans *et al.* 2008, abstract[21]	MP *vs* MPT	45 *vs* 66%	11 *vs* 15	31 *vs* 40
San Miguel *et al.* 2008[27]	MP *vs* MPV	35 *vs* 71%	16.6 *vs* 24	Not reached at 16.3 months follow-up

ORR=overall response rate; EFS=event-free survival, PFS=progression-free survival; OS=overall survival; ns=not significant, MP=melphalan prednisone; T=thalidomide; V=bortezomib.

Dexamethasone-based regimens

Dexamethasone is not commonly used in the treatment of elderly patients due to its high toxicity in this patient group. In a recent study by Ludwig *et al.*, MP was compared with thalidomide 50 to 400 mg daily combined with dexamethasone 40 mg on days 1-4 and 15-18 in even cycles and on days 1-4 in odd cycles.[28] Patients received nine cycles and responding patients underwent a second randomisation to maintenance treatment with thalidomide or interferon-α. The median age of the patients was 72 years (range 54 to 86) and 10% were older than 80 years. Although the TD arm resulted in a better RR of 68 *vs* 50% (p=0.002), the PFS was similar in both groups. However, median OS was significantly shorter in the TD group of 41.5 months compared with 49.4 months in the MP arm (p=0.024) due to more treatment-related deaths. Especially in elderly patients (>75 years) with a poor performance status, this dexamethasone regimen clearly resulted in higher toxicity and should be avoided.

This was also very recently demonstrated by Rajkumar *et al.* who included newly diagnosed patients without age limit in an open-label randomised phase III trial comparing lenalidomide 25 mg on days 1-21 in a 28-day cycle with high-dose dexamethasone or low-dose dexamethasone.[29] High-dose dexamethasone consisted of 40 mg on days 1-4, 9-12 and 17-20 or low-dose dexamethasone of 40 mg only once a week. The primary endpoint of the trial was response rate after four cycles of treatment. More than 50% of patients were ≥65 years, maximum age was 87 years. Comparable with the Ludwig trial, response rates were better in the high-dose group, 81 and 70% respectively (p=0.009), but OS was not. The one-year OS was 96% in the low-dose group compared with 87% in the high-dose group (p=0.0002) and this difference was even more pronounced in the patients >65 years, 94 and 83% respectively. Because of this difference in OS the study was stopped on the recommendation of the independent data monitoring committee and patients in the high-dose group were instructed to cross over.

The most common causes of treatment-related mortality (TRM) were venous thrombotic events despite prophylaxis (9 *vs* 2%), infection (7 *vs* 3%), and cardiac complications (11 *vs* 4%) in the high-dose and the low-dose group, respectively.

Conclusion

After years of absence of improvement in elderly MM patients, addition of novel agents to old regimens finally resulted in better patient outcome in elderly patients, however at the expense of an increase in side effects. Recently the new HOVON 87 elderly study opened, a phase III study comparing MPT *vs* MP-lenalidomide. Patients who cannot participate in this trial should be treated with MPT, or MPV in those patients who cannot be treated with thalidomide.

RELAPSE TREATMENT

The introduction of thalidomide, lenalidomide, and bortezomib, used either as a single agent or in combination with other drugs, has improved the median survival of patients relapsing after autologous stem cell transplantation from one to two years after relapse.[30] No clear superiority of one novel agent over the other has been demonstrated in relapsed/refractory MM in the absence of a randomised study. In addition, because of patient heterogeneity it is difficult to directly compare the results of the different studies. Since thalidomide is recommended for use in first-line treatment, only lenalidomide and bortezomib are discussed.

Lenalidomide

Lenalidomide is an amino-substituted derivative of thalidomide, which has more potent biological activity. Richardson *et al.* showed that lenalidomide monotherapy was effective and well tolerated in relapsed/refractory patients who received a median of three prior regimens. The maximum tolerated dose was 25 mg and 29% of the patients obtained at least PR.[31] Responses were also observed in thalidomide-exposed patients. In contrast to thalidomide, lenalidomide was associated with a low incidence of somnolence, rash, constipation, and peripheral polyneuropathy. Most common adverse events included neutropenia and thrombocytopenia, which were manageable with dose reduction or granulocyte colony stimulating factor (G-CSF) support. Single agent lenalidomide did not significantly increase the risk of venous thromboembolism. Other studies confirmed the effectiveness and good tolerability of single agent lenalidomide.[32]

Lenalidomide-based combinations

Laboratory studies demonstrated that dexamethasone enhances the antimyeloma effects of lenalidomide. Based on these preclinical data and results of single agent lenalidomide, two randomised phase III trials compared lenalidomide (25 mg on days 1-21 of a 28-day cycle) plus dexamethasone (40 mg on days 1-4, 9-12, and 17-20 for the first four cycles and thereafter 40 mg on days 1-4) with placebo plus dexamethasone in relapsed/refractory patients who had received a median of two previous therapies. Dimopoloulos *et al.* demonstrated superior efficacy of the study arm, in terms of higher overall RR (60.2 *vs* 24.0%, p<0.001), CR rate (15.9 *vs* 3.4%, p<0.001), and median OS (not reached and 20.6 months, p=0.03).[24] In the

other study by Weber comparable results were reported.[23] Adverse events associated with lenalidomide therapy were neutropenia, thrombocytopenia, and thromboembolic complications in both studies. The rate of grade 3 and 4 thromboembolic events varied between 11.4 and 14.7%, which is significantly higher when compared with lenalidomide monotherapy. Importantly, the introduction of prophylactic treatment with aspirin significantly reduced the rate of thromboembolic events induced by lenalidomide containing regimens.

To further improve efficacy of lenalidomide-based regimens, various other combinations have been studied. Lenalidomide in conjunction with adriamycin and dexamethasone (RAD) in refractory and relapsed patients resulted in a high response rate of 73% including 15% CR, with mainly haematological toxicity and infections as side effects.[33]

We recently demonstrated that the combination of low-dose oral cyclophosphamide and prednisone with lenalidomide has a remarkably high activity (CR in 14.3% and ≥ minimal response in 64.3%) with good tolerability in relapsed patients who were refractory to lenalidomide-dexamethasone combinations (van de Donk NWCJ *et al.*, BJH in press).

Bortezomib monotherapy

Several phase I and II studies have demonstrated that the potent reversible proteasome inhibitor bortezomib induces clinically significant responses with acceptable toxicity in relapsed/refractory MM.[34,35] In a phase III randomised trial (APEX study) bortezomib was compared with high-dose dexamethasone in patients who relapsed after a median of two treatment regimens.[36] Bortezomib (1.3 mg/m^2) was administered by intravenous bolus on days 1, 4, 8, 11 for 8 three-week cycles followed by treatment on days 1, 8, 15, and 22 for 3 five-week cycles. Dexamethasone (40 mg) was administered on days 1-4, 9-12, 17-20 for 4 five-week cycles, followed by treatment on days 1-4 for 5 four-week cycles. In the bortezomib group the RR was 38% including 6% CR, whereas it was 18% (<1% CR) in the dexamethasone group (p<0.001) One-year survival was 80 and 66% in the bortezomib and dexamethasone groups, respectively. Gastrointestinal events, herpes zoster infection, peripheral polyneuropathy, neutropenia, and thrombocytopenia were more common in the bortezomib-treated patients. Importantly, bortezomib was not associated with an elevated risk of deep venous thrombosis or pulmonary embolism. Bortezomib clearance is independent of renal function and dose adjustments are not required for patients with renal insufficiency. Various studies have shown that bortezomib or bortezomib-based combinations result in rapid responses independent of renal function and improvement of renal function with tolerability comparable with that seen in patients with normal renal function.[37,38]

Bortezomib-based combinations

Addition of dexamethasone to bortezomib treatment, in patients with relapsed/refractory MM who had progressive or stable disease during bortezomib monotherapy, resulted in improved responses without altering the type and incidence of adverse events.[39]

A phase III randomised clinical study tested the combination of bortezomib with or without pegylated liposomal doxorubicin (PLD; 30 mg/m^2 on day 4) in relapsed/refractory MM.[40] A modest improvement in overall RR was observed when PLD was added; however, there was superior efficacy, in terms of longer median TTP (time to progression) (9.3 *vs* 6.5 months, p<0.001) and 15-month survival rate (76 *vs* 65%, p=0.03). The combination arm had higher incidences of neutropenia, thrombocytopenia, and gastrointestinal events.

Conclusion

At the moment, there is no generally accepted standard treatment for relapsed patients. Choice of therapy depends on various factors including age, performance status, prior therapies, response to prior therapies, bone marrow reserve, presence of polyneuropathy, risk for thromboembolism, and renal function. Lenalidomide may be indicated in case of pre-existing peripheral neuropathy, or when a history of thromboembolism may contraindicate its use. On the other hand, bortezomib rapidly reduces tumour load in patients with renal insufficiency and is not associated with increased risk of thromboembolism. It is advised to combine both drugs with dexamethasone for higher efficacy. Prospective randomised studies are needed to determine the best salvage regimens.

ALLOGENEIC STEM CELL TRANSPLANTATION

Allogeneic stem cell transplantation (allo-SCT) is probably the only treatment for MM with a curative potential due to the graft-*vs*-MM effect (GVM) which was proven by the achievement of sustained complete remissions by donor lymphocyte infusions (DLI) without any other therapy in patients with a relapse after allo-SCT.[41] Clinical responses to DLI after myeloablative and nonmyeloablative conditioning have been reported in up to 50% of patients, including 20% of patients with a CR. In several patients these CR lasted for more than ten years. Chemo-sensitive disease and the occurrence of chronic Graft versus Host Disease (cGvHD) were associated with response to DLI.[42,43]

The role of allo-SCT in MM, however, is debated due to the high mortality and morbidity related with this procedure. Even as part of first-line therapy with myeloablative conditioning the TRM exceeded 30% and survival was

inferior to a matched group of patients receiving HDM with autologous PBSCT.[44]

Reduced intensity conditioning

The initial promising results of transplantations with reduced intensity conditioning (RIC) renewed the interest in allo-SCT as a treatment option. The pioneering studies were performed by the Seattle group who showed that donor engraftment could be achieved with the combination of low-dose TBI only (2 Gy) and high-dose immune suppressive drugs cyclosporine and mycophenolic acid.[45] They introduced the strategy of an autologous PBSCT followed two to four months later by a RIC allograft. In 52 patients treated with this tandem modality, a CR was achieved in 48% of patients and PFS and OS at 48 months were 48 and 69% respectively. A wide variety of conditioning regimens for MM have since been pioneered and in a previous review 26 different conditioning schemes with and without T cell depletion were identified.[46] No definite conclusions could be drawn from these studies but the best outcome after RIC was seen in those patients transplanted in first remission with less than two previous autologous PBSCT. Post transplant factors for prolonged PFS were achievement of CR and the occurrence of chronic GvHD.

