Netherlands
The Journal of Medicine

MISSION STATEMENT

The mission of the journal is to serve the need of the internist to practise up-to-date medicine and to keep track with important issues in health care. With this purpose we publish editorials, original articles, reviews, controversies, consensus reports, papers on speciality training and medical education, book reviews and correspondence.

EDITORIAL INFORMATION

Editorial office
Academic Medical Centre,
Department of Medicine (E2-126)
Meibergdreef 9
1105 AZ Amsterdam
The Netherlands
Tel.: +31 (0)20-566 21 71
Fax: +31 (0)20-691 96 58
E-mail: m.m.levi@amc.uva.nl
http://mc.manuscriptcentral.com/nethjmed

CITED IN

Biosis database; embase/excerpta medica; index medicus (medline) science citation index, science citation index expanded, isi alerting services, medical documentation services, current contents/clinical medicine, PubMed.

ISSN: 0300-2977

Subscriptions
General information
An annual subscription to The Netherlands Journal of Medicine consists of 10 issues. Issues within Europe are sent by standard mail and outside Europe by air delivery. Cancellations should be made, in writing, at least two months before the end of the year.

Subscription fee
The annual subscription fee within Europe is € 798, for the USA € 836 and for the rest of the world € 957. Subscriptions are accepted on a prepaid basis only and are entered on a calendar year basis.

Payment method
Please make your cheque payable to Van Zuiden Communications B.V., PO Box 2122, 2400 CC Alphen aan den Rijn, the Netherlands or you can transfer the fee to ING Bank, account number 67.87.10.872, Castellumstraat 1, Alphen aan den Rijn, the Netherlands, swift-code: ING BNL 2A. Do not forget to mention the complete address for delivery of the Journal.

Claims
Claims for missing issues should be made within two months of the date of dispatch. Missing issues will be mailed without charge. Issues claimed beyond the two-month limit must be prepaid at back copy rates.

Orders, preprints, advertising, changes in address, author or general enquiries
Please contact the publisher.

VAN ZUIDEN communications

Van Zuiden Communications B.V.
PO Box 2122
2400 CC Alphen aan den Rijn
The Netherlands
Tel.: +31 (0)172-47 61 91
Fax: +31 (0)172-47 18 82
E-mail: kapteyn@vanzuidencommunications.nl
Internet: www.njm-online.nl

Contents

Netherlands
The Journal of Medicine

EDITORIAL

Predicting mortality in the critically ill: a tricky enterprise

N.P. Juffermans

Department of Intensive Care, Academic Medical Center, Amsterdam, the Netherlands,
e-mail: n.p.juffermans@amc.uva.nl

Red blood cell distribution width (RDW) represents the size variation of all red blood cells in a patient. An increase in RDW can result from any disease process that causes the premature release of reticulocytes into the circulation. Thereby, RDW is determined by a large variety of conditions, including erythropoiesis, erythrocyte clearance, iron status, other nutritional deficiencies, renal insufficiency and haemodilution. Inflammation is also thought to increase RDW, due to suppression of erythrocyte maturation and possibly due to enhanced clearance of erythrocytes.[1]

RDW is given routinely as part of a complete blood count panel by an automated flow cytometry machine, rendering RDW an easily acquired and cheap analysis. Thereby, it is advantageous to explore the utility of this test in clinical practice. In this issue, Dr. Meynaar and colleagues report on results of a study on the performance of RDW to predict mortality in a cohort of almost 3000 critically ill patients.[2] They found that RDW is an independent predictor of mortality, which remains after correcting for the most important confounders of mortality. This confirms other findings of an association between RDW and mortality in the critically ill.

An association between RDW and inflammation (reflected by an increase in CRP and leukocyte count) was not found here. This result differs from a previous study performed in >50,000 ICU patients, which employed the same study design, but found that RDW was correlated with infection.[3] This may perhaps be due to a difference in case mix. In the present study, half of the patients were elective surgery patients, who may have experienced blood loss with subsequent iron deficiency. Iron status was not determined in the present study.

Regardless of the mechanism, it is intriguing that the association between RDW and mortality is found not only in cohorts of critically ill patients, which may differ in case mix between centres, but also in a wide variety of other patient populations, including myocardial infarction, heart failure and the general hospital population. This universal finding suggests that RDW may reflect a common pathway in the chain of events that lead to death.

Predicting chances of survival is important in clinical care. It helps physicians to determine futility of treatment and to provide an outlook for patient and relatives on chances of recovery. However, although research in this field is plenty, not many predictors of mortality are used clinically in the decision to withhold or withdraw treatment, including the use of disease severity scores. Consequences of these decisions are irreversible, which leaves no room for doubt on the performance of predictors of mortality. Also, most studies focus on mortality predictors measured at admission, while discussions on futility of treatment typically occur later in the course of ICU treatment. Thereby, with possibly the sole exception of the sensory evoked potential test, prediction scores are not commonly used in discussions about end-of-life decisions.

In my opinion, as long as we do not understand the mechanisms of dying, determining predictors of mortality are useful to gain understanding of how humans die and to stimulate research in this field, but not to guide a treatment decision. Both statements certainly apply to RDW.

REFERENCES

1. Scharte M, Fink MP. Red blood cell physiology in critical illness. Crit Care Med. 2003;31:S651-7.
2. Meynaar I, Knook AHM, Coolen S, et al. Red cell distribution width as a predictor for mortality in critically ill patients. Neth J Med. 2013;71: 488-93.
3. Bazick HS, Chang D, Mahadevappa K, Gibbons FK, Christopher KB. Red cell distribution width and all-cause mortality in critically ill patients. Crit Care Med. 2011;39:1913-21.

Netherlands
The Journal of Medicine

REVIEW

Current concepts in the management of diabetic nephropathy

F. Waanders*, F.W. Visser, R.O.B. Gans

Department of Internal Medicine, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands, *corresponding author: tel.: +31 (0)50 3616161, fax: +31 (0)50 3619310, e-mail: f.waanders@umcg.nl

ABSTRACT

Although much progress has been made in slowing the progression of diabetic nephropathy, renal dysfunction and development of end-stage renal disease (ESRD) remain major concerns in diabetes. In addition, diabetic patients with microalbuminuria have an increased cardiovascular mortality. Therefore, new treatment modalities or strategies are needed to prevent or slow the progression of diabetic nephropathy and prevent cardiovascular disease in diabetes. In this review we describe current concepts in pathophysiology, treatment goals and we discuss future developments in the treatment of diabetic nephropathy.

Common risk factors for diabetic nephropathy and its progression are longer duration, poor glycaemic control, hypertension and the presence of albuminuria. Available treatment options, especially renin-angiotensin aldosterone system (RAAS) blockade, but also better blood pressure and blood glucose control, decrease the incidence of cardiovascular disease and renal disease in diabetes. It is important that treatment goals are tailored to the individual patient with individual treatment goals of glycaemic control and blood pressure, depending on age, type of diabetes and diabetes duration. Aggressive treatment of glucose control and blood pressure might not always be best practice for every patient. Since the proportion of ESRD due to diabetic nephropathy remains high, optimisation of RAAS blockade is advocated and can be achieved by adequate sodium restriction and/or diuretic treatment. Moreover, aldosterone blockade might be a valuable strategy, which has potency to slow the progression of diabetic renal disease. Other possible future interventions are under investigation, but large clinical trials have to be awaited to confirm the safety and efficacy of these drugs.

KEYWORDS

Diabetic nephropathy, albuminuria, renin-angiotensin aldosterone system, sodium restriction, tailored treatment

INTRODUCTION

Diabetic nephropathy is one of the leading causes of end-stage renal disease (ESRD) in the Netherlands. In 2011, 15.6% of the 1950 patients developed ESRD due to diabetic nephropathy (www.renine.nl). When taking the group with missing primary diagnoses into account, this number might even increase to 19.5%. Between 2011 and 2030 the expected number of patients with type 2 diabetes in the Netherlands will increase by 35% (RIVM. Nationaal Kompas Volksgezondheid, sectie: Hoe vaak komt diabetes mellitus voor en hoeveel mensen sterven eraan? Webpage www.nationaalkompas.nl. June 2013), leading to an increase in patients reaching ESRD due to diabetic nephropathy. Moreover, diabetic subjects with microalbuminuria have an increased mortality risk, especially due to cardiovascular disease.[1] Both renal function decline and albuminuria are, with a strong synergistic interaction, of prognostic value for both progression to ESRD and death.[2,3]

The natural history of diabetic nephropathy has mainly been studied in type I diabetes, since in type I diabetic patients, the time of onset of diabetic disease is usually evident. For patients with type I diabetes with a duration of more than five years, the presence of sustained microalbuminuria (30-300 mg urinary albumin excretion per day for at least three months) is associated with the development of diabetic nephropathy.[4] In 20-30% of type I diabetic patients persistent microalbuminuria appears within the first 15 years of diabetes.[5] Microalbuminuria precedes macroalbuminuria (>300 mg of urinary albumin excretion per day) in both type 1 and type 2 diabetes. Renal endpoints (ESRD or doubling of serum creatinine) generally occur within ten years in approximately 20% of microalbuminuric patients, but in 60% of macroalbuminuric patients.[6]

In contrast to type I diabetics, the exact duration of diabetes is unclear in type 2 diabetic patients as time to diagnosis usually takes 5-7 years. Thus, sustained microalbuminuria in type 2 diabetes may even be present at diagnosis. However, only 20% of type 2 diabetic patients with microalbuminuria

progress to overt nephropathy after ten years of follow-up in contrast to over 80% of type 1 diabetic patients.[1] The rate of development of renal complications, however, is thought to be more or less similar in type 1 and 2 diabetes. Glomerular hyperfiltration is present in patients with non-insulin dependent diabetes from the onset of the disease until the time macroalbuminuria appears.[7] After the development of macroalbuminuria, the glomerular filtration rate in these patients declines at least as rapidly as has been reported in patients with insulin-dependent diabetes.[7] This pattern is consistent with the hypothesis that glomerular hyperfiltration causes progressive glomerular damage. Compared with type 1 diabetic patients, type 2 diabetic patients with microalbuminuria have different patterns of renal damage.[8] In type 1 diabetes 'typical' pathological changes include expansion of mesangium, due to accumulation of extracellular matrix protein. These lesions are most closely related to the decline in renal function in type 1 diabetes as was shown by quantitative morphometric studies.[9] Moreover, arteriolar hyalinosis affecting both afferent and efferent glomerular arterioles, ensuing thickening of glomerular and tubular basement membranes, tubulointerstitial fibrosis and glomerulosclerosis occurs in diabetic nephropathy. 'Typical' diabetic nephropathy patterns are seen in a minority of microalbuminuric type 2 diabetic patients and only in those patients with (proliferative) retinopathy and a normal body mass index (BMI), while 'atypical' patterns of renal injury (severe tubulointerstitial and/or vascular lesions disproportionate to the mild glomerular involvement) are more common among those with increased BMI and background or no retinopathy.[8] Finally, microalbuminuria is a reflection of generalised endothelial dysfunction in a subset of microalbuminuric type 2 diabetic patients who have near-normal renal structure.[8]

Although much progress has been made in slowing the progression of diabetic nephropathy, renal dysfunction and development of ESRD remain major concerns in diabetes. In addition, diabetic patients with microalbuminuria have an increased cardiovascular mortality compared with normoalbuminuric patients.[1] Therefore, new treatment modalities or strategies are needed to prevent or slow the progression of diabetic nephropathy, decrease albuminuria and prevent cardiovascular disease in diabetes. In this review we describe current concepts in pathophysiology, treatment goals and we discuss future developments in the treatment of diabetic nephropathy.

PATHWAYS AND MECHANISMS OF RENAL INJURY

The exact pathogenesis of diabetic nephropathy is complex and not completely understood. Pathogenetic factors include hyperglycaemia, increased activity of the renin-angiotensin aldosterone system (RAAS) and increased intraglomerular and systemic blood pressure. Moreover, several cytokines and growth factors, metabolic and haemodynamic factors, which have complex mutual interactions, have been identified and may cause damaging effects on the kidney. Renal response on these noxious effects further enhances renal damage.