Prospective studies of RIC allo-SCT as part of first-line therapy

The definite value of allo-SCT should be determined by prospective phase III studies with newly diagnosed patients that include a donor *vs* no donor comparison. Three such studies have been published. In the French IFM study, patients with an HLA-identical sibling donor and high-risk MM defined by B2 microglobulin >3 mg/l and deletion of chromosome 13 were candidates for autologous PBSCT followed by RIC allo-SCT ('auto-allo') with busulfan, fludarabine and a 5-day course of antithymocyte globulin (ATG).[47] Patients without a sibling donor were treated with double autologous PBSCT ('double auto'). The intention-to-treat analysis showed no significant difference in event-free survival (EFS) and OS. A major drawback of this study was the use of high-dose ATG included in the conditioning which resulted in profound *in vivo* T-cell depletion. The beneficial effects of this *in vivo* T-cell depletion are the low incidence of acute and chronic GvHD, the detrimental effect is the elimination of the desired GvM effect.

Also the Spanish PETHEMA study could not find a difference in EFS and OS between patients receiving a 'double auto' PBSCT compared with patients treated with 'auto-allo' SCT, despite higher response rates in the 'auto-allo'group.[48] A more positive result was published by Bruno *et al.*[49] In this study, 58 patients with an HLA identical sibling donor assigned to be treated with 'auto-allo' (conditioning low-dose TBI only) not only achieved more CR but also significantly prolonged EFS and OS as compared with the 59 patients assigned to be treated with the 'double auto' arm. Limitations of this study were the small number of patients and the relative inferior outcome of the double autologous PBSCT arm. What is encouraging is that the TRM of RIC allo-SCT in the upfront setting was strongly reduced to 11%. However, these studies cannot be compared due to differences in patient selection and conditioning regimens. A more definite conclusion about the role of allo-SCT in MM may come from two other prospective donor *vs* no donor studies with larger groups of patients in both arms that were performed by HOVON and the European Group for Blood & Marrow Transplantation (EBMT). In the HOVON 54 study patients with an HLA identical sibling donor could proceed to RIC allo-SCT between two and six months after HDM and autologous PBSCT. On the basis of an intention-to-treat analysis no difference in PFS and OS was found during an interim analysis that included 126 patients with a donor and 141 patients without a donor. The final analysis of both studies, expected in 2010, has to be awaited for definite conclusion. Until that time, allo-SCT should be offered to patients only in the context of clinical studies, such as the HOVON 76 study.

Treatment with allo-SCT is also possible for relapsed patients with chemo-sensitive disease.[50] However, no good-quality prospective clinical studies exist and therefore HOVON will start a randomised phase II trial in the near future for patients who relapsed after autologous PBSCT.

GENERAL CONCLUSION

After years of relative stagnation in the treatment of MM, much progress is now being made. For younger patients the introduction of autologous PBSCT has been important and all patient groups have benefitted from the introduction of novel drugs.[51] The use of these drugs has moved from the relapsed setting to the front-line setting and also combinations with the older chemotherapeutic drugs can be very active. Developments in the treatment will not stop here and already new drugs are being tested in relapsed MM patients. Therefore it is very important to try to include patients in clinical trials which hopefully will again lead to further improvement in the prognosis for MM patients.

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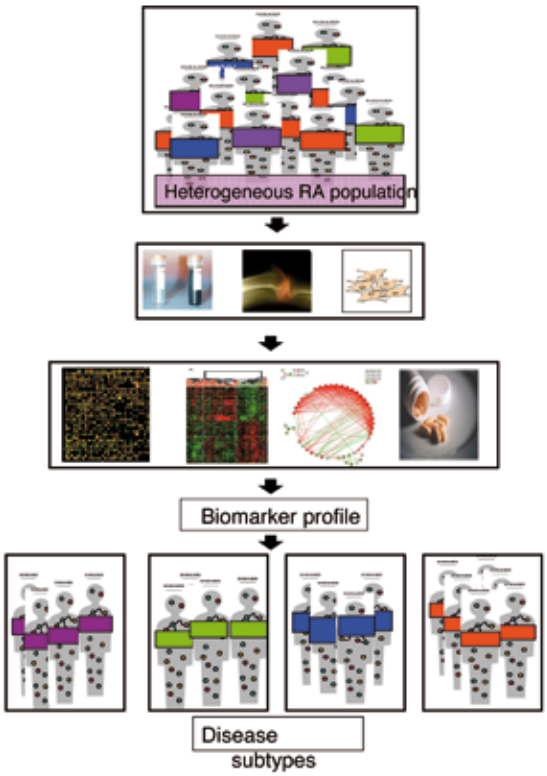
ERRATUM

Unfortunately in the article 'Transcript profiling towards personalised medicine in rheumatoid arthritis' by Verweij CL, which was published in Neth J Med. 2009;67(11):364-71, an error was made in printing *figure 1*. The correct figure is printed here.

We apologise for any inconvenience.



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CASE REPORT

A retroperitoneal mass with elevated alpha-1-fetoprotein: not always a testicular carcinoma

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ABSTRACT

High levels of alpha-1-fetoprotein are usually associated with nonseminoma carcinoma of the testis or hepatocellular carcinoma of the liver. We describe a male patient with extrahepatic hepatocellular carcinoma who presented with a large retroperitoneal mass and extremely high alpha-1-fetoprotein levels. The importance of taking an adequate biopsy specimen cannot be emphasised enough since both prognosis and treatment are completely different.

KEYWORDS

Alpha-1-fetoprotein, ectopic tissue, hepatocellular carcinoma

INTRODUCTION

Hepatocellular carcinoma (HCC) is known for its high mortality. It also demonstrates a high variability in incidence around the world. Areas such as sub-Saharan Africa and Eastern Asia have an incidence up to ten times higher than the incidence in, for example, Europe.[1] This is probably due to the local prevalence of risk factors as hepatitis B and C virus. A known yet very rare phenomenon is hepatocellular carcinoma in ectopic liver tissue. The incidence of ectopic liver tissue is estimated at 0.1 to 0.5%.[2] Described locations include the gallbladder, pancreas, diaphragm, thorax and testis. Virtually all cases of ectopic HCC involve the Asian population,[3] although three Caucasian patients have been described.[4] We present a Caucasian male with no relevant medical history and no risk factors for HCC with a retroperitoneal HCC without lesions in his liver.

CASE

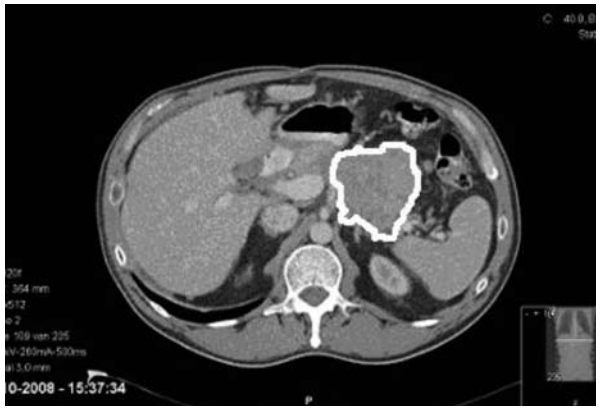
A 46-year-old Dutch male visited our emergency department after referral by his general practitioner with symptoms of stomach ache in his umbilical region that had been progressive over the last three months. Over that period his bodyweight had dropped about 5 kg. He complained of some diarrhoea without blood loss. His medical history comprised a surgical correction of a pyloric stenosis in his first year. He was not on any medication, did not smoke and used alcohol in moderate quantities.

On physical examination, a pale man was seen, in no acute distress with normal vital signs. There were no further abnormalities, aside from some peri-umbilical tenderness on palpation. There was no palpable mass in his testicles.

Biochemical investigation showed a microcytic anaemia (Hb 4.2 mmol/l; MCV 65 fl) and moderately elevated aspartate aminotransferase and alanine aminotransferase (76 U/l and 147 U/l, respectively). Ultrasonography of the abdomen revealed a soft-tissue mass in the upper abdomen. A computed tomography (CT) scan of the abdomen demonstrated a normal liver, but a large retroperitoneal mass in the upper abdomen (*figure 1*) with multiple enlarged lymph nodes in the mesentery. Magnetic resonance imaging did not show any hepatic lesions either. Because a testicular carcinoma was considered a possible diagnosis, an alpha-1-fetoprotein (α-1-FP) level was determined. This was extremely elevated at 24,000 kU/l (reference value <7 kU/l). The ultrasound investigation of his testes, however, was normal.

For further investigation the patient underwent an ultrasound-guided biopsy of the tumour. Microscopic examination revealed a tumour growing in trabeculae and some tubular structures with an aspect of hepatocytes. Some cells showed a positive alpha-1-fetoprotein (α-1-FP) immunostain. No bile production was seen. Further staining showed pankeratin

Figure 1. *Retroperitoneal mass (within white contour) at the location of the pancreatic tail*



and keratin-7 positive cells, suggesting epithelial tissue. Keratin-18, frequently present in normal hepatocytes, and keratin-19, usually absent in hepatocytes, were both negative. Beta-HCG was negative. The sample was concluded to be a poorly differentiated hepatocellular carcinoma. Pathological revision at the Department of Pathology of the University Hospital Nijmegen confirmed the conclusion. After evaluation of the radiological images we concluded the primary tumour to be irresectable. In addition, there were clearly metastases to abdominal lymph nodes; therefore the patient was treated with sorafenib (400 mg twice daily) for a metastasised ectopic HCC. After one week his level of α-1-FP dropped to 13,000 kU/l. During that week he visited the emergency department for the second time. On this occasion he complained of severe abdominal pain and diarrhoea. A CT scan showed an avascular necrosis of the head of the pancreas. The treatment with sorafenib was interrupted. After one week his pain had gone and the treatment with sorafenib was continued at a lower dose (200 mg twice daily). Until now, six months after diagnosis, his condition is stable and repeated CT scans have not revealed any signs of progression. Drug-related side effects are limited to fatigue and mild well-manageable diarrhoea.

DISCUSSION

Although the presence of ectopic liver tissue is rare (estimations below 0.5%) we have to bear in mind that this tissue is probably at a higher risk of developing into a carcinoma. All reviewed cases of ectopic HCC (all Japanese) show no primary hepatic lesions. In addition, more patients with ectopic HCC do not have the usual risk factors (such as HBV infection or cirrhosis) than patients with HCC in normal liver tissue.[2,3] Reasons for this higher carcinogenic tendency are not fully understood. It is suggested that impaired vascularisation or continuous cholestasis contributes to the development of HCC in ectopic liver tissue, although in this case no bile production was observed in the tissue sample. The phenomenon of ectopic liver tissue developing into carcinoma remains a diagnostic challenge. Learning more about the mechanisms leading to this higher carcinogenecity might lead to a better understanding of the principles behind the origin of hepatocellular carcinoma.

Until recently no proven or standard systemic treatment for advanced HCC was available. The SHARP trial, a randomised phase III trial in HCC, performed in the Western population, compared sorafenib 400 mg twice daily with placebo. Sorafenib is an oral multikinase inhibitor with antiproliferative and antiangiogenic effects. Treatment with sorafenib showed a progression-free survival (PFS) benefit of 2.4 months compared with placebo (PFS 5.2 *vs* 2.8 months in sorafenib and placebo respectively). The overall survival (OS) was 10.7 months *vs* 7.9 months in sorafenib and placebo, respectively.[5] Cheng *et al.* also showed a survival and PFS benefit in sorafenib *vs* placebo in 271 patients from 23 centres in China, South Korea and Taiwan (PFS 2.8 *vs* 1.4 and OS 6.5 *vs* 4.2 months in sorafenib and placebo, respectively).[6] The effect of systemic treatment of extrahepatic HCC is unknown. Sorafenib is generally well tolerated although side effects are sometimes severe and dose limiting. The most common drug-related adverse events include hand-food syndrome, diarrhoea, alopecia, fatigue, rash or desquamation and hypertension.

CONCLUSION

A retroperitoneal mass with elevated levels of α-1-FP and no liver lesions does not always mean a testicular carcinoma. Even in otherwise healthy Caucasian patients, ectopic hepatocellular carcinoma is a possibility, although a rare one. The importance of having a good enough biopsy specimen of the tumour for determining its origin cannot be emphasised enough.

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CASE REPORT

Fatal cerebral oedema in adult diabetic ketoacidosis

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ABSTRACT

In this report, a case of adult onset fatal cerebral oedema as a rare complication of diabetic ketoacidosis (DKA) is described and confirmed at post-mortem pathological examination. The pathogenesis of cerebral oedema due to DKA is still unknown. Potential mechanisms include the administration of sodium bicarbonate leading to intracellular acidosis, excessive fluid infusion causing swelling of brain tissue, or reduction of plasma osmolarity by a rapid fall in glucose levels causing osmotic swelling.

KEYWORDS

Diabetic ketoacidosis, cerebral oedema, pyrexia

INTRODUCTION

Cerebral oedema is a rare but severe complication of diabetic ketoacidosis (DKA), mainly seen in young children and adolescents, which may result in death. In young adults cerebral oedema due to DKA has only occasionally been reported.[1-4]

We present a case of a 31-year-old male who died of cerebral herniation due to cerebral oedema caused by DKA as the initial presentation of diabetes mellitus. Potential mechanisms and pathological data are discussed.

CASE

A 31-year-old comatose male presented at the emergency department. He was born in Zambia and had lived as a student in our country for three months. He had no known relevant previous medical history. His family history was negative for diabetes mellitus. Since three weeks he had complained of fatigue. Recent symptoms were dry cough, fever and vomiting. On physical examination, the patient was comatose ($E_1M_1V_1$), had dilated pupils with a very slow reaction to light, intact cornea reflexes, and no meningeal signs. His temperature was 38.5°C, blood pressure 70/30 mmHg, with a regular and equal pulse of 132 beats/min and a respiratory rate of 30 breaths/min. Auscultation of the heart and lungs was normal. Examination of the abdomen was unremarkable. Urine output was absent, even after catheterisation.

Laboratory investigations revealed: haemoglobin 11.4 mmol/l (8.5 to 11.0 mmol/l), haematocrit 0.59 l/l (0.40 to 0.52 l/l), leucocytes 13.3 x 10^3/l (4-11 x 10^3/l), urea 33.8 mmol/l (3.0 to 7.0 mmol/l), creatinine 768 µmol/l (60 to 110 µµol/l), sodium 153 mmol/l (135 to 145 mmol/l), potassium 5.0 mmol/l (3.5 to 4.7 mmol/l), chloride 107 mmol/l (97 to 107 mmol/l), glucose 84.9 mmol/l (4 to 10 mmol/l) and C-reactive peptide of 3 mg/l (0 to 17 mg/l). Liver enzymes were normal. Arterial blood gas analysis showed metabolic acidosis: pH 7.112 (7.35 to 7.45), pCO_2 6.7 kPa (4.5 to 6.0 kPa), pO_2 40.5 kPa (9.5 to 13.0 kPa), $HCO3^-$ 15.4 mmol/l (22 to 26 mmol/l), base excess -14.0 mmol/l (-2-2 mmol/l) and oxygen saturation 99% on oxygen (15 l/min) via non-rebreathing mask. Lactate was 4.2 mmol/l (0.5 to 1.7 mmol/l). Anion gap was 30. Urine was positive for ketones and negative for nitrite. Chest X-ray was normal.

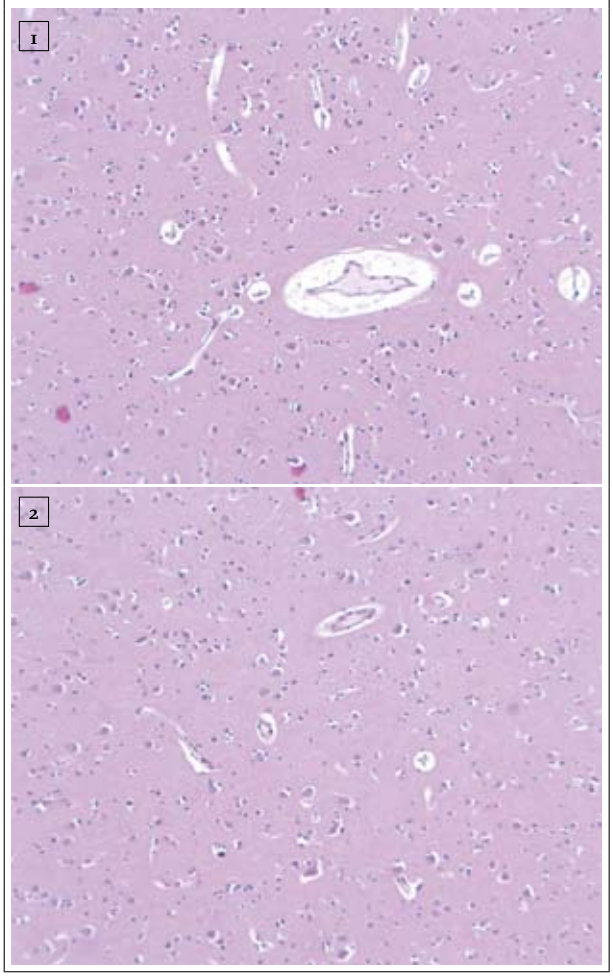
Patient was admitted to the intensive care unit (ICU) with severe hyperglycaemia with severe metabolic (lactic) acidosis and coma, diagnosed as DKA, based on a high anion gap, high glucose and ketones in urine. In addition, oliguric renal failure probably due to dehydration, electrolyte abnormalities and fever of unknown origin were noted. He was treated for DKA according to the national guidelines with fluids and insulin IV.[5] NaCl 0.9% at a rate of 0.5 l/h was infused. Insulin was administered as a bolus of 10 IUs followed by 7 IUs/h. Earlier, in the emergency

department, the patient had already received 3x 500 ml hydroxyl-ethyl starch solution infusion (Na 154 mmol/l). Cultures were taken and tests for TBC, HIV and malaria performed. The results were negative. Intravenous amoxicillin/clavulanic acid was started empirically. Serum sodium, potassium, glucose and arterial blood gasses were measured hourly (*table 1*). Norepinephrine was started because of hypotension, not responsive to fluid resuscitation.

Initially, the patient started to wake up after metabolic correction (max. $E_2M_3V_2$). Maximal urine output was 20 ml/h. Four hours after admission to the ICU he suddenly developed an acute rise in temperature up to 42°C and epileptic seizures. His coma score deteriorated again to $E_1M_1V_1$. Laboratory results at that moment were sodium 169 mmol/l, potassium 3.6 mmol/l, glucose 38 mmol/l, pH 7.034, pCO_2 8.9 kPa, and HCO_3^- 16.9 mmol/l. Neurological examination by the consulted neurologist confirmed the comatose state and an urgent CT scan of the brain was requested to rule out cerebral oedema or infection. Before CT scanning could be performed, the patient developed a sudden cardiac arrest with asystole. Cardiac resuscitation was immediately initiated. Return of spontaneous circulation was quickly established; however, the patient developed recurrent episodes of ventricular tachycardia, ventricular fibrillation and pulse-less electrical activities with asystole. External pacing was initiated with inadequate cardiac output. Cardiac resuscitation was finally discontinued 75 minutes after the start of cardiopulmonary resuscitation.

Autopsy showed extensive perivascular, pericellular and interstitial oedema of the brain (*figures 1* and *2*), and cerebral herniation was confirmed. Furthermore, pulmonary oedema was seen compatible with acute lung injury (ALI). Surprisingly, there were no signs of infection, bowel ischaemia or organ failure. All post-mortem cultures were negative.