Longstanding exposure to hyperglycaemia results in renal damage due to metabolic effects as well as direct haemodynamic effects. In experimental human studies in patients with type 1 diabetes mildly elevated serum glucose values resulted in increased blood pressure and volume expansion.[10] It is thought that these effects lead to renal hyperperfusion, glomerular hypertension and subsequent hyperfiltration. Necessary triggers for hyperfiltration and subsequent kidney growth in experimental diabetes are hyperglycaemia and a (transient) increase in growth factors as insulin-like growth factor-1 (IGF-1), TGF-beta and VEGF.[11,12] Indeed, large kidneys and a supranormal glomerular filtration rate (GFR), i.e. hyperfiltration, confer an increased risk for the development of diabetic nephropathy. Patients with type 1 diabetes and an increased kidney volume have an increased risk of developing microalbuminuria, as was shown in a cohort study.[13] Furthermore, kidney volume and hyperfiltration have an association with the rate of decline in renal function.[14] The exact molecular and cellular mechanisms linking hyperglycaemia and hyperfiltration to renal damage are only partially understood. However, glomerulosclerosis seems to develop from a cascade with mesangial matrix hypertrophy, proliferation, contraction and extracellular matrix accumulation. Of note, expansion of the mesangium, due to accumulation of extracellular matrix protein, is most closely related to the decline in renal function in type 1 diabetes, as was shown by quantitative morphometric studies.[15] Upregulation of the RAAS and endothelin-1, upregulated growth factors, oxidative stress and AGE formation all seem to be involved. The RAAS is the main homeostatic system for regulation of extracellular fluid volume, systemic and renal haemodynamics. Diabetic nephropathy is characterised by an upregulation of the RAAS, with glomerular hypertension as a key feature. However, the RAAS also seems to have non-haemodynamic effects in the development of diabetic nephropathy. In experimental studies, long-term hyperglycaemia increases the formation of mesangial angiotensin II.[16] Mesangial angiotension II has been implicated in glomerulosclerosis because it stimulates synthesis of mesangial matrix proteins, as shown by increases in growth factors as TGF-beta and inhibition of mesangial matrix degradation, as shown by decreased collagenase.[17] Probably not only angiotensin II, but also other vasoconstrictors may promote proliferative actions of growth factors. Endothelin-1 is one of the

most potent vasoconstrictors which also exerts its action on water and sodium excretion and acid-base balance. Stimulation of endothelin-1 receptors in mesangial cells may lead to mesangial cell proliferation and hypertrophy.[18] Mechanisms of renal damage in type 1 and 2 diabetes probably have multiple similarities. However, as stated previously, patterns of renal damage differ. Due to differences in patient characteristics and comorbidity between type 1 and type 2 diabetes, differences in pathophysiology of diabetic nephropathy exist. Type 1 diabetic patients are generally non-obese and young, and renal damage is mainly due to long-term exposure to hyperglycaemia. The onset of hypertension is closely associated with the onset of diabetic nephropathy in type 1 diabetes.[19,20] Interestingly, a familial predisposition to arterial hypertension increases the risk for development of diabetic nephropathy in type 1 diabetic patients.[19] Type 2 diabetic patients are generally older, have a higher BMI and more frequent additional morbidities as hypertension and dyslipidaemia. These comorbidities are thought to play an additional pathophysiological role in the development of diabetic nephropathy. Conversely, kidneys affected by these comorbidities may be more vulnerable to the detrimental effects of hyperglycaemia. Obesity itself is directly linked to renal dysfunction, independent of diabetes or hypertension. One of several possible mechanisms is that lower levels of adiponectin in obese patients result in increased oxidative stress. Oxidative stress causes podocyte damage and fusion of foot processes leading to the development of albuminuria.[21] Interestingly, obese type 2 diabetics are characterised by very low adiponectin levels, which are lower than in obesity per se.[22]

BIOMARKERS FOR DIABETIC NEPHROPATHY AND THE IMPORTANCE OF THE TUBULOINTERSTITIUM

Albuminuria is the most important biomarker for diabetic nephropathy, reflecting the extent of glomerular damage and mesangial matrix expansion. Albuminuria is a strong predictor for progression of renal disease and cardiovascular disease and mortality in diabetes.[23-26] Lowering albuminuria reduces renal and cardiovascular risk.[23-26] However, albuminuria is not a perfect biomarker. The randomised placebo-controlled Renin-Angiotensin System Study (RASS) investigated whether treatment with an AT1 receptor blocker (losartan) would prevent diabetic kidney disease in type 1 diabetic patients.[27] In this study kidney biopsies were taken at baseline and after five years of treatment. Although there was more occurrence of microalbuminuria during the study in the losartan group (17%) compared with the placebo group (6%), mesangial glomerular volume changes were similar in all groups as was the decline in renal function. The onset of microalbuminuria did not adequately reflect structural renal disease in this study. Experimental data in rats show that in spite of a reduction in albuminuria and blood pressure, pronounced progression of renal interstitial damage can be present.[28] And it is the extent of tubulointerstitial injury that ultimately determines the rate of renal function decline.[29] Of note, some diabetic patients develop biopsy-proven diabetic nephropathy in the absence of (micro)albuminuria.[30] Therefore, in some cases, therapy response to albuminuria and blood pressure can dissociate from renal interstitial damage. This indicates that better or additional tools for monitoring therapy response in diabetic kidney disease are needed. Biomarkers for interstitial damage could be valuable for this purpose and for predicting renal outcome. The predictive value of tubular injury markers for the onset of microalbuminuria or macroalbuminuria was evaluated in patients with type 1 diabetes mellitus participating in the Diabetes Control and Complication Trial (DCCT) in a nested case-control study.[31] Baseline urinary N-acetyl-β-D-glucosaminidase (NAG) as well as a rise in NAG were independently associated with the subsequent occurrence of both microalbuminuria and macroalbuminuria. This suggests that tubular alterations might either be a first sign of diabetic kidney involvement, and/or play a pathogenetic role in the development of diabetic nephropathy.

CARDIO-RENAL INTERACTION

Cardiovascular mortality and morbidity is the major threat for patients with microalbuminuria and diabetic nephropathy. An integrated approach for treating the heart and kidney dysfunction is advocated, since important interactions exist; these interactions are addressed as the cardio-renal syndrome.

The cardio-renal syndrome is a disorder of the heart and kidneys whereby acute or chronic dysfunction in one organ may induce acute or chronic dysfunction in the other organ, irrespective of the original cardiac or renal disease.[32] The association between heart and kidney failure is multifactorial, but haemodynamic factors play a major role as both organs are involved in homeostasis and management of extracellular and circulating fluid volume. On the one hand, the kidneys play a pivotal role in maintaining volume homeostasis via both vascular and tubular mechanisms. Low renal perfusion pressure activates baroreceptors in the vascular part of the juxtaglomerular apparatus in the kidneys, which activates the RAAS by stimulating these cells to release the enzyme renin. On the tubular level, volume depletion is detected by low sodium and chloride delivery to the macula densa. The macula densa in turn induces afferent

vasodilation, reducing vascular resistance and increasing renal perfusion (tubuloglomerular feedback mechanism). Sympathetic activation also stimulates renin release, leading to RAAS activation. On the other hand, reduction in cardiac output due to heart failure leads to a reduction in renal perfusion pressure. The kidneys respond by sodium and water retention, thereby increasing extracellular fluid volume and hence venous return. The increased venous return increases cardiac output (Frank-Starling mechanism), which restores renal perfusion pressure at the expense of volume retention. Furthermore, decreased cardiac output activates baroreceptors in the carotid sinus stimulating beta-adrenergic activity and thereby activating the RAAS. In addition, the cardio-renal syndrome may be aggravated by atherosclerotic changes and vascular damage in both the heart and kidneys as well as by hormonal and immunological factors.

TREATMENT GOALS

Treatment in diabetic nephropathy is aimed at deceleration of renal function loss as well as prevention or treatment of its (cardiovascular) complications. In current clinical practice, treatment is multifactorial and involves prevention or treatment of cardiovascular and renal risk factors (hypertension, albuminuria, glycaemic control, overweight, smoking, dyslipidaemia)[33] and secondary metabolic complications of renal failure (anaemia, mineral and bone disease, acidosis, malnutrition). Here we discuss some specific treatment goals for diabetic nephropathy (*table 1*).

Table 1. *Main pillars in the prevention and treatment of diabetic nephropathy*

Blood pressure (BP)	Type 1 (and young): the lower the BP the better with orthostatic symptoms limiting further decrease in BP
	Type 2 (and atherosclerosis), systolic BP between 120 and 130 mmHg
Albuminuria	Start treatment in patients with sustained urinary albumin excretion of >30 mg/day In patients with overt proteinuria treatment goal is <0.5 g/day
Interventions to reduce BP and albuminuria	
1.	Dietary sodium restriction: <100 mmol/day, < 6 gram salt/day
2.	Start RAAS blockade with ACEi or AT1 receptor blockade; aim for maximal dosage
3.	Addition of diuretic therapy: first choice loop diuretics, second choice thiazides (metabolic side effects as dyslipidaemia and hyperglycaemia)
4.	When proteinuria goal is not reached: add aldosterone receptor blockade (with strict monitoring of potassium)
5.	When blood pressure goal is not reached: add calcium re-entry blockers, beta-blockers, (alpha-blockers).
Glycaemic control	Young, well-informed patients, free of symptomatic atherosclerosis: HbA1c <6.5%
	Older, advanced renal damage, cardiovascular disease: HbA1c 7-7.5%
Dyslipidaemia	Total cholesterol <4.5 mmol/l
	eGFR >15 ml/min/1.73m^2: advise lipid-lowering treatment for reduction of cardiovascular events
	eGFR <15 ml/min/1.73m^2: lipid-lowering treatment not advised
Overweight	Advise all diabetic patients to lose weight if BMI >25 kg/m^2
Smoking	Advise all diabetic patients to quit smoking
Exercise	>30 minutes of exercise per day is recommended for all diabetic patients

Reduction of albuminuria

Diabetic patients have a substantial prevalence of (micro) albuminuria. Sustained microalbuminuria is associated with increased renal endpoints as well as cardiovascular morbidity and mortality in patients with longstanding type 1 diabetes (>5 years) or type 2 diabetes of any duration.[4] In line with this, lowering albuminuria predicts better renal outcomes in patients with diabetic kidney disease treated with RAAS blocking agents.[23-26]

Prevention of albuminuria, useful?

Because albuminuria predicts higher cardiovascular and renal risks and lowering existing albuminuria decreases this risk, one could hypothesise that preventing albuminuria lowers cardiac and renal risk. Therefore, trials have been undertaken to determine whether treating diabetic patients without albuminuria or signs of renal disease with RAAS-inhibiting agents will lower the risk of developing albuminuria and subsequently prevent cardiovascular and renal endpoints.

One such recent study was the European Randomized Olmesartan and Diabetes Microalbuminuria Prevention (ROADMAP) trial, which was performed in patients with type 2 diabetes.[34] New microalbuminuria developed in 8.2% of type 2 diabetic patients randomised to olmesartan compared with 9.8% of those receiving placebo, and the time to onset was also significantly longer in the olmesartan group. However, more cardiovascular events occurred in the olmesartan group. Lower blood pressure perhaps played a contributory role and we assume that some of these type 2 diabetic patients already had coronary atherosclerosis, which was as yet undetected. Prevention of albuminuria at the expense of too low blood pressures is thus undesirable in patients with type 2 diabetes. Therefore, RAAS-inhibiting agents in type 2 diabetic patients with normal blood pressure levels and without albuminuria are not indicated.

In this respect, type 2 diabetic patients may differ from type 1 diabetic patients, who might benefit from treatment to prevent microalbuminuria. As hypertension usually develops with the onset of kidney disease in type 1

diabetes, all primary prevention studies in type 1 diabetes have been performed in normotensive patients. Ramipril reduced the development of microalbuminuria compared with placebo in a subgroup of patients with type 1 diabetes from the micro-HOPE (Heart Outcomes Prevention Evaluation) study.[35] Moreover, perindopril prevented the development of microalbuminuria in normoalbuminuric patients with type 1 diabetes, even without effects on blood pressure.[36] However, as mentioned earlier, losartan did not prevent the development of microalbuminuria or mesangial glomerular volume changes, or the decline in renal function in type 1 diabetic patients (RASS study).[27] Thus, RAAS-inhibiting agents for primary prevention in normotensive normoalbuminuric type 1 diabetic patients is not justified. In theory, it may not be unreasonable to consider RAAS-inhibiting agents in a small subset of normotensive normoalbuminuric type 1 diabetic patients who are at high risk of developing nephropathy, such as those with a strong family history of diabetic nephropathy or hypertension in both parents. Future studies need to address this issue.

Glycaemic control

Long-term control of hyperglycaemia delays or prevents development of albuminuria and overt proteinuria.[37] Intriguingly, after pancreas transplantation, lesions of diabetic nephropathy can be reversed, although this takes more than five years of normoglycaemia.[38] The beneficial effects of strict glycaemic control persist in the long term even when glucose control is relaxed. This fascinating concept of metabolic memory is reported in trials as the Diabetes Control and Complications Trial (DCCT)[37] and the observational Epidemiology of Diabetes Interventions and Complications (EDIC) study.[39,40] Four years after finishing the DCCT study with a follow-up of 6.5 years, the difference in the median glycosylated haemoglobin values between the conventional-therapy and intensive-therapy groups (average, 9.1% and 7.2%, respectively) narrowed during follow-up (median during four years, was 8.2% and 7.9%, respectively; p<0.001). Nevertheless, the proportion of patients with new onset of microalbuminuria was still significantly lower in the former intensive-therapy group (5% of 601 patients versus 11% of 573 patients in the former conventional-therapy group).[40]

The other way around, it is believed that short-term hyperglycaemia, for example brief postprandial high elevations of glucose, may be sufficient to provoke renal injury in diabetes, probably also based on 'metabolic memory'. Recently, a potential role for epigenetic mechanisms in this metabolic memory was suggested.[41] Preliminary work in endothelial cells shows that transient episodes of hyperglycaemia can induce changes in gene expression that are dependent on modifications to histone tails (for example, methylation), and that these changes persist after return to normoglycaemia.[41] Moreover, clamping plasma glucose for just two hours at a high level results in increased urinary excretion of isoprostanes and TGF-beta, suggesting that the kidney undergoes oxidative damage and increased profibrotic growth factor expression.[42] The biochemical mechanism for this memory is not completely understood, but accumulation of advanced glycation end products (AGEs) as one of the carriers of metabolic memory and oxidative stress is possibly involved.[43] AGE accumulation can be assessed noninvasively by measuring skin autofluorescence and it predicts the development of diabetic nephropathy.[44,45] The cell biological basis of metabolic memory as well as the long-term effects of repetitive short-term peaks in blood glucose need further investigation.