Figures 1 and 2. *Slides of the brain showing vascular congestion, perivascular and pericellular oedema*



DISCUSSION

We have described a case of adult-onset fatal cerebral oedema as a rare complication of DKA, which could be confirmed at post-mortem pathological examination. The pathogenesis of cerebral oedema due to DKA is still unknown. Three possible mechanisms have been suggested but never proven: 1) administration of sodium bicarbonate leading to intracellular acidosis,[6,7] 2) excessive fluid infusion causing swelling of brain tissue,[8,9] and 3) reduction of plasma osmolarity by a rapid fall in glucose levels causing osmotic swelling.[6,10-12]

Two of these mechanisms could have played a role in our case. First, we noted a rapid fall in glucose levels due to

Table 1. *Laboratory test results during treatment for diabetic ketoacidosis*

Time	Reference value	12:13*	14:03	15:37	17:23#	17:54	18:08	18:22	18:42
Urea	3.0-7.0 mmol/l	33.8	34.8						
Creatinine	60-110 umol/l	768	762						
Sodium	135-145 mmol/l	153	155	158	163	169	177	176	176
Potassium	3.5-4.7 mmol/l	5.0	3.3	2.2	2.1	3.6	2.1	3.5	2.7
Glucose	4-10 mmol/l	84.9	74.2	59.7	43.2	38	38	33	39
Effective serum osmolality[1]		391	384	376	369	376	392	385	391

***Presentation and initiation of treatment; #clinical deterioration; [1]effective serum osmolality = 2 x $[Sodium]_{serum}$ + glucose.**

rehydration and insulin administration. Potentially this may have caused swelling of the brain. However, the rapid reduction in glucose levels was accompanied by a simultaneous rise in plasma sodium, resulting in a modest reduction of plasma osmolality from 391 to 369. Second, we infused large amounts of fluids according to and not more than is suggested in the practice guidelines. Third, although the metabolic acidosis slightly improved, the persistent acidosis was caused by ongoing hypercarbia and respiratory acidosis. Increased dead space ventilation due to ALI or neurogenic pulmonary oedema may have played a role in the persistent combined acidosis. Pulmonary oedema could be demonstrated post-mortem. The increase in pCO_2 levels may have caused intracellular acidosis and mimic the same situation as has been observed in mechanism 1 after sodium bicarbonate administration. However, in this patient sodium bicarbonate was not administered.

We speculate that another mechanism may have played a role. Fever could have contributed to the cerebral deterioration. We have previously demonstrated that in a patient resuscitated after cardiac arrest and showing initial improvement, pyrexia resulted in fatal cerebral oedema.[13] After cardiac arrest and in several neurological conditions mild therapeutic hypothermia and fever control proved to be protective.[14] Possibly, this observation could be translated to other clinical settings like our case.

Temperatures exceeding 40°C cause transient vasoparalysis in humans, resulting in cerebral metabolic uncoupling and loss of pressure-flow autoregulation. These findings may be related to the development of brain oedema, intracerebral haemorrhage, and intracranial hypertension observed after prolonged therapeutic hyperthermia. Furthermore, deliberate hyperthermia critically worsens the extent of histopathological damage in animal models of traumatic, ischaemic and hypoxic brain injury. However, it is unknown whether these findings translate into episodes of spontaneous fever in neurologically injured patients.[15]

On the other hand, fever may have also been a symptom of cerebral oedema and imminent cerebral herniation.

The progressive hypernatriaemia was not completely understood. Fluids infused had maximum sodium levels of 154 mmol/l and loss of water through renal elimination is not relevant at a urine output of 20 ml/h, such as may be seen in diabetes insipidus. Most likely, third-spacing of hypotonic fluids due to gastrointestinal paralysis or capillary leakage – as was seen in the lungs – may have caused a further rise in sodium levels.

CONCLUSION

In conclusion, adults presenting with DKA may develop fatal cerebral oedema, although this is rare. Rapid correction of hyperglycaemia and osmolality and persistent respiratory acidosis due to ALI or neurogenic lung oedema may have contributed to the fatal outcome.

Fever may be seen as a symptom of neurological disease or could have contributed further to the final outcome. Therefore we feel that also in DKA, fever control is of pivotal importance to prevent further damage due to fever.

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CASE REPORT

Renal abnormalities in a family with Alagille syndrome

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INTRODUCTION

Alagille syndrome is largely unknown to the general internist because the diagnosis is usually made by a paediatrician. Nevertheless, it is important to be aware of this syndrome because it sometimes manifests later in life with a great variability in clinical presentation and important consequences for the individual patient. We therefore discuss this syndrome using a patient with the usual characteristics of this syndrome.

KEYWORDS

Alagille syndrome, renal failure, dysmorphic feautures

CASE

A 38-year old woman was referred to our outpatient clinic because of hypertension. She felt otherwise well. On physical examination she had a peculiar face with a prominent forehead, straight nose and a pointed chin. Her blood pressure was 180/70 mmHg, with a regular pulse rate of 80 beats/min. She had a systolic ejection murmur, crescendo-decrescendo, grade II/VI at the left upper sternal border.

Her previous medical history revealed a heart operation because of pulmonary artery stenosis, and jaundice during her childhood. She had also had four miscarriages in the past. The diagnosis of Alagille syndrome was established. Two sisters and two brothers had already been diagnosed with Alagille syndrome.

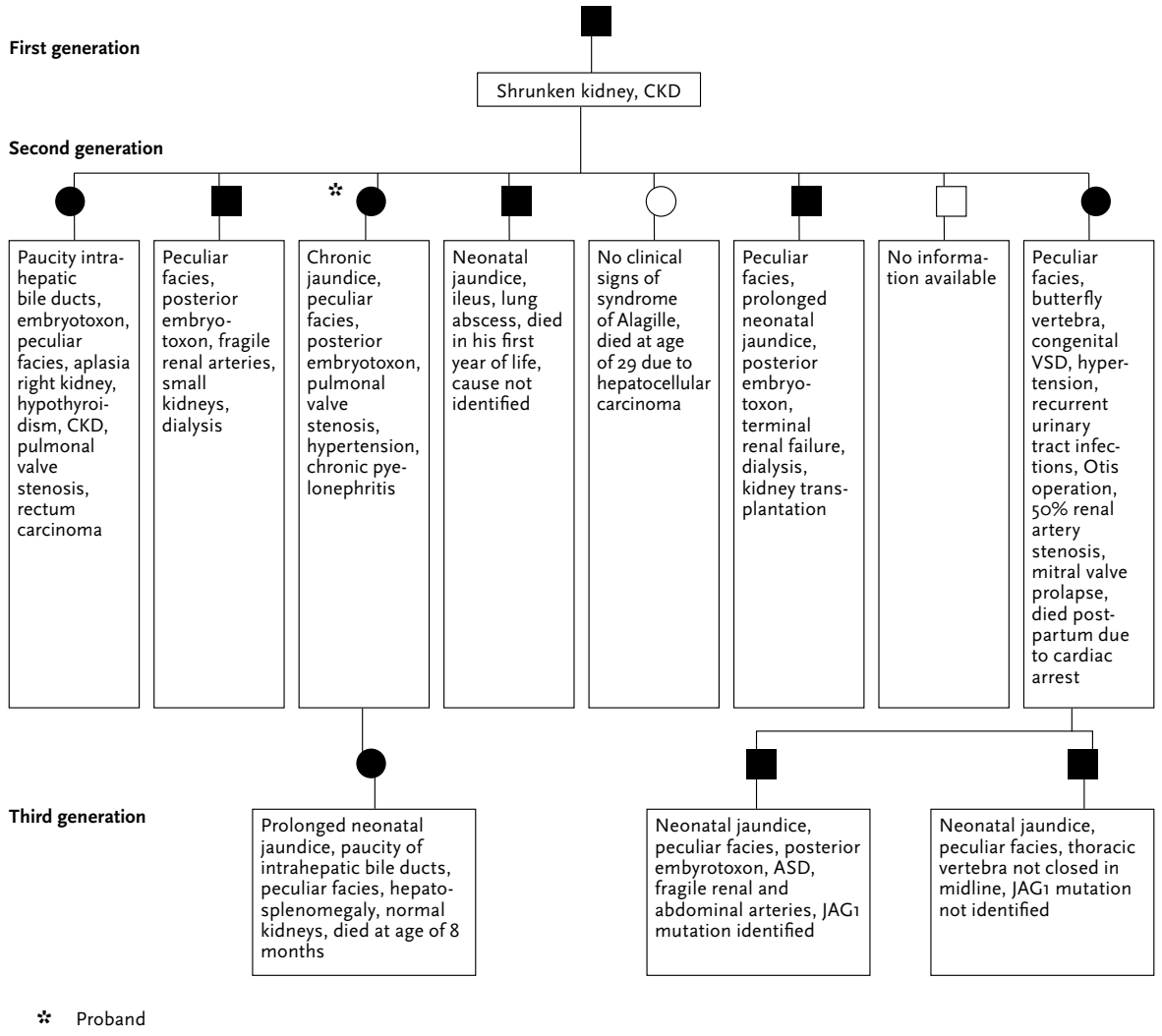
Initial biochemistry results were normal with an isolated high gamma-glytamyl transpeptidase of 172 U/l. Ultrasound examination of the kidneys showed that she only had one kidney on the left side. Further analysis with a digital subtraction angiography showed a shrunken kidney on the right side and evidence of a hypoplastic left renal artery and abdominal aorta with 50% concentric narrowing. Ophthalmological examination confirmed a posterior embryotoxon. She was treated for her hypertension with a calcium channel blocker and follow-up was organised at our outpatient clinic. After 13 years she underwent a successful living kidney donor transplantation because of terminal renal insufficiency due to slowly progressive kidney failure.