The importance of tight glycaemic control once diabetic nephropathy has occurred is less straightforward. In a Canadian cohort study of 23,296 diabetic subjects with chronic kidney disease with a median follow-up time of 48 months, the association between levels of HbA1c stratified to the level of GFR was studied. In subjects with an eGFR between 30-60 ml/min/1.73m^2 an HbA1c <7% was associated with a 22% lower event rate of reaching ESRD compared with subjects with an HbA1c between 7-9%. In subjects with an eGFR between 15-30 ml/min/1.73m^2 no significant benefit of tight glycaemic control in subsequent subgroups was noted. Interestingly in this study high HbA1c levels of >9%, but also low levels <6.5% were associated with increased mortality rates.[46] The finding that a too strict glycaemic control is associated with increased mortality is in line with the results of a large intervention trial aiming at tight glycaemic control: the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial. In this study in type 2 diabetic patients with high cardiovascular risk, tight glycaemic control did not induce beneficial outcomes, but was associated with increased mortality.[47] It is assumed that these patients with established type 2 diabetes and major cardiovascular risk factors were more susceptible to the adverse consequences of hypoglycaemia. A subsequent analysis on renal endpoints at ACCORD's end showed that intensive glycaemic control resulted in a 20-30% reduction in the risk of new-onset microalbuminuria and macroalbuminuria, but without a reduction in the risk of doubling in serum creatinine or the development of ESRD.[48] Similar results were recently obtained by a post-hoc analysis of the Action in Diabetes and Vascular Disease (ADVANCE) study, in which 11,140 type 2 diabetic patients with an increased cardiovascular risk were included. Intensive glucose control reduced the risk of ESRD (20 compared with 7 events), new-onset microalbuminuria by 9% (1298 compared with 1410 patients), and new-onset macroalbuminuria by 30% (162 compared with 231 patients).[49] The most recent randomised

trial of intensified glycaemic control, the Veterans Affairs Diabetes Trial (VADT), enrolled 1791 military veterans with longstanding type 2 diabetes (mean duration 11.5 years), 40% of whom had known cardiovascular disease.[50] Patients were randomised to standard therapy versus intensified glycaemic control (HbA1c of 8.4% versus 6.9%). After a median of 5.6 years, there was no difference in the risk of mortality or microvascular endpoints, other than a reduced risk of progression of albuminuria; the risks of doubling of serum creatinine or ESRD were similar between groups.[50] In dialysis patients glycaemic control, measured as HbA1c level, is not associated with mortality.[51]

A recently published interesting editorial addresses the issue of the effects of glycaemic control on diabetic nephropathy in type 2 diabetes, and, more broadly, on patient survival and cardiovascular events.[52] As discussed, high-quality data from ACCORD, VADT and ADVANCE all demonstrate that tight glucose control (HbA1c 6-7%) will improve albuminuria-based surrogate outcomes, but only the post hoc analysis of ADVANCE[49] suggests that such an intensive strategy may reduce the clinically relevant outcome of ESRD. In contrast, tight glucose control (HbA1c <6.5%) increased mortality risk in the ACCORD study, probably related to the adverse effects of hypoglycaemia in patients with a high cardiovascular risk.[53] Taken together, intensive glycaemic control appears to have both risks and benefits. Tailored treatment with a more personalised approach aimed at the individual patient seems to be important. Tight glucose control is important in newly diagnosed type 1 diabetic patients, since they have a long time to develop diabetic kidney damage. Moreover, tight glucose control should only be advised to well-informed type 1 or 2 diabetic patients who are younger, at lower risk for hypoglycaemia, and free of symptomatic cardiovascular disease.[52] This is in contrast to older diabetics or subjects who already have more advanced renal and cardiovascular disease, in whom an HbA1c target of 7.5% might be sufficient.

Blood pressure

The ideal systolic blood pressure for patients with diabetes and chronic kidney disease would be between 120-130 mmHg. Systolic blood pressure is correlated with progressive decline in kidney function in patients with diabetes as shown by the UK Prospective Diabetes Study (UKPDS). There was a reduction of 13% in microvascular complications for every 10 mmHg decrease in systolic blood pressure.[54] However, the Irbesartan in Diabetic Nephropathy Trial (IDNT) showed an increase in all-cause mortality in patients with a systolic blood pressure below 120 mmHg.[55] The ACCORD trial, in patients with diabetes and pre-existing peripheral arterial or cardiovascular disease, showed no benefit but more adverse effects when aiming at strict blood pressure control of a systolic blood pressure below 120 mmHg.[53] The relation between blood pressure and cardiovascular morbidity and mortality in diabetic nephropathy seems to be J-curved. The worse outcome in diabetic subjects with low blood pressure is probably a reflection of relative hypoperfusion and/or hypoxia to major vascular beds as the heart and brain. Concerning the kidney, a decrease in blood pressure and thus glomerular pressure contributes to renoprotection by reduction of albuminuria and glomerular damage. However, too low perfusion pressure may result in renal hypoxia, tubulo-interstitial damage and as result in renal function decline. The optimal level of blood pressure lowering, where renal protection is outweighed by renal damage, has to be established.

How to treat diabetic patients with adequately controlled blood pressure but high residual albuminuria? Further lowering of albuminuria decreases cardiovascular risk. As for glycaemic control, tailored treatment with a more personalised approach aimed at the individual patient seems to be important. We propose that in young patients, especially those with type 1 diabetes: the lower the blood pressure, the better, with orthostatic symptoms limiting further increase in antiproteinuric treatment. On the other hand, especially in older patients with type 2 diabetes and a higher risk of atherosclerosis, systolic blood pressure targets should be higher. For whom and at which blood pressure level the downside of a low blood pressure outweighs the benefit of reduction of albuminuria is unknown. Unfortunately (as far as we know) this issue is not answered in subgroup analyses of these large trials aiming at strict blood pressure control.

Dyslipidaemia

Both diabetes and chronic kidney disease are associated with increased incidence of cardiovascular disease, with dyslipidaemia being an important factor in these associations. Current guidelines recommend treating nearly all diabetic patients with statins (HMG-CoA reductase inhibitors). The use of statin therapy in primary prevention in diabetics without elevated LDL levels has been studied in the Collaborative Atorvastin Diabetics Study (CARDS), showing a 37% rate reduction in cardiovascular events in 3.9 years median follow-up.[56] In patients with chronic kidney disease, a subgroup routinely excluded in large statin trials, it has long been debated whether statins should be used in primary prevention. Especially Die Deutsche Diabetes Dialysis (4D) study[57] and Aurora trial,[58] showing no benefit of statin use compared with placebo on cardiovascular outcomes in patients on dialysis, outlined the need for studies in subjects with chronic kidney disease. The Study of Heart and Renal Protection (SHARP) investigated whether simvastatin plus ezetimibe reduced renal and cardiovascular risk in 3023 patients on dialysis and 6247 patients with chronic kidney

disease in a setting of primary prevention.[59] During the median follow-up period of 4.9 years a 17% proportional reduction on major cardiovascular events was seen in the lipid-lowering group, with no evident differences in outcome between patients on dialysis and those who were not.[59] Subsequently performed meta-analysis shows some evidence that lipid-lowering therapy is effective in reduction of cardiovascular events in patients with chronic kidney disease at levels of eGFR >15 ml/min/1.73m.[2,60] In levels below that and in dialysis patients results are conflicting and lipid-lowering therapy is not advised.

In theory, use of statins could prevent renal damage by its antiatherosclerotic, anti-inflammatory and antioxidant effects.[61] However, secondary outcome measures in diverse statin trials show conflicting results regarding effects of statins on albuminuria and rate of renal function decline. The SHARP trial is the only large randomised controlled trial with prespecified renal endpoints. The use of lipid-lowering treatment did not reduce the rate of renal function decline nor progression to end-stage renal disease.[59] In summary, there is no evidence to support the use of statins for renoprotection only. A concern with statins that has recently been raised is their association with an increased risk of developing diabetes in the Women's Health Initiative cohort study.[62] Although bias by indication plays a role, this issue deserves more attention and further investigation.

CURRENT AND EMERGING TREATMENT APPROACHES

Based on the above-mentioned treatment goals a multifactorial approach in the treatment of diabetes and diabetic nephropathy could be advocated (*table 1*). The Steno-2 trial showed the success of such a multifactorial approach in patients with type 2 diabetes and microalbuminuria.[63] In this trial patients were treated during a mean treatment period of 7.8 years with either conventional therapy or an intensive regimen consisting of tight glucose control (target HbA1c <6.5%), RAAS blockade (regardless of blood pressure; target <130/80 mmHg), aspirin and lipid-lowering agents (target total cholesterol <4.5 mmol/l, fasting triglycerides <1.7 mmol/l). After 13.3 years of follow-up, the intensive regimen not only resulted in a 20% absolute mortality risk reduction, also diabetic nephropathy developed in fewer subjects (20 versus 37 patients), and ESRD in only one versus six patients. These results show that a multifactorial approach can slow the progression of diabetic nephropathy.

RAAS inhibition

Agents intervening in the RAAS are the mainstay in the management of diabetic nephropathy. Major evidence for this strategy is provided by the Captopril study in patients with type 1 diabetes and kidney disease[26] and the Reduction of Endpoints in NIDDM with Angiotensin II Antagonist Losartan (RENAAL)[24] and IDNT[25] study in patients with type 2 diabetes and kidney disease. The long-term benefits of ACEi and AT1 receptor blockade are mainly mediated by the reduction in systemic and intraglomerular blood pressure. In addition, these agents are thought to exert a specific antiproteinuric effect that cannot be fully attributed to the reduction in blood pressure.

Since inhibition of the RAAS by a single agent is incomplete, blocking the system at different levels simultaneously could be advocated. Dual blockade with both ACEi and AT1 receptor blockade or with a renin inhibitor further decreases albuminuria and has therefore temporarily been advocated as treatment for slowing renal disease. However, the ONTARGET study, a large (n=25,620) long-term follow-up (56 months) clinical trial recently reported the effects of dual RAAS blockade with ramipril and telmisartan on renal endpoints in patients with cardiovascular disease and a low renal risk. Despite a beneficial effect on microalbuminuria, dual blockade was associated with worse renal outcomes.[64] This may have been due to more hypotension.[64] Furthermore dual RAAS blockade induced more hyperkalaemia.[64] Recently the ALTITUDE study was stopped prematurely due to safety concerns.[65] This study evaluated the role of renin inhibition with aliskiren in addition to RAAS blockade in the prevention of cardiovascular events and hard renal endpoints in more than 8000 type 2 diabetic patients. Although blood pressure and albuminuria were reduced by aliskiren, the incidence of hyperkalaemia (potassium levels of >6 mmol/l) was higher. In addition, significant effects on primary cardiovascular and renal outcomes were lacking.[65] Based on the aforementioned studies, dual blockade with ACEi, AT1 receptor antagonists, or renin inhibitors is not recommended.

Several studies have shown that the addition of aldosterone receptor blockade to ACEi or AT1 receptor blockade can lead to further reduction in albuminuria. Classically, aldosterone exerts its effects on volume status by the regulation of sodium reabsorption through the mineralocorticoid receptor (MR) on epithelial sodium channels, which are located on cortical collecting duct cells in the distal nephron. Additionally, there is increasing evidence that aldosterone is directly involved in the development and progression of renal disease via nonepithelial MR-mediated effects. Aldosterone exerts profibrotic effects through increased production of TGF-beta, reactive oxygen species, PAI-1 and increased collagen gene expression and synthesis, which can be abolished by MR blockade.[66] RAAS blockade initially decreases circulating aldosterone levels, but suppression will not be sustained in 10-50% of patients,

a phenomenon called aldosterone breakthrough.[67] This is particularly the case during long-term treatment or during sodium restriction, which potentiates the adrenal response to angiotensin II. Thus many patients are exposed to high levels of a hormone with known profibrotic effects on the kidney. Furthermore, aldosterone breakthrough is associated with a poor response to antiproteinuric treatment and an enhanced decline of renal function in patients with diabetic nephropathy.[68,69] It is suggested that the worse clinical prognosis of patients with aldosterone breakthrough is due to direct effects of aldosterone. In line with this hypothesis, studies in patients with chronic kidney disease and early diabetic nephropathy show that MR blockade on top of ACEi and/or AT1 receptor blockade exerts added renoprotective effects.[68-70] Interestingly, the reduction in albuminuria induced by the addition of spironolactone to ACEi was related to aldosterone levels.[68] This suggests that aldosterone is a component of the renal damage that is associated with chronic kidney disease and that its inhibition by RAAS blockade can be incomplete. In spite of these encouraging results on albuminuria, long-term data on the efficacy of MR blockade on hard endpoints, for example the development of ESRD or patient survival, are still lacking.