DISCUSSION

This patient has four characteristic hallmarks of Alagille syndrome: peculiar facies, posterior embryotoxon, chronic cholestasis and pulmonary valve stenosis. Patients with Alagille syndrome have five main features, four of which were demonstrated by our patient. The usual fifth feature is the existence of vertebral arch defects. Her family history revealed four siblings diagnosed with Alagille syndrome. The unusual findings in this family are agenesis of the kidney, kidney dysfunction and renal artery stenosis. One brother also underwent successful kidney transplantation due to terminal renal failure. The complete history of the proband and her family is summarised in *figure 1*.[1]

In 1969, Daniel Alagille, a French paediatric liver specialist, described a new and distinct form of cholestasis in infancy in association with cardiac abnormalities, vertebral malformations and a peculiar facies. We now know that Alagille syndrome is a genetic syndrome due to a mutation of the *JAG1* gene on chromosome 20p12 that encodes Jagged 1, which has an important role in cell fate determination. Mutations in the *JAG1* gene are associated with developmental problems in especially the cardiovascular and bile duct system, but also in other organ systems.[2]

Figure 1. *This figure shows three generations of the family of the proband (*). The relevant medical history of each family member is described shortly*



CKD = chronic kidney disease; VSD = ventricular septal defect; ASD = atrial septal defect.

Alagille syndrome is diagnosed almost exclusively in children in the setting of predominant liver manifestations. It remains a 'paediatric diagnosis' largely unknown to practitioners who deal with adult patients. Although the diagnosis is made on clinical grounds, genetic testing is possible. There is an autosomal dominant inheritance with a high variability of expression and a high rate of new mutations, approximately 35%. In approximately 70% of the patients a *JAG1* mutation can be identified. The inability to identify a mutation in the remaining 30% is thought to be because of technical limitations in testing this fairly large gene.[3,4]

We would like to conclude that renovascular and renal problems are not uncommon in Alagille syndrome as a review of the literature and the family history of our patient demonstrated. We believe that Alagille syndrome is probably underdiagnosed in adult patients, especially in the absence of severe hepatic disease and/or a positive familial history. Because of the high frequency of new mutations and differences in the clinical features in affected families, it is important to look for renovascular abnormalities in a patient with Alagille syndrome.

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PHOTO QUIZ

A 29-year-old male with right-sided chest pain

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CASE

A 29-year-old male, with no relevant medical history, presented to the emergency department because of an acute right-sided chest pain after taking a shower. The pain increased during exercise. Patient was a current smoker and denied any previous trauma. He had no apparent risk factors for venous thromboembolism. He was afebrile, normotensive and the oxygen saturation was 97%, without additional oxygen supply. Medical examination was unremarkable.

Routine chest X-ray (*figure 1A*) showed 'a pleural line' and was suggestive of a small pneumothorax at the top of the right lung, according to the radiologist. The patient was treated conservatively and was discharged from hospital with an appointment for the outpatient clinic, and was advised to stop smoking.

At follow-up ten days after the visit to the emergency department, a chest X-ray was taken, which showed no radiographic improvement. Thoracic computed tomography was performed to confirm or exclude the diagnosis of pneumothorax, since the patient wanted to travel on an airplane for vacation (*figure 1B*).

Figure 1A. *A chest X-ray on presentation*

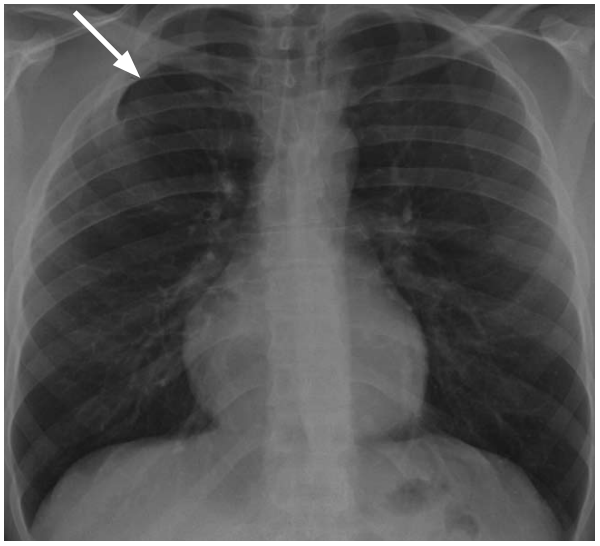
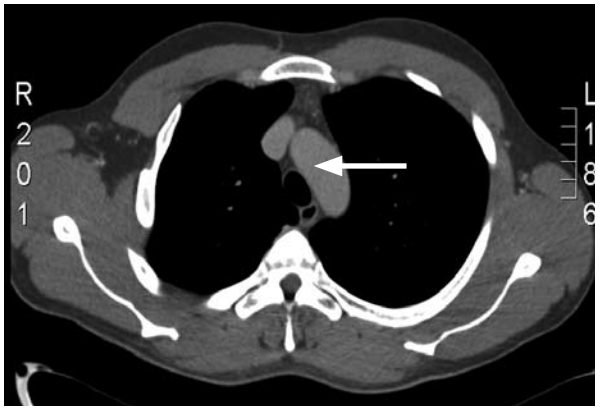


Figure 1B. *Computed tomography performed ten days later*



WHAT IS YOUR DIAGNOSIS?

See page 43 for the answer to this photo quiz.

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PHOTO QUIZ

A family with skin lesions

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A 45-year-old woman presented with a skin lesion on her right zygoma. Her 10-year-old daughter and 8-year-old son had also skin lesions on the left zygoma and left auricle, respectively *(figure 1, arrows; figures 2, 3,* and *4)*. The mother described a small acne-like lesion on her right zygoma. Since it persisted, she was given cream antibiotics. The lesion progressed despite the therapy and she noted her son's and daughter's lesions.

WHAT IS YOUR DIAGNOSIS?

See page 44 for the answer to this photo quiz.

Figure 1

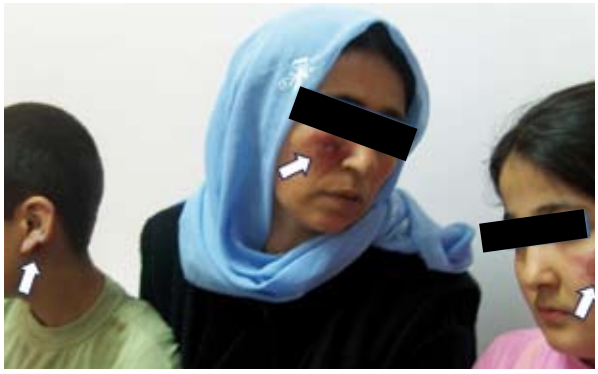

Figure 2

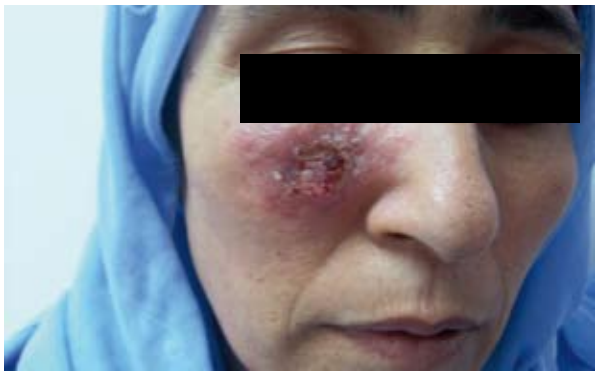

Figure 3

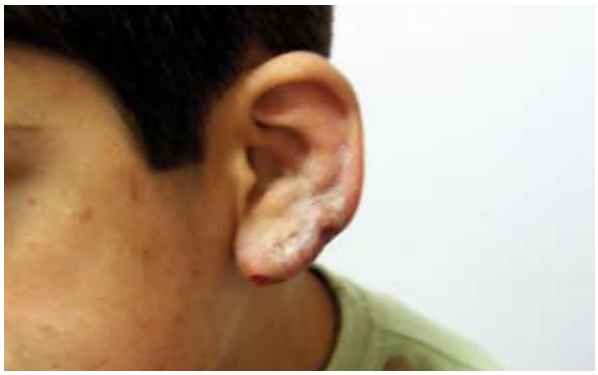

Figure 4

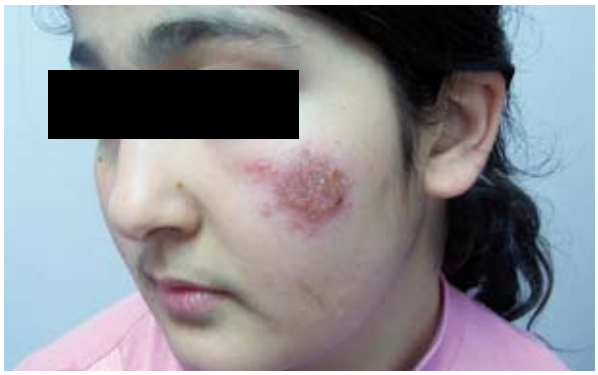

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PHOTO QUIZ

An unusual cause of upper gastrointestinal bleeding

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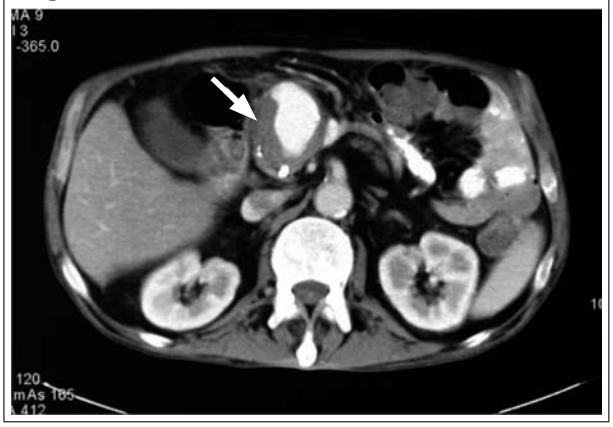
CASE

A 56-year-old man was admitted to the hospital with anaemic syndrome and an episode of melaena. His past medical story was remarkable for chronic ethanol abuse, chronic liver disease with previous ascites, chronic calcifying pancreatitis and chronic obstructive pulmonary disease. Previous surgical procedures included perforated peptic ulcer and funduplicature for hiatal hernia. Exploration disclosed a normal blood pressure, skin pallor, tachycardia, and was otherwise within normal limits. Laboratory revealed a normocytic anaemia with a high reticulocyte count. Gastroscopy did not show macroscopic lesions. Contrast-enhanced abdominal computed tomography (*figure* 1) showed features of chronic pancreatitis and a homogenously enhancing mass (arrow) within a 5-cm pancreatic pseudocyst in the head of the pancreas.

Figure 1. *Contrast-enhanced abdominal computed tomography scan. Homogenously enhancing lesion (arrow) within a pancreatic pseudocyst in the head of the pancreas*



WHAT IS YOUR DIAGNOSIS?

See page 45 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 40)

A 29-YEAR-OLD MALE WITH RIGHT-SIDED CHEST PAIN

DIAGNOSIS

Rib anomaly mimicking pneumothorax.
Bridging between 2nd and 3rd right-sided rib with pseudoarticulation.

Computed tomography of the thorax did not show a pneumothorax; however, there was deformation of the 2nd and 3rd right-sided ribs with bridging and pseudoarticulation, which might have caused the pain (*figures 2 and 3*).
The patient was informed about the diagnosis, reassured and discharged from follow-up. And naturally, no objections were made to his planned journey.
A rib normally develops from the costal process of the developing thoracic vertebrae.[1] Structural and numerical rib anomalies occur in approximately 2% of individuals, although the reported incidence of this condition varies between the series (from 0.15 to 3.4%).[2] These anomalies are generally more common in females than in males, occur more frequently on the right side and they are usually asymptomatic. The most important clinical exception is a cervical rib which may cause a thoracic outlet syndrome by compression of the brachial plexus or subclavian vessels. Because of that, a cervical rib is of particular interest to surgeons.[3]
The most frequent anomaly in Wattanasirichaigoon's series was a fusion (72%), followed by bifid (28%) and hypoplastic rib (26%).[2] Most of these abnormalities are isolated findings but they could also occur in combination with other congenital abnormalities.
Bone bridging, as in our patient, may be posttraumatic or a congenital anomaly and pseudoarthrosis may be present. Bridging can be seen anywhere along the ribs. There is no predilection site for bridging.[4]
Rib anomalies could be easily overlooked initially and the knowledge of rib anomalies is essential for the differential diagnosis with other thoracic abnormalities (such as pneumothorax in the reported patient).
In conclusion, the reported patient shows that diagnosis of a pneumothorax or rib anomaly can be difficult and can present a diagnostic challenge, especially in symptomatic patients. Careful evaluation is therefore warranted.

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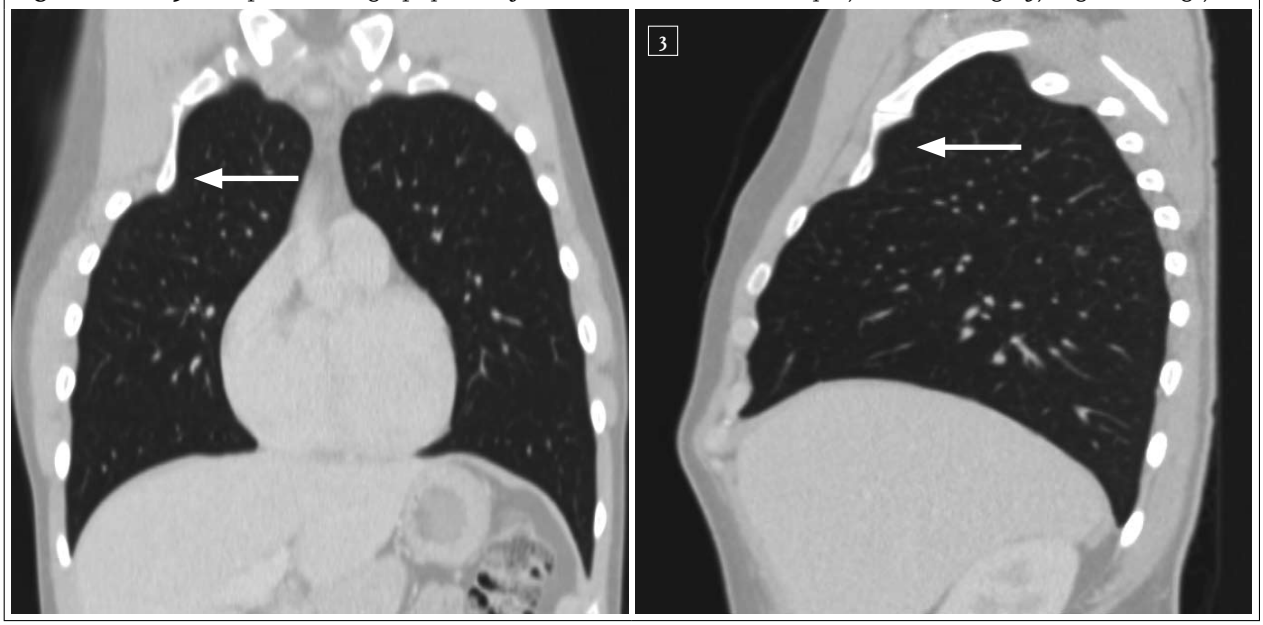
Figures 2 and 3. *Computed tomography scan of the chest shows rib anomaly; 2) coronal image, 3) sagittal image)*



ANSWER TO PHOTO QUIZ (PAGE 41)

A FAMILY WITH SKIN LESIONS

This family was from Southeast Anatolia, which is an endemic area for leishmaniasis. Biopsies of the skin lesions revealed amastigotes in the tissue. The cultures on specific *Leishmania* media (Nicolle Novy MacNeal -NNN) showed promastigotes *(figure 5)*.

Leishmania infection can be divided into cutaneous, mucocutaneous, or visceral disease. Cutaneous leishmaniasis is the most common form of leishmaniasis caused by a single-celled parasite. It is transmitted by sandfly bites. Cutaneous lesions tend to occur on exposed areas of skin. It begins as a red papule, enlarges to form an ulcer with granulomatous tissue at the base and raised, heaped up margins. The ulcers are characteristically painless unless secondarily infected.[1] Cutaneous leishmaniasis lesions typically undergo spontaneous resolution varying according to the infecting *Leishmania* species and the immune reaction of the host. A residual hypopigmented, depressed scar at the site is common following curation. Cutaneous leishmaniasis is treated to accelerate curation to reduce scarring, especially in cosmetic sites, and to prevent parasite dissemination (i.e. mucosal leishmaniasis) or relapse.[2]

The leishmaniases are caused by *Leishmania*, a protozoon transmitted by the bite of a tiny 2 to 3 millimetre-long insect vector, the phlebotomine sandfly *(figure 6)*.

Cutaneous leishmaniasis is endemic in more than 70 countries worldwide, and 90% of cases occur in Afghanistan, Algeria, Brazil, Pakistan, Peru, Saudi Arabia, and Syria *(figure 7)*.[3] There are about 1.5 million cases of cutaneous leishmaniasis worldwide each year and according to the World Health Organisation, leishmaniasis is endemic in 88 countries, with a total of 350 million people at risk.[4] It is a public health concern in most countries bordering the Mediterranean littoral[5] and also the southeast region of Turkey.[6] In relation to the recent increase of international military activity in Southwest and Central Asia, cutaneous leishmaniasis has become an increasing problem in military personnel. During deployment in northern Afghanistan, a total of 172 Dutch military personnel and three civilians embedded with the armed forces were infected with *L. major*.[7]

The development of one or more chronic skin lesions with the appropriate characteristics and a history of exposure in an endemic area suggest cutaneous leishmaniasis.

Figure 5

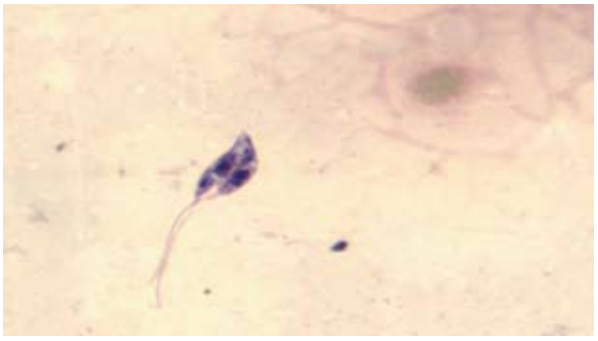


Figure 6

source: http://www.who.int/leishmaniasis/leishmaniasis_maps/en/index.html

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Figure 7

source: http://www.who.int/leishmaniasis/disease_epidemiology/en/index.html

DISCUSSION

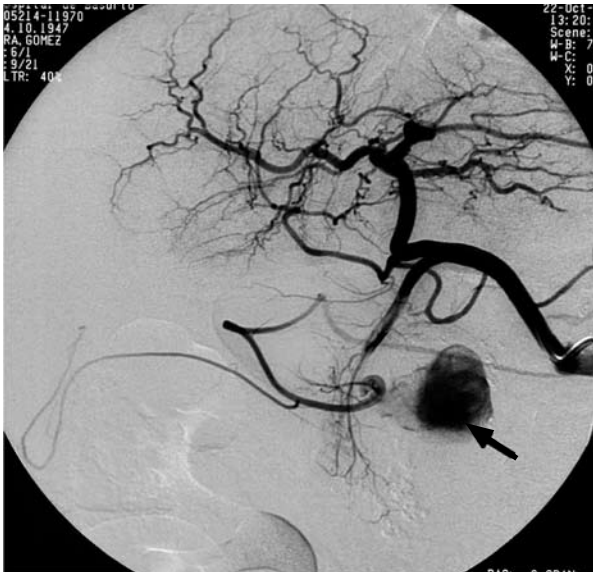
Arteriography (*figure 2*) revealed a pseudoaneurysm of the gastroduodenal artery (arrow) that was successfully embolised with thrombin. Abdominal colour Doppler ultrasonography performed one and six months after the procedure did not identify anomalous blood flow.