Dietary sodium restriction and diuretic therapy

Despite the proven efficacy of RAAS blockade, the risk of renal and cardiovascular disease remains high, which is likely due to high residual blood pressure and/or albuminuria. Sodium status is an important determinant of the responses of blood pressure and albuminuria to RAAS blockade. ACEi and AT1 receptor blockers are largely ineffective during states of volume excess, either due to renal dysfunction, nephrotic syndrome or to increased sodium intake. Sodium restriction and diuretic treatment increase the top of the dose-response curve to RAAS blockade, and therefore a larger maximum response can be obtained.[71] Correction of volume overload, or induction of mild volume depletion by dietary sodium restriction, diuretic treatment or their combination, increases the therapeutic efficacy of RAAS blockade.[72,73] Interestingly, the addition of dietary sodium restriction to ACEi is considerably more effective than dual RAAS blockade with ACEi plus AT1 receptor blockade for the reduction of albuminuria and blood pressure in patients with overt proteinuria.[74] In this study sodium intake was reduced from a level that equals the prevailing sodium intake in the renal and general population (160-200 mmol sodium/day corresponding with 9.6-12 g salt/day) to a level conform current recommendations (<100 mmol/day, <6 g salt/day).[74,75] Accordingly, it should be feasible to implement the benefits of dietary sodium restriction in daily clinical practice. A recent study showed an association of sodium status with hard renal endpoints, which substantiates these short-term beneficial effects of dietary sodium restriction on albuminuria and blood pressure.[76] Data from the RENAAL and IDNT trials were merged and analysed retrospectively, showing that during AT1 receptor blockade lower dietary sodium intake was associated with lower albuminuria, less progression to ESRD and fewer cardiovascular events in patients with diabetic nephropathy.[76] Furthermore, compared with non-RAAS blockade based therapy, the positive treatment effects of AT1 receptor blockade on hard renal and cardiovascular outcomes were completely annihilated in subjects with the highest sodium intake.[76] This implicates that interventions to reduce dietary sodium intake will have the potential to greatly improve long-term renal and cardiovascular outcome in patients with diabetic nephropathy, particularly those patients who are treated with RAAS blockade.

Although the importance of sodium restriction seems to be evident, its importance is debated by some. A recent meta-analysis from Italy suggested more hospitalisation and increased mortality in patients with heart failure on a very strict sodium diet. However, while originally published in a high-impact journal, this paper was retracted due to incomplete and non-retraceable raw data.[77] We are not aware of any other report showing detrimental effects of a low-sodium diet.

Diuretic therapy with increased furosemide dosage together with the avoidance of excessive salt intake on top of dual RAAS blockade by ACEi and AT1 receptor blockade has been shown to decrease proteinuria in nephrotic patients (7 out of 18 had diabetic nephropathy).[78] Moreover, in albuminuric patients during high sodium intake the antiproteinuric as well as the blood pressure response to ACE inhibition was blunted, but could be restored by the addition of hydrochlorothiazide.[79] However, the practical problems with thiazides are their metabolic side effects, such as dyslipidaemia and hyperglycaemia, which is clearly undesirable in diabetic patients with an increased cardiovascular risk profile.

There is some evidence that the effects of sodium restriction and hydrochlorothiazide might not be equivalent, despite the fact that both act on sodium status. Thiazides mainly exert their antihypertensive effect by specific vascular changes rather than by volume depletion.[80] In an experimental study in rat, sodium restriction, but not diuretic therapy, diminished renal hypertrophy while blood pressure was similar.[81] Different modes of action of diuretics and sodium restriction are suggested by a randomised placebo-controlled study in 33 proteinuric patients.[72] Whereas, the addition of sodium restriction or hydrochlorothiazide to losartan was equally effective in reducing proteinuria, the effect of hydrochlorothiazide was associated with blood pressure response, while this was not the case for sodium restriction.[72]

NOVEL THERAPIES

In experimental studies several new targets and drugs for the treatment of diabetic nephropathy are being developed. It is beyond the scope of this review to provide a complete overview of these novelties. However, we want to highlight some of the most promising possible interventions.

Low vitamin D levels are associated with faster renal function decline. Experimental studies show that treatment with paricalcitol, a selective activator of the vitamin D receptor, decreases urinary albumin excretion and slows progression of kidney injury. Mechanisms probably include suppression of the RAAS, and anti-inflammatory and antifibrotic effects of vitamin D.[82,83] Recent experimental work shows that these beneficial effects especially exert a protective role in the apoptotic response of podocytes to hyperglycaemia.[84] The VITAL study, a short-term randomised controlled trial, investigated the effect of paricalcitol 1 or 2 μg per day on microalbuminuria in diabetic patients. After 24 weeks the patients using paracalcitol 2 μg showed a significant reduction in albuminuria ranging from -18% to -28%, with a favourable short-term safety profile.[85] Long-term studies with a special focus on long-term safety issues (risk for adynamic bone disease) are awaited.

The renal endothelin-1 system is activated in patients with diabetic nephropathy, linked to renal damage by its action on mesangial cell proliferation and hypertrophy.[18] In experimental studies, blocking endothelin-1 exerted renoprotective effects and a reduction in albuminuria was seen.[86] The endothelin-1 antagonist avosentan indeed reduced albuminuria in a randomised controlled trial in almost 1400 diabetic patients.[87] However, this trial was prematurely ended after a follow-up period of four months because of an increased incidence of cardiovascular adverse events, especially fluid overload and congestive heart failure.[87] Other endothelin-1 antagonists as atrasentan and sitaxsentan have been developed and appear to have fewer side effects and comparable albuminuria-reduction abilities. Additional trials using this class of drugs in diabetic nephropathy are ongoing.[88]

We have discussed the role of advanced glycation end products (AGEs) in the development of diabetic nephropathy. The AGE inhibitor pimagedine showed promising results in animal models and in a human study. In the ATION 1 study, 454 type I diabetic patients with nephropathy and retinopathy were treated with pimagadine during a follow-up period of 2-4 years. Pimagedine reduced albuminuria and the rate of renal function decline.[89] However, pimagedine did not reduce renal endpoints (doubling of serum creatinine). Further development of this AGE inhibitor was stopped due to safety concerns, primarily based on the non-specific actions of pimagedine.[90] Pyridoxine, another AGE inhibitor, has been tested in experimental studies, and seems to be well tolerated in humans.[91] The results of more trials using pyridoxine are being awaited. AGE crosslink breakers, a linked class of drugs able to cleave preformed AGE crosslinks, show promising results in experimental studies by showing improvement in structural morphological cardiac[92] and glomerular damage.[93] In small clinical trials the AGE-crosslink breaker alagebrium improved endothelial function and arterial compliance.[94] Clinical trials confirming these potential beneficial effects are needed.

CONCLUSION

To prevent diabetic nephropathy and cardiovascular mortality in patients with diabetes it is important to tailor treatment to the individual patient with individual treatment goals, depending on age, type of diabetes and diabetes duration. Aggressive treatment of glucose control and blood pressure might not always be best practice for every patient. The three main pillars in the treatment of diabetic nephropathy are albuminuria, blood pressure and glycaemic control with RAAS blockade being the cornerstone of treatment. To potentiate the effects of RAAS blockade, sodium restriction and/or diuretics can be added. Moreover, positive effects of aldosterone blockade have been shown.

REFERENCES

1. Mogensen CE. Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. N Engl J Med. 1984;310:356-60.
2. Amin AP, Whaley-Connell AT, Li S, et al. The synergistic relationship between estimated GFR and microalbuminuria in predicting long-term progression to ESRD or death in patients with diabetes: results from the Kidney Early Evaluation Program (KEEP). Am J Kidney Dis. 2013;61:S12-23.
3. Berhane AM, Weil EJ, Knowler WC, Nelson RG, Hanson RL. Albuminuria and estimated glomerular filtration rate as predictors of diabetic end-stage renal disease and death. Clin J Am Soc Nephrol. 2011;6:2444-51.
4. Groop PH, Thomas MC, Moran JL, et al. The presence and severity of chronic kidney disease predicts all-cause mortality in type 1 diabetes. Diabetes. 2009;58:1651-8.
5. Orchard TJ, Dorman JS, Maser RE, et al. Prevalence of complications in IDDM by sex and duration. Pittsburgh Epidemiology of Diabetes Complications Study II. Diabetes. 1990;39:1116-24.
6. Hovind P, Rossing P, Tarnow L, Smidt UM, Parving HH. Progression of diabetic nephropathy. Kidney Int. 2001;59:702-9.
7. Nelson RG, Bennett PH, Beck GJ, et al. Development and progression of renal disease in Pima Indians with non-insulin-dependent diabetes mellitus. Diabetic Renal Disease Study Group. N Engl J Med. 1996;335:1636-42.
8. Fioretto P, Mauer M, Brocco E, et al. Patterns of renal injury in NIDDM patients with microalbuminuria. Diabetologia. 1996;39:1569-76.
9. Mauer SM, Steffes MW, Ellis EN, et al. Structural-functional relationships in diabetic nephropathy. J Clin Invest. 1984;74:1143-55

10. Jacobsen P, Rossing K, Hansen BV, Bie P, Vaag A, Parving HH. Effect of short-term hyperglycaemia on haemodynamics in type 1 diabetic patients. J Intern Med. 2003;254:464-71.

11. Bak M, Thomsen K, Christiansen T, Flyvbjerg A. Renal enlargement precedes renal hyperfiltration in early experimental diabetes in rats. J Am Soc Nephrol. 2000;11:1287-92.

12. Wolf G, Ziyadeh FN. Molecular mechanisms of diabetic renal hypertrophy. Kidney Int. 1999;56:393-405.

13. Zerbini G, Bonfanti R, Meschi F, et al. Persistent renal hypertrophy and faster decline of glomerular filtration rate precede the development of microalbuminuria in type 1 diabetes. Diabetes. 2006;55:2620-5.

14. Cherney DZ, Miller JA, Scholey JW, et al. Renal hyperfiltration is a determinant of endothelial function responses to cyclooxygenase 2 inhibition in type 1 diabetes. Diabetes Care. 2010;33:1344-6.

15. Mauer SM, Steffes MW, Ellis EN, Sutherland DE, Brown DM, Goetz FC. Structural-functional relationships in diabetic nephropathy. J Clin Invest. 1984;74:1143-55.

16. Singh R, Singh AK, Alavi N, Leehey DJ. Mechanism of increased angiotensin II levels in glomerular mesangial cells cultured in high glucose. J Am Soc Nephrol. 2003;14:873-80.

17. Singh R, Alavi N, Singh AK, Leehey DJ. Role of angiotensin II in glucose-induced inhibition of mesangial matrix degradation. Diabetes. 1999;48:2066-73.

18. Sorokin A, Kohan DE. Physiology and pathology of endothelin-1 in renal mesangium. Am J Physiol Renal Physiol. 2003;285:F579-89.

19. Earle K, Viberti GC. Familial, hemodynamic and metabolic factors in the predisposition to diabetic kidney disease. Kidney Int. 1994;45:434-7.

20. Ayodele OE, Alebiosu CO, Salako BL. Diabetic nephropathy--a review of the natural history, burden, risk factors and treatment. J Natl Med Assoc. 2004;96:1445-54.

21. Sharma K, Ramachandrarao S, Qiu G, et al. Adiponectin regulates albuminuria and podocyte function in mice. J Clin Invest. 2008;118:1645-56.

22. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest. 2006;116:1784-92.

23. Eijkelkamp WB, Zhang Z, Remuzzi G, et al. Albuminuria is a target for renoprotective therapy independent from blood pressure in patients with type 2 diabetic nephropathy: post hoc analysis from the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) trial. J Am Soc Nephrol. 2007;18:1540-6.

24. Brenner BM, Cooper ME, de Zeeuw D, et al. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. N Engl J Med. 2001;345:861-9.

25. Lewis EJ, Hunsicker LG, Clarke WR, et al. Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. N Engl J Med. 2001;345:851-60.

26. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. N Engl J Med. 1993;329:1456-62.

27. Mauer M, Zinman B, Gardiner R, et al. Renal and retinal effects of enalapril and losartan in type 1 diabetes. N Engl J Med. 2009;361:40-51.

28. Hamming I, Navis G, Kocks MJ, van Goor H. ACE inhibition has adverse renal effects during dietary sodium restriction in proteinuric and healthy rats. J Pathol. 2006;209:129-39.