Bleeding pseudoaneurysm is an uncommon but life-threatening complication of acute and chronic inflammatory pancreatic processes.[1-3] In chronic pancreatitis prevalence of pseudoaneurysms may reach 3 to 10%.[1] Pathogenesis involves destruction of vessel walls exposed to proteolytic pancreatic enzymes that lead to pseudoaneurysm formation or haemorrhage into a pre-existing pseudocyst.[2] Affected vessels are in close proximity to the pancreas; the splenic artery is the most commonly affected, followed by pancreatoduodenal and gastroduodenal arteries. Other vessels are involved with less frequency.[4]

Gastrointestinal haemorrhage, due to rupture of a pseudoaneurysm to the gastrointestinal tract or biliopancreatic duct (haemosuccus pancreaticus),[5] is the most frequent form of presentation in patients with vascular complications of pancreatitis. However, bleeding to the retroperitoneum, peritoneal cavity, aorta and portal vein may occur.[1] Mortality with actual diagnostic and therapeutic procedures is approximately 20%, although several case series report mortality rates over 50% that may reach 90% in untreated patients.[2]

The diagnostic approach in patients with gastrointestinal haemorrhage should include fibreoptic endoscopy to rule out other causes of bleeding. Contrast-enhanced computed tomography scan and Doppler ultrasound usually detect the pseudoaneurysm. Arteriography is the diagnostic gold standard, which confirms the diagnosis and allows therapeutic embolisation of the pseudoaneurysm, with coils or thrombin.[3,6] Success rates of arteriographic embolisation range from 75 to 100% in the literature.[3,7] The role of surgery is limited to patients with ongoing bleeding and embolisation failure,[6] although some authors suggest that effective embolisation should be followed by surgical vessel ligation with pseudocyst drainage or partial pancreatectomy.[7]

Figure 2. *Abdominal arteriography. Pseudoaneurysm of gastroduodenal artery (arrow)*



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SPECIAL ARTICLE

Are claims of advertisements in medical journals supported by RCTs?

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ABSTRACT

Background: Claims made in advertisements in medical journals might not always be supported by high-quality evidence, and referenced studies may have been sponsored by the pharmaceutical industry itself. We studied to what extent randomised controlled trials (RCTs) support the claims in advertisements in leading medical journals.

Methods: Consecutive unique advertisements were selected from nine different medical journals, and evaluated by 250 medical students using a standardised score form. The quality of RCTs that were referenced in these advertisements was assessed with an instrument based on the Chalmers' score.

Results: 158 RCTs from 94 advertisements were used in the study. In total 55% of the RCTs had a high-quality score, 44% intermediate, and <1% had a low-quality score. Almost 40% of the RCTs had a high-quality score and at the same time supported the claim for which they were cited, while only 17% were also not sponsored by a pharmaceutical company.

Conclusion: RCTs used to support claims in medical advertisements are often not a high-quality and independent source of evidence. This distracts from the credibility of claims in advertisements, even in the high-ranked journals.

KEYWORDS

Advertisements, claims, RCT, support

INTRODUCTION

Pharmaceutical companies make use of advertisements in medical journals to promote their products. To increase credibility, claims made in the advertisements are frequently accompanied by references to sources of evidence. However, these sources may vary in quality and are not always readily accessible. In addition, these studies may have been sponsored by the pharmaceutical industry itself, leading to a potential conflict of interest.

Several studies have assessed the validity of claims in advertisements in medical journals.[1-6] Villanueva *et al.* assessed claims about efficacy, safety, convenience, or cost of antihypertensive and lipid-lowering drugs included in advertisements of six Spanish medical journals. Of the 102 references they were able to trace, 44% did not support the promotional statement and 40% of the references were sponsored by the pharmaceutical industry that made the claim. The authors concluded that doctors should not automatically believe seemingly evidence-based claims made in medical advertisements.[1]

Evaluation of advertisements has been done in various specialities such psychiatry, orthopaedics, cardiology and rheumatology.[1,2,5,6] In 2006, van Winkelen *et al.* assessed advertisements in four leading journals of rheumatology. They selected 84 unique advertisements and 300 references belonging to them. Five percent of these advertisements were supported by high-quality evidence and they concluded, in line with the conclusions made by Villanueva, that few advertisements in journals of rheumatology are entirely evidence-based.[2]

While the results from inventories in specialised journals are not very promising, readers of high-ranking general medical journals might expect that their journals do better in this respect. Leading medical journals are influential and it seems reasonable to believe that they not only publish scientific papers of outstanding quality, but also contain high-level evidence-based advertisements with claims accompanied by valid sources of reference. As there is not much evidence to support this expectation, we studied to what extent randomised trials support the claims of advertisements in leading general medical journals.

MATERIALS AND METHODS

Advertisements were extracted from seven leading medical journals and two specialised journals (*American Journal of Medicine, Annals of Internal Medicine, Archives of Internal Medicine, British Medical Journal, European Heart Journal, Journal of the American Medical Association, Lancet, New England Journal of Medicine*, and *Nephrolopy Dialysis Transplantation*) published between 2003 and 2005. All unique advertisements were included if they made a claim regarding the effect of a drug.

For all claims in the advertisements, references, if any, were checked. Only references to RCTs were included in this study. All publically available RCTs (in digital libraries) were reviewed by a group of 250 medical students following a three-week regular course of 'Scientific Education' in the second year of the medical curriculum at the Leiden University Medical Center. After specific training and small group exercises in critical appraisal, each student independently assessed two RCTs and the advertisements to which the RCTs belonged using a set questionnaire. RCTs were randomly distributed among the students.

The quality of the RCTs was assessed with a modified instrument based on the Chalmers' score.[7] This score measures validity by three subscores: 1) method of treatment assignment (randomisation), 2) control of selection bias after treatment assignment (intention-to-treat principle) and 3) blinding of participants. The score ranges from 0-9 points, with 3 points possible per subscore. The total score is evaluated as low (0-2 points), intermediate (3-5 points) or high (6-9 points).

Truly randomised, blind and the method is described (3 points), the study was stated to involve blind randomisation without a (valid) method (2 points), randomisation without blinding and methods (1 point), randomisation was not mentioned explicitly (0 points).

All patients entered the trial and received assigned treatment (3 points), withdrawals listed and remaining patients analysed by original treatment assignment (2 points), the reports did not mention withdrawals or results had been analysed by received treatment only (1 point), no description of withdrawals and results analysed by received treatment (0 points).

Double-blinded such that patients and caregivers and investigators are all kept unaware of treatment assignment (3 points), only two out of three categories had been blinded or the methods for blinding are not mentioned (2 points), blinding was impossible or it was impossible to judge whether it had been attempted (1 point), could have been conducted as double blinded but had not been (0 points).

The students also evaluated whether the claim in the advertisement was supported by the referenced RCT (i.e., whether the conclusion of the RCT matched with the claim in the advertisement and concerned the same patient population). Furthermore, they recorded whether the RCT was sponsored by the pharmaceutical industry or not.

Analysis

Two to six students assessed each referenced RCT. For each RCT, a final Chalmers' score was calculated, i.e. the mean of the two to six individual scores. In case of a discrepancy of 4 or more points between individual student assessments, a panel of four researchers independently assessed these articles and the new score was discussed in the panel to increase the inter-rater reliability.

The decision whether or not a claim was supported by the referenced RCTs was based on the decision made by the majority of student assessments. In the absence of a majority, the panel reassessed claims and references. The new score was discussed to increase inter-rater reliability. The same protocol was used for the assessment of pharmaceutical sponsorship.

An RCT with a high Chalmers' score that also supported the claim in the advertisement resulted in an evidence-based claim. A claim was classified as not evidence-based when it had a negative Chalmers' score *or* it was not supported by the referenced RCT. A claim was classified as intermediate when the Chalmers' score was intermediate while the RCT supports the claim made in the advertisement. Additionally, the results were related to sponsorship by the pharmaceutical industry.

RESULTS

From the sample of nine medical journals published between 2003 and 2005, 189 unique advertisements were obtained, in which 614 claims were made. Fifty-two claims had no references. From the 562 claims with a reference, 255 references were unusable for our study because they were not RCTs (instead they were reviews, meta-analyses, observational studies or case reports) and 128 were not publically available in digital libraries or untraceable. The remaining references and advertisements were distributed among the students at the Leiden University Medical Center. During the evaluation, 21 RCTs were not rated by the students because of random allocation of the RCTs and because each student rated only two RCTs. In addition, some students stopped active participation in the course completely, did not do their examinations or did not send in their results. In the end, the analysis comprised 158 RCTs from 94 advertisements. The panel had to reassess 40 RCTs.

In total 88 of these RCTs (55.7%) had a high Chalmers' score, 69 (43.7%) had an intermediate score and 1 (0.6%) scored low. Without taking into account the quality of the

RCT, students evaluated 91 RCTs (57.6%) to be supportive for the claims for which they were referenced.

To determine whether the claim was evidence-based, the Chalmers' score was combined with the question whether the RCT supported the claim. In total 62 (39.2%) RCTs with a high Chalmers' score supported the claim in the advertisement, while 29 (18.4%) RCTs with an intermediate Chalmers' score supported the claim. One RCT had a low Chalmers' score, which makes it unsupportive of the claim. This means that 62 claims (39.2%) were supported with high-level evidence. RCTs supporting the claim made in the advertisement were of higher quality than RCTs not supporting the claim (RR for positive Chalmers' score 1.76; 95% CI 1.26 to 2.45).

In total 68 referenced RCTs (43%) were reported to be sponsored by the pharmaceutical industry. Study quality, as measured by Chalmers' score, was not related to being sponsored (RR 1.01; 95% CI 0.76 to 1.33). Of all the RCTs, only 35 (22.2%) had a high Chalmers' score, supported the claim and were not sponsored by the pharmaceutical industry. Only 27 (17.1%) of the high Chalmers' score and supporting RCTs were sponsored (*table 1*).

Table 1. *Chalmers' score combined with the questions whether the RCT supported the claim and whether the RCT was sponsored*

		Supporting the claim in the advertisement	
Chalmers' score	**Sponsored**	**Yes**	**No**
High	Yes	27 (17.1%)	11 (7.0%)
Intermediate		14 (8.9%)	15 (9.5%)
Low		0 (0.0%)	1 (0.6%)
High	No	35 (22.2%)	15 (9.5%)
Intermediate		15 (9.5%)	25 (25.8%)
Low		0 (0.0%)	0 (0.0%)

DISCUSSION

We studied to what extent advertisements in leading medical journals are supported by a high level of evidence. Our results show that almost 40% of the RCTs with a high Chalmers' score supported the claim for which they were cited. Only 17% of the RCTs were of good quality, supporting the claim in the advertisement, and not sponsored by the company itself. In addition 18.4% of RCTs with an intermediate Chalmers' score supported their claim, while 42.4% of RCTs did not support the claim for which they were cited.