29. Tang SC, Lai KN. The pathogenic role of the renal proximal tubular cell in diabetic nephropathy. Nephrol Dial Transplant. 2012;27:3049-56.

30. Caramori ML, Fioretto P, Mauer M. Low glomerular filtration rate in normoalbuminuric type 1 diabetic patients: an indicator of more advanced glomerular lesions. Diabetes. 2003;52:1036-40.

31. Kern EF, Erhard P, Sun W, Genuth S, Weiss MF. Early urinary markers of diabetic kidney disease: a nested case-control study from the Diabetes Control and Complications Trial (DCCT). Am J Kidney Dis. 2010;55:824-34.

32. Ronco C, McCullough P, Anker SD, et al. Cardio-renal syndromes: report from the consensus conference of the acute dialysis quality initiative. Eur Heart J. 2010;31:703-11.

33. Altemtam N, Russell J, El Nahas M. A study of the natural history of diabetic kidney disease (DKD). Nephrol Dial Transplant. 2012;27:1847-54.

34. Haller H, Ito S, Izzo JL Jr, et al. Olmesartan for the delay or prevention of microalbuminuria in type 2 diabetes. N Engl J Med. 2011;364:907-17.

35. Gerstein HC, Mann JF, Pogue J, et al. Prevalence and determinants of microalbuminuria in high-risk diabetic and nondiabetic patients in the Heart Outcomes Prevention Evaluation Study. The HOPE Study Investigators. Diabetes Care. 2000;23(Suppl 2):B35-9.

36. Kvetny J, Gregersen G, Pedersen RS. Randomized placebo-controlled trial of perindopril in normotensive, normoalbuminuric patients with type 1 diabetes mellitus. QJM. 2001;94:89-94.

37. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. N Engl J Med. 1993;329:977-86.

38. Fioretto P, Steffes MW, Sutherland DE, Goetz FC, Mauer M. Reversal of lesions of diabetic nephropathy after pancreas transplantation. N Engl J Med. 1998;339:69-75.

39. Epidemiology of Diabetes Interventions and Complications (EDIC). Design, implementation, and preliminary results of a long-term follow-up of the Diabetes Control and Complications Trial cohort. Diabetes Care. 1999;22:99-111.

40. Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. N Engl J Med. 2000;342:381-9.

41. Tonna S, El-Osta A, Cooper ME, Tikellis C. Metabolic memory and diabetic nephropathy: potential role for epigenetic mechanisms. Nat Rev Nephrol. 2010;6:332-41.

42. McGowan TA, Dunn SR, Falkner B, Sharma K. Stimulation of urinary TGF-beta and isoprostanes in response to hyperglycemia in humans. Clin J Am Soc Nephrol. 2006;1:263-8.

43. Genuth S, Sun W, Cleary P, et al. Glycation and carboxymethyllysine levels in skin collagen predict the risk of future 10-year progression of diabetic retinopathy and nephropathy in the diabetes control and complications trial and epidemiology of diabetes interventions and complications participants with type 1 diabetes. Diabetes. 2005;54:3103-11.

44. Gerrits EG, Lutgers HL, Kleefstra N, et al. Skin autofluorescence: a tool to identify type 2 diabetic patients at risk for developing microvascular complications. Diabetes Care. 2008;31:517-21.

45. Smit AJ, Gerrits EG. Skin autofluorescence as a measure of advanced glycation endproduct deposition: a novel risk marker in chronic kidney disease. Curr Opin Nephrol Hypertens. 2010;19:527-33.

46. Shurraw S, Hemmelgarn B, Lin M, et al. Association between glycemic control and adverse outcomes in people with diabetes mellitus and chronic kidney disease: a population-based cohort study. Arch Intern Med. 2011;171:1920-7.

47. ACCORD Study Group, Gerstein HC, Miller ME, et al. Long-term effects of intensive glucose lowering on cardiovascular outcomes. N Engl J Med. 2011;364:818-28.

48. Ismail-Beigi F, Craven T, Banerji MA, et al. Effect of intensive treatment of hyperglycaemia on microvascular outcomes in type 2 diabetes: an analysis of the ACCORD randomised trial. Lancet. 2010;376:419-30.

49. Perkovic V, Heerspink HL, Chalmers J, et al. Intensive glucose control improves kidney outcomes in patients with type 2 diabetes. Kidney Int. 2013;83:517-23.

50. Duckworth W, Abraira C, Moritz T, et al. Glucose control and vascular complications in veterans with type 2 diabetes. N Engl J Med. 2009;360:129-39.

51. Shurraw S, Majumdar SR, Thadhani R, Wiebe N, Tonelli M, Alberta Kidney Disease Network. Glycemic control and the risk of death in 1,484 patients receiving maintenance hemodialysis. Am J Kidney Dis. 2010;55:875-84.

52. Shurraw S, Tonelli M. Intensive glycemic control in type 2 diabetics at high cardiovascular risk: do the benefits justify the risks? Kidney Int. 2013;83:346-8.

53. ACCORD Study Group, Cushman WC, Evans GW, et al. Effects of intensive blood-pressure control in type 2 diabetes mellitus. N Engl J Med. 2010;362:1575-85.

54. Bretzel RG, Voigt K, Schatz H. The United Kingdom Prospective Diabetes Study (UKPDS) implications for the pharmacotherapy of type 2 diabetes mellitus. Exp Clin Endocrinol Diabetes. 1998;106:369-72.

55. Pohl MA, Blumenthal S, Cordonnier DJ, et al. Independent and additive impact of blood pressure control and angiotensin II receptor blockade on renal outcomes in the irbesartan diabetic nephropathy trial: clinical implications and limitations. J Am Soc Nephrol. 2005;16:3027-37.

56. Colhoun HM, Betteridge DJ, Durrington PN, et al. Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomised placebo-controlled trial. Lancet. 2004;364:685-96.

57. Wanner C, Krane V, Marz W, et al. Randomized controlled trial on the efficacy and safety of atorvastatin in patients with type 2 diabetes on hemodialysis (4D study): demographic and baseline characteristics. Kidney Blood Press Res. 2004;27:259-66.

58. Fellstrom BC, Jardine AG, Schmieder RE, et al. Rosuvastatin and cardiovascular events in patients undergoing hemodialysis. N Engl J Med. 2009;360:1395-407.

59. Baigent C, Landray MJ, Reith C, et al. The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and Renal Protection): a randomised placebo-controlled trial. Lancet. 2011;377:2181-92.

60. Upadhyay A, Earley A, Lamont JL, Haynes S, Wanner C, Balk EM. Lipid-lowering therapy in persons with chronic kidney disease: a systematic review and meta-analysis. Ann Intern Med. 2012;157:251-62.

61. Kassimatis TI, Konstantinopoulos PA. The role of statins in chronic kidney disease (CKD): friend or foe? Pharmacol Ther. 2009;122:312-23.

62. Culver AL, Ockene IS, Balasubramanian R, et al. Statin use and risk of diabetes mellitus in postmenopausal women in the Women's Health Initiative. Arch Intern Med. 2012;172:144-52.

63. Gaede P, Lund-Andersen H, Parving HH, Pedersen O. Effect of a multifactorial intervention on mortality in type 2 diabetes. N Engl J Med. 2008;358:580-91.

64. ONTARGET Investigators, Yusuf S, Teo KK, et al. Telmisartan, ramipril, or both in patients at high risk for vascular events. N Engl J Med. 2008;358:1547-59.

65. Parving HH, Brenner BM, McMurray JJ, et al. Cardiorenal Endpoints in a Trial of Aliskiren for Type 2 Diabetes. N Engl J Med. 2012;367:2204-13.

66. Waanders F, de Vries LV, van Goor H, et al. Aldosterone, from (patho) physiology to treatment in cardiovascular and renal damage. Curr Vasc Pharmacol. 2011;9:594-605.

67. Bomback AS, Kshirsagar AV, Amamoo MA, Klemmer PJ. Change in proteinuria after adding aldosterone blockers to ACE inhibitors or angiotensin receptor blockers in CKD: a systematic review. Am J Kidney Dis. 2008;51:199-211.

68. Sato A, Hayashi K, Naruse M, Saruta T. Effectiveness of aldosterone blockade in patients with diabetic nephropathy. Hypertension. 2003;41:64-8.

69. Chrysostomou A, Pedagogos E, MacGregor L, Becker GJ. Double-blind, placebo-controlled study on the effect of the aldosterone receptor antagonist spironolactone in patients who have persistent proteinuria and are on long-term angiotensin-converting enzyme inhibitor therapy, with or without an angiotensin II receptor blocker. Clin J Am Soc Nephrol. 2006;1:256-62.

70. Schjoedt KJ, Jacobsen P, Rossing K, Boomsma F, Parving HH. Dual blockade of the renin-angiotensin-aldosterone system in diabetic nephropathy: the role of aldosterone. Horm Metab Res. 2005;37(Suppl 1):4-8.

71. Heeg JE, de Jong PE, van der Hem GK, de Zeeuw D. Efficacy and variability of the antiproteinuric effect of ACE inhibition by lisinopril. Kidney Int. 1989;36:272-9.

72. Vogt L, Waanders F, Boomsma F, de Zeeuw D, Navis G. Effects of dietary sodium and hydrochlorothiazide on the antiproteinuric efficacy of losartan. J Am Soc Nephrol. 2008;19:999-1007.

73. Houlihan CA, Allen TJ, Baxter AL, et al. A low-sodium diet potentiates the effects of losartan in type 2 diabetes. Diabetes Care. 2002;25:663-71.

74. Slagman MC, Waanders F, Hemmelder MH, et al. Moderate dietary sodium restriction added to angiotensin converting enzyme inhibition compared with dual blockade in lowering proteinuria and blood pressure: randomised controlled trial. BMJ. 2011;343:d4366.

75. Kidney Disease Outcomes Quality Initiative (K/DOQI). K/DOQI clinical practice guidelines on hypertension and antihypertensive agents in chronic kidney disease. Am J Kidney Dis. 2004;43:S1-290.

76. Lambers Heerspink HJ, Holtkamp FA, Parving HH, et al. Moderation of dietary sodium potentiates the renal and cardiovascular protective effects of angiotensin receptor blockers. Kidney Int. 2012;82:330-7.

77. Dinicolantonio JJ, Pasquale PD, Taylor RS, Hackam DG. Low sodium versus normal sodium diets in systolic heart failure: systematic review and meta-analysis. Heart. 2013; March 12. [Epub ahead of print].

78. Esnault VL, Ekhlas A, Delcroix C, Moutel MG, Nguyen JM. Diuretic and enhanced sodium restriction results in improved antiproteinuric response to RAS blocking agents. J Am Soc Nephrol. 2005;16:474-81.

79. Buter H, Hemmelder MH, Navis G, de Jong PE, de Zeeuw D. The blunting of the antiproteinuric efficacy of ACE inhibition by high sodium intake can be restored by hydrochlorothiazide. Nephrol Dial Transplant. 1998;13:1682-5.

80. Jones B, Nanra RS. Double-blind trial of antihypertensive effect of chlorothiazide in severe renal failure. Lancet. 1979;2:1258-60.

81. Benstein JA, Feiner HD, Parker M, Dworkin LD. Superiority of salt restriction over diuretics in reducing renal hypertrophy and injury in uninephrectomized SHR. Am J Physiol. 1990;258:F1675-81.

82. Alborzi P, Patel NA, Peterson C, et al. Paricalcitol reduces albuminuria and inflammation in chronic kidney disease: a randomized double-blind pilot trial. Hypertension. 2008;52:249-55.

83. Tan X, Li Y, Liu Y. Paricalcitol attenuates renal interstitial fibrosis in obstructive nephropathy. J Am Soc Nephrol. 2006;17:3382-93.

84. Wang Y, Deb DK, Zhang Z, et al. Vitamin D Receptor Signaling in Podocytes Protects against Diabetic Nephropathy. J Am Soc Nephrol. 2012;23:1977-86.

85. de Zeeuw D, Agarwal R, Amdahl M, et al. Selective vitamin D receptor activation with paricalcitol for reduction of albuminuria in patients with type 2 diabetes (VITAL study): a randomised controlled trial. Lancet. 2010;376:1543-51.

86. Cosenzi A, Bernobich E, Trevisan R, Milutinovic N, Borri A, Bellini G. Nephroprotective effect of bosentan in diabetic rats. J Cardiovasc Pharmacol. 2003;42:752-6.

87. Mann JF, Green D, Jamerson K, et al. Avosentan for overt diabetic nephropathy. J Am Soc Nephrol. 2010;21:527-35.

88. Kohan DE, Pollock DM. Endothelin antagonists for diabetic and non-diabetic chronic kidney disease. Br J Clin Pharmacol. 2012; doi: 10.1111/bcp.12064 [Epub ahead of print].

89. Bolton WK, Cattran DC, Williams ME, et al. Randomized trial of an inhibitor of formation of advanced glycation end products in diabetic nephropathy. Am J Nephrol. 2004;24:32-40.

90. Williams ME. Clinical studies of advanced glycation end product inhibitors and diabetic kidney disease. Curr Diab Rep. 2004;4:441-6.

91. Williams ME, Bolton WK, Khalifah RG, Degenhardt TP, Schotzinger RJ, McGill JB. Effects of pyridoxamine in combined phase 2 studies of patients with type 1 and type 2 diabetes and overt nephropathy. Am J Nephrol. 2007;27:605-14.

92. Kranstuber AL, Del Rio C, Biesiadecki BJ, et al. Advanced glycation end product cross-link breaker attenuates diabetes-induced cardiac dysfunction by improving sarcoplasmic reticulum calcium handling. Front Physiol. 2012;3:292.

93. Watson AM, Gray SP, Jiaze L, et al. Alagebrium reduces glomerular fibrogenesis and inflammation beyond preventing RAGE activation in diabetic apolipoprotein E knockout mice. Diabetes. 2012;61:2105-13.

94. Zieman SJ, Melenovsky V, Clattenburg L, et al. Advanced glycation endproduct crosslink breaker (alagebrium) improves endothelial function in patients with isolated systolic hypertension. J Hypertens. 2007;25:577-83.

Netherlands
The Journal of Medicine

REVIEW

Intestinal cholesterol secretion: future clinical implications

L. Jakulj[1]*, J. Besseling[1], E.S.G. Stroes,[1] A.K. Groen[2]

[1]Department of Vascular Medicine, Academic Medical Centre, Amsterdam, the Netherlands, [2]Departments of Paediatrics and Laboratory Medicine, University Medical Centre Groningen, the Netherlands, corresponding author: tel: +31 (0)20-5993503, fax: +31(0)20-5993523, e-mail: l.jakulj@olvg.nl

ABSTRACT

Together with the liver, the intestine serves as a homeostatic organ in cholesterol metabolism. Recent evidence has substantiated the pivotal role of the intestine in reverse cholesterol transport (RCT). RCT is a fundamental antiatherogenic pathway, mediating the removal of cholesterol from tissues in the body to the faeces. In humans, faecal cholesterol elimination via the RCT pathway is considered to be restricted to excretion via the hepatobiliary route. Recently, however, direct trans-intestinal excretion of plasma-derived cholesterol (TICE) was shown to contribute substantially to faecal neutral sterol (FNS) excretion in mice. TICE was found to be amenable to stimulation by various pharmacological and dietary interventions in mice, offering new options to target the intestine as an inducible, cholesterol-excretory organ. The relevance of TICE for cholesterol elimination in humans remains to be established. There is, however, emerging evidence for the presence of TICE in human (patho) physiology. This review discusses our current understanding of TICE and its novel therapeutic potential for individuals at increased risk of cardiovascular disease.

KEYWORDS

Reverse cholesterol transport, intestine, cardiovascular disease

INTRODUCTION

In the human body, cholesterol homeostasis is tightly regulated. This is not only of physiological importance, but also bears clinical relevance, since excessive cholesterol accumulation in the arterial wall invariably leads to the development of atherosclerotic cardiovascular disease (CVD). Although the inhibition of cholesterol synthesis by statins has resulted in a powerful reduction of CVD risk with a mean relative reduction of 25%,[1] there is still an unmet need for additional effective therapies to further reduce the residual CVD risk. In the past decade, research has mainly focussed on high-density lipoprotein cholesterol (HDL-C) raising therapies,[2,3] because of the strong inverse relationship between plasma HDL-C concentrations and CVD risk in epidemiological studies.[4-7] However, recent studies, aimed at increasing plasma HDL-C concentrations, have not substantiated a significant CVD risk reduction.[7-10] Hence, rather than aiming for an increase of HDL-C concentration, current research is focussed on elucidating and, if feasible, quantifying the mechanisms contributing to the atheroprotective properties of HDL-C.

The most established protective function of HDL-C is its role in the reverse cholesterol transport (RCT). This process was originally defined as the efflux of cholesterol from peripheral tissues, including arterial intra-plaque macrophages, subsequent transport in the plasma and uptake by the liver, followed by biliary secretion and elimination via the faeces.[11] Faecal excretion is the predominant way for eliminating cholesterol, because apart from conversion to bile acids, cholesterol cannot be catabolised to a significant extent within the human body. The classical RCT concept rests on two principles: 1. HDL-C is the primary lipoprotein involved in RCT and 2. biliary secretion is the sole route for intestinal elimination of plasma-derived cholesterol. In view of recent findings, both of these principles need to be reconsidered. The first is beyond the scope of this review. In short, in contrast to the current consensus, several studies have shown that plasma HDL-C levels do not determine biliary or faecal excretion of cholesterol in mice,[12-14] whereas

studies in humans have yielded conflicting results.[15-18] This review handles the second paradigm, the obligatory role of hepatobiliary cholesterol secretion in RCT. This historical concept has recently been challenged by studies in mice, indicating the existence of direct trans-intestinal cholesterol excretion (TICE) as an alternative cholesterol-eliminating pathway.

TRANS-INTESTINAL CHOLESTEROL EXCRETION: ANIMAL STUDIES

Cholesterol destined for hepatobiliary cholesterol secretion is taken up at the basolateral side of the hepatocyte via a number of lipoprotein receptors and is subsequently secreted at the canalicular membrane by a not fully elucidated secretion process, mediated for the largest part by the ATP-binding cassette G5/G8 (abcg5/g8) transporter.[19] If hepatobiliary cholesterol secretion were the primary route for cholesterol elimination, then inhibition of abcg5/g8 could be expected to result in extreme reductions of faecal neutral sterol (FNS) excretion. Interestingly, *abcg5* and *abcg8* double-knockout mice did not show these expected reductions in FNS loss.[20,21] Similar observations were made in other murine models of impaired hepatobiliary cholesterol secretion.[22-25] These studies unambiguously point towards the existence of an alternative, non-biliary cholesterol excretion pathway, at least in mice with genetically hampered hepatobiliary cholesterol secretion.

The concept of a non-biliary cholesterol excretion route is not novel. Already in 1927, it was demonstrated that FNS loss was paradoxically increased in dogs undergoing surgical bile diversion, as compared with control dogs.[26] These early findings were confirmed in a replication study in 1973,[27] as well as in studies in bile-diverted rats.[28,29] Similar observations have been made in the human situation, as described below.

More recently, additional murine intestinal perfusion studies and *in vivo* stable isotope studies substantiated that this alternative TICE route is also present in mice with intact hepatobiliary secretion and enterohepatic cycling.[30,31] In these studies, TICE accounted for roughly 20-33% of FNS loss. Moreover, the intestinal perfusion studies showed that plasma cholesterol can directly traverse the small intestine in a basolateral to apical direction, stimulated by the luminal presence of bile salt and phospholipid acceptors.[32] Furthermore, TICE was found to occur predominantly in the proximal part of the small intestine.[25,30] Importantly, a recent study showed that faecal excretion of macrophage-derived cholesterol can also proceed in the absence of biliary sterol secretion, suggesting that TICE can also mediate reverse cholesterol transport from cholesterol-loaded macrophages.[33] This implies that TICE may have antiatherogenic effects. However, a similar study could not confirm these results, for as yet unknown reasons.[34] Hence, it remains to be established whether induction of cholesterol elimination via TICE results in inhibition of atherosclerosis progression.

The molecular mechanisms underlying TICE are not fully understood. Hence, it is not known whether TICE is an active, transporter-mediated metabolic process. In order to effectively target this pathway, the following items need to be addressed: characterisation of plasma donor particles delivering cholesterol to the intestine for subsequent excretion via TICE; identification of transporters located at the basolateral membrane of intestinal cells, involved in the uptake of cholesterol destined for intestinal excretion; elucidation of intracellular trafficking mechanisms by which cholesterol is transported towards the apical membrane of enterocytes; identification of all apically located transporters and potential luminal acceptors which facilitate the excretion of cholesterol to the enteric lumen.

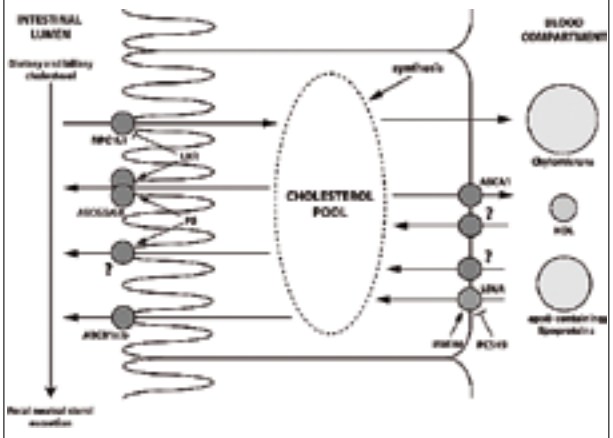
Thus far, some progress has been made, which has been the focus of recent comprehensive reviews.[35-37] The most important findings are summarised below (see also *figure 1*).

Despite its classical role in RCT, several studies indicate that plasma HDL is not the donor particle delivering plasma cholesterol for elimination via TICE. *Abca1* and *apoA1*-deficient mice, expressing negligible plasma HDL-C concentrations, exhibit normal or increased FNS excretion under normal conditions[12-14] and intestinal secretion of radiolabelled plasma-derived cholesterol was unaltered in *abca1*-/- mice as compared with their wild-type littermates.[38]

Instead, a recent review provides evidence to show that very-low-density lipoprotein (VLDL) remnants or further catabolic products of VLDL may serve as plasma donor particles delivering cholesterol for TICE.[35] In line, previous kinetic studies established a relatively high uptake rate of LDL cholesterol (LDL-C) into the intestine.[39] The LDL receptor (LDL-R) or one of the other receptors in the LDL-R family have been proposed as the basolateral transporters that mediate TICE.[35,40] However, experiments in *ldl-r*-/- mice failed to substantiate this concept.[25,35] Further studies are required to elucidate the intracellular itinerary of cholesterol destined for excretion via TICE.

The abcg5/g8 transporter, located at the brush border membrane of the small intestine, is likely to facilitate the last step of TICE. This is supported by several murine studies using various methodologies to quantify TICE.[31,41-43] In contrast, abcg5/g8 function was not found to affect TICE as measured in intestinal perfusion,[30] most likely reflecting methodological differences. Hence, TICE cannot be fully attributed to the activity of abcg5/g8, as a significant amount of TICE is still present in *abcg5* or *abcg8*-deficient

Figure 1. *Schematic representation of cholesterol fluxes in enterocytes related to the TICE pathway. The cholesterol pool of the intestinal cell is fuelled by uptake from the intestinal lumen via apically localised NPC1L1, endogenous synthesis, and via uptake of cholesterol from HDL and apoB-containing lipoproteins on the basolateral side (blood compartment). Apically, the main contributor to TICE is the ABCG5/G8 heterodimer; ABCB1a/b might also play a role as well as an additional route that has not yet been identified. On the basolateral side, the main cholesterol donors for TICE seem to be apoB-containing lipoproteins in a pathway that is likely to involve modulation of LDL-R expression. TICE can be increased by LXR activation as well as dietary plant sterols, partly dependent on functional ABCG5/G8 expression. The role of different intracellular pathways of cholesterol trafficking connecting basolateral uptake and apical secretion is currently unclear*



ABC = ATP-binding cassette; apoB = apolipoprotein B; HDL = high-density lipoprotein; LDL-R = low-density lipoprotein receptor; LXR = liver X receptor; NPC1L1 = Niemann-Pick C1 Like 1; PCSK9 = pro-protein convertase subtilisin kexin type 9; PS = plant sterols; TICE = transintestinal cholesterol excretion. (Adapted from: Tietge UW, Groen AK. Role the TICE?: Advancing the concept of transintestinal cholesterol excretion. Arterioscler Thromb Vasc Biol. 2013;33:1452-3).

mice. Other apically located proteins are likely to be involved. In fact, a recent report suggests that the abcb1a/b protein may serve as an apical excretory transporter in TICE.[40] Finally, it is plausible that acceptors in the intestinal lumen are required for cholesterol excretion via TICE. Bile acids and phospholipids in the intestinal lumen have been shown to stimulate the amount of cholesterol excreted via TICE.[30,32] The dependence on phospholipids is analogous to hepatic cholesterol secretion into the bile.[23] In the absence of these acceptors, only a small mass of TICE could be observed, which was probably attributable to shedding of enterocytes. Finally, the Niemann-Pick C2 (NPC2) protein was recently found to stimulate abcg5/g8-dependent biliary cholesterol secretion without affecting the abcg5/g8-independent pathway.[44,45] Although speculative, NPC2 might function as an acceptor for TICE mediated by abcg5/g8. Additional studies focusing on these acceptors might yield therapeutic interventions that would not require systemic distribution.