Our results are in line with the first available study conducted in this specific field by Villanueva *et al.*[1] They reported that 44.1% of the claims in advertisements in Spanish medical journals were not supported by their reference. It should be noted that a direct comparison between the two studies is difficult, because of the different ways of assessing the references and because they used all references and not just RCTs. Furthermore we used three endpoints (evidence-based, not evidence-based or intermediate) where Villanueva *et al.* used two endpoints (not supported and could-be supported). While Villanueva studied only Spanish medical journals, we found that advertisements in the leading medical journals of the world are not much better.

A recent study on the accuracy of psychiatric medication advertisements reported that claims about efficacy are supported by references 53% of the time.[5] They only used advertisements on psychiatric medications in four journals and the methods to assess whether a claim was supported by a reference were also different. They coded the claims into types and after that their accuracy was evaluated. The evaluators were a professor of psychology and two undergraduate students. This study used other methods to assess claims and their proportion of reliable claims is higher, but they conclude that increased regulation of advertisements is warranted.[5]

Another similar study was performed by van Winkelen *et al.*[2] Only 17% of the claims in this study are supported, which seems low compared with the results in our study. Again the direct comparison is difficult because van Winkelen *et al.* used another classification of the endpoints (well supported, poorly supported and misleading). Furthermore, van Winkelen *et al.* not only used RCTs, but also systematic reviews and other types of studies extracted from journals regarding the subspeciality of rheumatology.

Of all the studies used, 68 (43%) were sponsored by the pharmaceutical industry. The majority of these studies (55.8%) had a high Chalmers' score. The study by van Winkelen *et al.* reported that 97% of the RCTs they used were sponsored by the pharmaceutical industry and that almost 70% of them had a high Chalmers' score and none of them had a low Chalmers' score. Even if an RCT is valid and supports a claim, the fact that it is sponsored by the industry leads to a potential conflict of interest.[8-12] Furthermore, our results are in line with the findings of Tricoci *et al.*[13] who reported that a large proportion (48%) of the practice guidelines of the ACC and the AHA were based on low levels of evidence, such as expert opinion. Although there is no industry funding for guideline development, recommendations based on expert opinion are prone to conflicts of interest. Clinical experts are likely to receive fees and honoraria from the pharmaceutical industry. They conclude that clinicians need to be careful considering recommendations not supported by solid evidence.

A study by Cooper *et al.*[8] determined that 58% of the studies cited in advertisements were sponsored by the pharmaceutical industry or had an author affiliated

with the manufacturer of the product. These results are not surprising knowing that research trials are very expensive and only pharmaceutical companies are able to finance them. Evidence shows that industry-sponsored studies more often report outcomes that benefit the sponsoring company. In 2003 Lexchin *et al.*[10] published a systematic review discussing sponsorship and research outcome. The article reported that research sponsored by the pharmaceutical industry was more likely to produce results favouring the product made by the industry than research funded by other sources (odds ratio 4.05). Other studies have similar conclusions.[9,11,12] Publication bias could be a partial explanation for this finding. Our study, as well as other studies,[9-11] found that methodological quality was not related to sponsorship and that only the outcomes were influenced.

Shortcomings

Assessing the methodological quality of RCTs, even using the Chalmers' score, has unequivocal subjective elements. We tried to minimise misclassification by doing multiple independent assessments of each RCT. Still discrepancies between assessors may arise. In case of discrepancy of four or more points on the Chalmers' score, the reference was assessed again by one of the researchers. The new score was discussed in a panel of the four researchers to increase the inter-rater reliability.

In this study only RCTs were rated. This means that claims with references to other sorts of studies were not assessed. In general, an RCT is the best way to investigate new drugs compared with placebos or older drugs. That is why we aimed to assess whether claims are evidence-based if they have an RCT in their reference. If we had included all the different types of studies, our numbers could have been different.

Not all the claims in the advertisements could be assessed because not all of the references were RCTs. That is why we do not draw numerical conclusions on the level of advertisements. Based on this study we only know more about the extent to which RCTs support claims in advertisements.

Because we failed to get the results from some students and because some RCTs could not be found, we were not able to assess all RCTs. Although our results could have been somewhat more extended we do not feel it influenced the results much, as we were able to draw a significant conclusion from the RCTs we did analyse.

CONCLUSION

Our study aimed to assess to what extent RCTs support claims in advertisements of leading medical journals. Even though an RCT seems a reliable type of study, they are not always of high quality. In our study only 39.2% are of high quality (i.e. has a high Chalmers' score) and at the same time support the claim for which they were cited for. Some RCTs are sponsored by the pharmaceutical industry, leading to a potential conflict of interest. Sponsorship does not affect the methodological quality of studies per se, but there is evidence that sponsorship is associated with a positive study outcome. By and large, only 17% of referenced RCTs are of good quality, supportive, and not sponsored by the company itself. As at least one out of two referenced RCTs are not perfect, our results suggest that physicians should always critically assess the RCTs mentioned in the references of claims before they use them as evidence to prescribe drugs.

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LETTER TO THE EDITOR

Microarray analysis as a helpful tool in identifying the primary tumour in cancer with an unknown primary site

Dear Editor,

A small proportion of patients (~4%) with metastatic cancer are diagnosed with cancer of unknown primary (CUP), for whom microarray gene expression analysis may play an important role. Our experience suggests that clinical management would have been changed in 12 out of 21 CUP cases if a 'site-of-origin' microarray result had been available.[1]

A 78-year-old woman underwent lumpectomy of the right breast and a sentinel node procedure for an upper outer quadrant tumour ($T_2N_oM_x$). Pathology revealed an atypical papillary largely necrotic lesion with a diameter of 1.8 cm, which was considered to be an intracystic papillary carcinoma with no signs of invasion and with a resection margin of 4 mm. In addition, a 3 mm focus of ductal carcinoma *in situ* was removed with a resection margin of 3 mm. Immunohistochemical analysis revealed that both the oestrogen receptor (ER) and progesterone receptor (PR) were strongly positive. The sentinel node and six resected lymph nodes were free of tumour.

Postoperatively, local radiotherapy was administered. A productive cough prompted the attending physician to order a chest radiograph. The X-ray was highly suggestive for multiple lung metastases and a computed tomography (CT) scan revealed paratracheal lymphadenopathy, as well. Bronchoscopy, radionuclide bone scan and an ultrasound of the liver revealed no abnormalities. Fine needle aspirate of one of the pulmonary lesions was performed revealing hormone receptor negative adenocarcinoma. These results are unsupportive for the diagnosis of metastatic mammary carcinoma. Furthermore, an invasive component had never been identified in the breast lesion.

To gain further insight into the site of origin of the pulmonary lesions, gene expression analysis using CupPrint™[2] (licensed by Aviara) was performed. The CupPrint classified this patient's pulmonary tumour as likely originating in the breast. Following the CupPrint test result, the patient was treated for pulmonary metastases of breast cancer with hormonal therapy using an aromatase inhibitor, anastrazole. This clinical decision would not have been anticipated without the CupPrint test result. A CT scan four months later revealed a partial response to therapy with a >50% reduction in the number and size of lung metastases. Three months later, a follow-up CT revealed a complete remission now lasting for >24 months with continuous hormonal therapy. This case illustrates how a dedicated gene expression array can redirect clinical decision making.

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WHAT WAS KNOWN ON THIS TOPIC?

Profiles have been shown to retain part of their gene expression pattern in the metastatic setting and can thus be used to determine the primary tissue of origin. The use of gene expression in the diagnostic setting is becoming more common.

WHAT DOES THIS ADD?

This letter is very illustrative of how gene expression profiling can change clinical management of a patient to a more tailored approach.

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STATEMENT OF CONFLICT OF INTERESTS

M. Soesan and J. Westerga declare no financial and personal relationship with other people or organisations that could inappropriately influence their work. R. Bender reports to be an employee of Agendia Inc. A. Floore and F. de Snoo report to be employees of Agendia BV.

LETTER TO THE EDITOR

A woman with a swollen neck

Dear Editor,

Van Kuilenburg *et al.* present an interesting case of Ludwig's angina.[1] In 1836 Ludwig described five patients with rapidly evolving submandibular infections and these infections are now known as Ludwig's angina. The medical history of a recent dental extraction and the swelling of the submandibular space points to a diagnosis of Ludwig's angina.

I would like to emphasise that it is important at diagnosis to define to which space this infection has spread. In this case, the computed tomography scan points to advanced spread of infection beyond the submandibular and parapharyngeal spaces to the retropharyngeal space. The retropharyngeal space lies near the 'danger space', which descends directly into the posterior mediastinum to the level of the diaphragm.[2]

Most cases of Ludwig's angina are caused by Gram-positive organisms, mostly *Streptococci* and anaerobes and not Gram-negative rods.[2] Furthermore, I would like to point out that the first choice of treatment in different guidelines (Dutch guideline available at www.swab.nl) is high-dose penicillin G in combination with metronidazole or broad-spectrum penicillin with a β-lactamase inhibitor instead of a cephalosporin with metronidazole.

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Aims and scope

The *Netherlands Journal of Medicine* publishes papers in all relevant fields of internal medicine. In addition to reports of original clinical and experimental studies, reviews on topics of interest or importance, case reports, book reviews and letters to the editor are welcomed.

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Manuscripts submitted to the Journal should report original research not previously published or being considered for publication elsewhere. Submission of a manuscript to this Journal gives the publisher the right to publish the paper if it is accepted. Manuscripts may be edited to improve clarity and expression.

Language

The language of the Journal is English. English idiom and spelling is used in accordance with the Oxford dictionary. Thus: Centre and not Center, Tumour and not Tumor, Haematology and not Hematology.

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A *Covering letter* should accompany the manuscript, identifying the corresponding person (with the address, telephone number, fax number and e-mail address). Conflicts of interest, commercial affiliations, consultations, stock or equity interests should be specified. In the letter one to three sentences should be dedicated to what this study adds. The letter should make it clear that the final manuscript has been seen and approved by all authors. All authors should sign the letter. The letter should either be submitted through http://mc.manuscriptcentral.com/nethjmed or faxed to the editorial office (+31 (0)20-691 96 58).

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Acknowledgement: All funding sources should be credited here. Also a statement of conflicts of interest should be mentioned.