TRANS-INTESTINAL CHOLESTEROL EXCRETION: HUMAN STUDIES

The extent to which TICE contributes to faecal cholesterol elimination in humans remains to be established. Until recently, indications of the presence of TICE in human physiology were predominantly based on studies in patients with bile fistulae. In patients with complete biliary obstruction, a substantial portion of faecal sterols was found to be of non-dietary origin[46] and in another study in bile-diverted patients, the intestinal mucosa was found to secrete 250-400 mg of cholesterol per day.[47] A human intestinal perfusion study corroborated the presence of TICE, showing that approximately 44% of total FNS output originated from non-biliary origin.[48] These and a number of other reports[49-51] were mostly disregarded, likely pertaining to the small series of observations and the study limitations of the bile-diversion conditions. These include hampered cholesterol absorption and strongly upregulated cholesterol and bile acid synthesis. Furthermore, limitations of intestinal perfusion studies may have contributed, such as the absence of food, biliary and pancreatic components in the rinsed and perfused intestinal segments, together with the specific composition of the perfusate, which may have influenced the excretory capacity of enterocytes. Hence, studies on the non-biliary cholesterol excretion route were not pursued, until the more recent animal studies described above. Moreover, recent *in vitro* experiments using jejunal explants from humans showed activity of the TICE pathway for the first time in humans.[40] In these experiments, TICE depended on the presence of oxygen and was significantly decreased at low temperatures, which suggests that TICE is an active metabolic process.

Although the currently available human data collectively lend support to the presence of TICE in human cholesterol metabolism, a definite answer to this question has remained elusive. This is largely due to the technical challenges faced to reliably estimate this flux in humans *in vivo*, which requires simultaneous assessment of cholesterol absorption, biliary secretion and FNS excretion. We have recently attempted to quantify TICE in a population of mildly hypercholesterolaemic humans, by combining our previous experience from validated stable cholesterol isotope methodologies in mice[31] and humans.[15,52,53] Our unpublished data indicate that TICE is indeed present in human physiology and that it is sensitive to pharmacological stimulation, as described below.

TRANS-INTESTINAL CHOLESTEROL EXCRETION: FUTURE THERAPEUTIC POTENTIAL

The TICE pathway was found to be sensitive to various forms of dietary and pharmacological activation. However, at present, this is mostly confined to preclinical studies.

Liver-X-receptor agonists

Liver X nuclear receptors (LXRs) play a central role in cholesterol metabolism. Upon activation, LXRs induce expression of a series of genes that are involved in cholesterol efflux, absorption, transport and excretion.[54] Consistently, LXRs limit the development of atherosclerosis in mice and are therefore considered promising therapeutic targets for CVD risk reduction.[55] However, activation of LXRs concurrently promotes hepatic *de novo* lipogenesis, steatosis, and hypertriglyceridemia via direct activation of the sterol regulatory element-binding protein-1c (*SREBP-1c*) gene and fatty acid synthesis pathways.[56] LXR agonists were found to stimulate TICE up to sixfold in murine studies using different experimental methodologies.[23,30,31]

Recently, intestine-specific LXR agonists, which evade the unfavourable LXR-mediated effects on hepatic lipogenesis, have been developed. Studies indicate that intestine-specific activation of LXR, either genetic[57] or pharmacological,[58] is crucial for LXR-induced atheroprotection. Although it is tempting to suggest that TICE underlies parts of these favourable sequelae, a study which directly shows that TICE is stimulated by intestine-specific LXR agonists has not yet been reported. Finally, although promising in animal studies, the development of LXR-targeted drugs has largely been discontinued due to observations of marked increases in plasma apoB containing lipoproteins and/or a marked liver-steatotic response. To the best of our knowledge, there are no ongoing trials with intestine-specific LXR agonists. Hence, clinical studies evaluating their effects on TICE and atherosclerosis are not expected in the very near future.

Ezetimibe

Ezetimibe inhibits intestinal cholesterol absorption[59] in both mice and men, accomplished through inhibition of the Niemann-Pick C1 Like 1 (NPC1L1) transporter.[60] Despite a compensatory increase in endogenous cholesterol biosynthesis,[60] ezetimibe monotherapy lowers plasma LDL-C concentrations by approximately 15-20%.[59]

Ezetimibe has been shown to stimulate RCT from macrophages in mice, via as yet unidentified mechanisms.[61,62] Furthermore, when assessing cholesterol balance in ezetimibe-treated mice, the enhancement in FNS excretion cannot be attributed to cholesterol absorption inhibition or increased biliary cholesterol secretion alone.[41] In line, it has been suggested that ezetimibe might stimulate FNS excretion through stimulation of TICE,[63] although this was contradicted by another murine intestinal perfusion study.[64] Yet, our unpublished results of *in vivo* stable isotope studies in both mice and men showed a striking effect of ezetimibe on TICE [unpublished results, Jakulj, Stroes, Groen].

The underlying mechanisms by which ezetimibe might stimulate TICE are unknown. We speculated that the inhibition of NPC1L1 disturbs normal intracellular vesicle trafficking leading to increased transport of cholesterol to the apical membrane of the enterocytes.[41] Another possibility is that ezetimibe exerts its stimulatory effect on TICE by manipulation of the intraluminal bile acid and phospholipid content.[32,65,66]

Although our findings suggest an alternative mode by which ezetimibe might reduce plasma cholesterol concentrations and possibly reduce CVD risk, the latter issue is still precarious. Despite preclinical evidence that ezetimibe is atheroprotective,[67] to date, clinical studies have not been able to substantiate this: ezetimibe failed to regress carotid intima media thickness (cIMT) progression in patients with familial hypercholesterolaemia in the ENHANCE trial[68] and was found to be inferior to niacin in patients with coronary heart disease in the ARBITER-6 HALTS trial.[69] Next to major methodological disadvantages,[68,70] several off-target effects,[71] as well as upregulation of HMG-CoA reductase expression,[72] have been proposed as potential explanations. However, in the ARBITER-6 HALTS study, ezetimibe did hamper cIMT progression in statin-treated patients with fairly low LDL-C concentrations, who would thereby not likely to be considered for ezetimibe add-on therapy.[69] Furthermore, not all cIMT trials investigating ezetimibe have been negative.[73] A large clinical study of 18,000 patients, the IMPROVE-IT trial, is underway to determine whether additional cholesterol lowering by ezetimibe on top of statins can be translated into a reduction in cardiovascular event rate.[74] Although this trial started in 2005 and the results were expected in 2011, outcomes are still awaited, supposedly due to recruitment of additional patients after an unfavourable interim analysis. This trial is conducted in patients who have suffered from an acute coronary syndrome and who expressed low LDL-C concentrations at baseline, as inclusion of patients with higher LDL-C concentrations would not have achieved guideline-recommended LDL-C concentrations under the trial protocol, which would have been ethically unacceptable. Hence, it is conceivable that no additional benefit can be gained in this population, if ezetimibe's effect on atherosclerosis is causally related to plasma LDL-C reductions alone. Release of the study outcomes has been postponed until September 2014 (ClinicalTrials.gov:NCT00202878).

PCSK9

Proprotein convertase subtilisin kexin type 9 (PCSK9) is a secreted protein that reduces the amount of LDL-R at the cell surface of primarily the liver. PCSK9 circulates in the blood and binds the extracellular domain of the LDL-R to produce post-translational down-regulation of this receptor in lysosomes.[75] Loss-of-function mutations in the *PCSK9* gene result in 15-18% reductions in plasma LDL-C concentrations and carriers of these mutations express a 47-88% reduction in CVD risk.[76]

Next to the liver, PCSK9 is abundantly expressed in the intestine and it has been shown that PCSK9 modulates cholesterol transport and metabolism, as well as production of apoB-containing lipoproteins, in intestinal cells.[77] A recent report by the same research group revealed that PCSK9 is a repressor of TICE and that acute repression depended on a functional LDL-R.[40]

These findings may be of clinical importance, as Phase I and II PCSK9-inhibiting treatment modalities, such as single-stranded antisense DNA-like oligonucleotides or double-stranded small interference RNA, have shown promising results in terms of LDL-C lowering.[75] Phase III trials with longer duration and larger patient populations are currently underway, which should also establish whether PCSK9 inhibition reduces cardiovascular event rate in humans.

Plant sterols

Plant sterols are not endogenously synthesised by humans, but are strictly derived from the diet. They perform functions in plant cells similar to those of cholesterol in mammalian cells. Campesterol and sitosterol are the most abundant ones. They share a high degree of structural similarity with cholesterol, but are much more hydrophobic. Plant sterols are present in small amounts of fruits, vegetables, nuts, seeds and edible oils; marketed sources are primarily derived from soybean and pine tree oil. Total dietary plant sterol consumption in the average Western diet is 150-350 mg per day.[78,79] Daily consumption of 2g of plant sterols is associated with LDL-C reductions varying from 4-15% in hypercholesterolaemic or normocholesterolaemic adults.[80]

Plant sterols are thought to displace cholesterol from incorporation into micelles, thereby limiting cholesterol absorption in the intestinal lumen by approximately 25-36%.[81] However, additional cholesterol-lowering mechanisms have been postulated.[82] Interestingly, the cholesterol-lowering effects of plant sterol consumption were recently ascribed to stimulation of intestinal cholesterol excretion via TICE, as plant sterol feeding resulted in a sixfold induction of TICE in wild-type mice.[43] This is supported by a recent crossover plant sterol feeding trial in 18 adults, who in random order consumed dietary plant sterols from negligible (0 mg) to high (2g) amounts, resulting in a dose-dependent increase in FNS output, which could not be explained by the corresponding reductions in measured cholesterol absorption.[83]

The mechanisms by which plant sterols could stimulate TICE are currently unknown. Several mechanisms have been suggested, including both LXR-dependent and -independent mechanisms.[82] It is less likely that the stimulation of TICE is LXR-mediated, as plant sterols did not alter LXR target genes in the study by Brufau *et al.*[43] and studies investigating plant sterols as possible ligands of LXR have been conflicting.[21,84,85] A possible LXR-independent mechanism might include interference of plant sterols with cholesterol trafficking within the enterocyte, as plant sterols have been shown to affect expression of genes encoding proteins of the annexin family, which are involved in the regulation of membrane properties.[86] Besides studies aiming to unravel the underlying molecular mechanisms, human studies to assess the effect of plant sterols on TICE are also lacking at present.

CONCLUSIONS

In conclusion, trans-intestinal cholesterol excretion might serve as an attractive future target for LDL-C lowering and CVD reduction, provided underlying molecular mechanisms are elucidated. Although promising, the therapeutic potential of targeting the TICE pathway is to date confined to preclinical studies and it is unknown whether pharmacological targeting of the TICE pathway will also yield a clinical benefit. Available interventions that have been shown to stimulate TICE and may therefore warrant further clinical evaluation include ezetimibe, PCSK9-inhibitors and plant sterols.

ACKNOWLEDGEMENTS

Dr Stroes has received lecture fees from Novartis, Merck, Roche, MSD, Genzyme and Boehringer Ingelheim.

REFERENCES

1. Baigent C, Blackwell L, Emberson J, et al. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet. 2010;376:1670-81.
2. Wright RS. Recent clinical trials evaluating benefit of drug therapy for modification of HDL cholesterol. Cur Opin Cardiol. 2013;28:389-98.
3. Bosch N, Frishman WH. Newer Therapeutic Strategies to Alter HDL Level and Function. Cardiol Rev. 2013 May 23.
4. Gordon DJ, Probstfield JL, Garrison RJ, et al. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. Circulation. 1989;79:8-15.

5. Assmann G, Schulte H, von Eckardstein A, Huang Y. High-density lipoprotein cholesterol as a predictor of coronary heart disease risk. The PROCAM experience and pathophysiological implications for reverse cholesterol transport. Atherosclerosis. 1996;124(Suppl):S11-20.

6. deGoma EM, DeGoma RL, Rader DJ. Beyond high-density lipoprotein cholesterol levels evaluating high-density lipoprotein function as influenced by novel therapeutic approaches. J Am Coll Cardiol. 2008;51:2199-211.

7. Barter PJ, Caulfield M, Eriksson M, et al. Effects of torcetrapib in patients at high risk for coronary events. New Engl J Med. 2007;357:2109-22.

8. Briel M, Ferreira-Gonzalez I, You JJ, et al. Association between change in high density lipoprotein cholesterol and cardiovascular disease morbidity and mortality: systematic review and meta-regression analysis. BMJ. 2009;338:b92.

9. Boden WE, Probstfield JL, Anderson T, et al. Niacin in Patients with Low HDL Cholesterol Levels Receiving Intensive Statin Therapy. New Engl J Med. 2011;365:2255-67.

10. Schwartz GG, Olsson AG, Abt M, et al. Effects of dalcetrapib in patients with a recent acute coronary syndrome. New Engl J Med. 2012;367:2089-99.

11. Glomset JA. The plasma lecithins:cholesterol acyltransferase reaction. J Lipid Res. 1968;9:155-67.

12. Groen AK, Bloks VW, Bandsma RH, Ottenhoff R, Chimini G, Kuipers F. Hepatobiliary cholesterol transport is not impaired in Abca1-null mice lacking HDL. J Clin Invest. 2001;108:843-50.

13. Jolley CD, Woollett LA, Turley SD, Dietschy JM. Centripetal cholesterol flux to the liver is dictated by events in the peripheral organs and not by the plasma high density lipoprotein or apolipoprotein A-I concentration. J Lipid Res. 1998;39:2143-9.

14. Xie C, Turley SD, Dietschy JM. ABCA1 plays no role in the centripetal movement of cholesterol from peripheral tissues to the liver and intestine in the mouse. J Lipid Res. 2009;50:1316-29.

15. Harchaoui K El, Franssen R, Hovingh GK, et al. Reduced fecal sterol excretion in subjects with familial hypoalphalipoproteinemia. Atherosclerosis. 2009;207:614-6.

16. Eriksson M, Carlson LA, Miettinen TA, Angelin B. Stimulation of fecal steroid excretion after infusion of recombinant proapolipoprotein A-I. Potential reverse cholesterol transport in humans. Circulation. 1999;100:594-8.

17. Nanjee MN, Cooke CJ, Garvin R, et al. Intravenous apoA-I/lecithin discs increase pre-beta-HDL concentration in tissue fluid and stimulate reverse cholesterol transport in humans. J Lipid Res. 2001;42:1586-93.

18. Holleboom AG, Jakulj L, Franssen R, et al. In vivo tissue cholesterol efflux is reduced in carriers of a mutation in APOA1. J Lipid Res. 2013;54:1964-71.

19. Berge KE, Tian H, Graf GA, et al. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science. 2000;290:1771-5.

20. Yu L, Hammer RE, Li-Hawkins J, et al. Disruption of Abcg5 and Abcg8 in mice reveals their crucial role in biliary cholesterol secretion. Proc Natl Acad Sci U S A. 2002;99:16237-42.

21. Plõsch T, Kok T, Bloks VW, et al. Increased hepatobiliary and fecal cholesterol excretion upon activation of the liver X receptor is independent of ABCA1. J Biol Chem. 2002;277:33870-7.

22. Temel RE, Tang W, Ma Y, et al. Hepatic Niemann-Pick C1-like 1 regulates biliary cholesterol concentration and is a target of ezetimibe. J Clin Invest. 2007;117:1968-78.

23. Kruit JK, Plösch T, Havinga R, et al. Increased fecal neutral sterol loss upon liver X receptor activation is independent of biliary sterol secretion in mice. Gastroenterology. 2005;128:147-56.

24. Schwarz M, Russell DW, Dietschy JM, Turley SD. Marked reduction in bile acid synthesis in cholesterol 7alpha-hydroxylase-deficient mice does not lead to diminished tissue cholesterol turnover or to hypercholesterolemia. J Lipid Res. 1998;39:1833-43.

25. Brown JM, Bell TA, Alger HM, et al. Targeted depletion of hepatic ACAT2-driven cholesterol esterification reveals a non-biliary route for fecal neutral sterol loss. J Biol Chem. 2008;283:10522-34.

26. Sperry W. Lipid Excretion IV. A study of the relationship of the bile to the fecal lipids with special reference to certain problems of sterol metabolism. J Biol Chem. 1927;71:351-78.

27. Pertsemlidis D, Kirchman EH, Ahrens EH. Regulation of cholesterol metabolism in the dog. I. Effects of complete bile diversion and of cholesterol feeding on absorption, synthesis, accumulation, and excretion rates measured during life. J Clin Invest. 1973;52:2353-67.

28. Dietschy JM, Siperstein MD. Cholesterol synthesis by the gastrointestinal tract: localization and mechanisms of control. J Clin Invest. 1965;44:1311-27.

29. Bandsma RH, Stellaard F, Vonk RJ, et al. Contribution of newly synthesized cholesterol to rat plasma and bile determined by mass isotopomer distribution analysis: bile-salt flux promotes secretion of newly synthesized cholesterol into bile. Biochem J. 1998;329(Pt 3):699-703.

30. Van der Velde AE, Vrins CLJ, van den Oever K, et al. Direct intestinal cholesterol secretion contributes significantly to total fecal neutral sterol excretion in mice. Gastroenterology. 2007;133:967-75.

31. Van der Veen JN, van Dijk TH, Vrins CLJ, et al. Activation of the liver X receptor stimulates trans-intestinal excretion of plasma cholesterol. J Biol Chem. 2009;284:19211-9.

32. Van der Velde AE, Vrins CLJ, van den Oever K, et al. Regulation of direct transintestinal cholesterol excretion in mice. Am J Physiol Gastrointest Liver Physiol. 2008;295:G203-G208.

33. Temel RE, Sawyer JK, Yu L, et al. Biliary sterol secretion is not required for macrophage reverse cholesterol transport. Cell Metab. 2010;12:96-102.

34. Nijstad N, Gautier T, Briand F, Rader DJ, Tietge UJF. Biliary sterol secretion is required for functional in vivo reverse cholesterol transport in mice. Gastroenterology. 2011;140:1043-51.

35. Temel RE, Brown JM. Biliary and nonbiliary contributions to reverse cholesterol transport. Curr Opin Lipidol. 2012;23:85-90.

36. Brufau G, Groen AK, Kuipers F. Reverse cholesterol transport revisited: contribution of biliary versus intestinal cholesterol excretion. Arterioscler Thromb Vasc Biol. 2011;31:1726-33.

37. Van der Velde AE, Brufau G, Groen AK. Transintestinal cholesterol efflux. Curr Opin Lipidol. 2010;21:167-71.

38. Vrins CLJ, Ottenhoff R, van den Oever K, et al. Trans-intestinal cholesterol efflux is not mediated through high density lipoprotein. J Lipid Res. 2012;53:2017-23.

39. Stange EF, Dietschy JM. Cholesterol synthesis and low density lipoprotein uptake are regulated independently in rat small intestinal epithelium. Proc Natl Acad Sci U S A. 1983;80:5739-43.

40. Le May C, Berger JM, Lespine A, et al. Transintestinal cholesterol excretion is an active metabolic process modulated by PCSK9 and statin involving ABCB1. Arterioscler Thromb Vasc Biol. 2013;33:1484-93.

41. Jakulj L, Vissers MN, van Roomen CP, et al. Ezetimibe stimulates faecal neutral sterol excretion depending on abcg8 function in mice. FEBS Lett. 2010;584:3625-8.

42. Yu L, York J, von Bergmann K, Lutjohann D, Cohen JC, Hobbs HH. Stimulation of cholesterol excretion by the liver X receptor agonist requires ATP-binding cassette transporters G5 and G8. J Biol Chem. 2003;278:15565-70.

43. Brufau G, Kuipers F, Lin Y, Trautwein EA, Groen AK. A reappraisal of the mechanism by which plant sterols promote neutral sterol loss in mice. PloS One. 2011;6:e21576.

44. Yamanashi Y, Takada T, Shoda J-I, Suzuki H. Novel function of Niemann-Pick C1-like 1 as a negative regulator of Niemann-Pick C2 protein. Hepatology. 2012;55:953-64.

45. Yamanashi Y, Takada T, Yoshikado T, Shoda J-I, Suzuki H. NPC2 regulates biliary cholesterol secretion via stimulation of ABCG5/G8-mediated cholesterol transport. Gastroenterology. 2011;140:1664-74.

46. Stanley M, Pineda E, Cheng S. Serum cholesterol esters and intestinal cholesterol secretion and absorption in obstructive jaundice due to cancer. New Engl J Med. 1959;261:368-73.

47. Cheng S, Stanley M. Secretion of cholesterol by intestinal mucosa in patients with complete common bile duct obstruction. Proc Soc Exp Biol Med. 1959;101:223-5.

48. Simmonds WJ, Hofmann AF, Theodor E. Absorption of cholesterol from a micellar solution: intestinal perfusion studies in man. J Clin Invest. 1967;46:874-90.

49. Deckelbaum RJ, Lees RS, Small DM, Hedberg SE, Grundy SM. Failure of complete bile diversion and oral bile acid therapy in the treatment of homozygous familial hypercholesterolemia. New Engl J Med. 1977;296:465-70.

50. Rosenfeld R, Hellman L. The relation of plasma and biliary cholesterol to bile acid synthesis in man. J Clin Invest. 1959;38:1334-8.

51. Hellman L, Rosenfeld R, Eidinoff M, et al. Isotopic studies of plasma cholesterol of endogenous and exogenous origins. J Clin Invest. 1955;34:48-60.

52. Stellaard F, Sackmann M, Sauerbruch T, Paumgartner G. Simultaneous determination of cholic acid and chenodeoxycholic acid pool sizes and fractional turnover rates in human serum using 13C-labeled bile acids. J Lipid Res. 1984;25:1313-9.

53. Jakulj L, Mohammed H, van Dijk TH, et al. Plasma plant sterols serve as poor markers of cholesterol absorption in man. J Lipid Res. 2013;54:1144-50.

54. Im S-S, Osborne TF. Liver x receptors in atherosclerosis and inflammation. Circ Res. 2011;108:996-1001.

55. Calkin AC, Tontonoz P. Liver x receptor signaling pathways and atherosclerosis. Arterioscler Thromb Vasc Biol. 2010;30:1513-8.

56. Bonamassa B, Moschetta A. Atherosclerosis: lessons from LXR and the intestine. Trends Endocrinol Metab. 2013;24:120-8.

57. Lo Sasso G, Murzilli S, Salvatore L, et al. Intestinal specific LXR activation stimulates reverse cholesterol transport and protects from atherosclerosis. Cell Metab. 2010;12:187-93.

58. Yasuda T, Grillot D, Billheimer JT, et al. Tissue-specific liver X receptor activation promotes macrophage reverse cholesterol transport in vivo. Arterioscler Thromb Vasc Biol. 2010;30:781-6.

59. Sudhop T, Lütjohann D, Kodal A, et al. Inhibition of intestinal cholesterol absorption by ezetimibe in humans. Circulation. 2002;106:1943-8.

60. Davis HR, Zhu L-J, Hoos LM, et al. Niemann-Pick C1 Like 1 (NPC1L1) is the intestinal phytosterol and cholesterol transporter and a key modulator of whole-body cholesterol homeostasis. J Biol Chem. 2004;279:33586-92.

61. Sehayek E, Hazen SL. Cholesterol absorption from the intestine is a major determinant of reverse cholesterol transport from peripheral tissue macrophages. Arterioscler Thromb Vasc Biol. 2008;28:1296-7.

62. Briand F, Naik SU, Fuki I, et al. Both the peroxisome proliferator-activated receptor delta agonist, GW0742, and ezetimibe promote reverse cholesterol transport in mice by reducing intestinal reabsorption of HDL-derived cholesterol. Clin Transl Sci. 2009;2:127-33.

63. Tang W, Ma Y, Jia L, Ioannou YA, Davies JP, Yu L. Genetic inactivation of NPC1L1 protects against sitosterolemia in mice lacking ABCG5/ABCG8. J Lipid Res. 2009;50:293-300.

64. Vrins CLJ, van der Velde AE, van den Oever K, et al. Peroxisome proliferator-activated receptor delta activation leads to increased transintestinal cholesterol efflux. J Lipid Res. 2009;50:2046-54.

65. Wang HH, Portincasa P, Mendez-Sanchez N, Uribe M, Wang DQ-H. Effect of ezetimibe on the prevention and dissolution of cholesterol gallstones. Gastroenterology. 2008;134:2101-10.

66. Zúñiga S, Molina H, Azocar L, et al. Ezetimibe prevents cholesterol gallstone formation in mice. Liver Int. 2008;28:935-47.

67. Davis HR, Lowe RS, Neff DR. Effects of ezetimibe on atherosclerosis in preclinical models. Atherosclerosis. 2011;215:266-78.

68. Kastelein JJP, Akdim F, Stroes ESG, et al. Simvastatin with or without ezetimibe in familial hypercholesterolemia. New Engl J Med. 2008;358:1431-43.

69. Taylor AJ, Villines TC, Stanek EJ, et al. Extended-release niacin or ezetimibe and carotid intima-media thickness. New Engl J Med. 2009;361:2113-22.

70. Bogiatzi C, Spence JD. Ezetimibe and regression of carotid atherosclerosis: importance of measuring plaque burden. Stroke. 2012;43:1153-5.

71. Villines TC, Stanek EJ, Devine PJ, et al. The ARBITER 6-HALTS Trial (Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol 6-HDL and LDL Treatment Strategies in Atherosclerosis): final results and the impact of medication adherence, dose, and treatment duration. J Am Coll Cardiol. 2010;55:2721-6.

72. Engelking LJ, McFarlane MR, Li CK, Liang G. Blockade of cholesterol absorption by ezetimibe reveals a complex homeostatic network in enterocytes. J Lipid Res. 2012;53:1359-68.

73. Descamps OS, De Sutter J, Guillaume M, Missault L. Where does the interplay between cholesterol absorption and synthesis in the context of statin and/or ezetimibe treatment stand today? Atherosclerosis. 2011;217:308-21.

74. Califf RM, Lokhnygina Y, Cannon CP, et al. An update on the IMProved reduction of outcomes: Vytorin Efficacy International Trial (IMPROVE-IT) design. Am Heart J. 2010;159:705-9.

75. Petrides F, Shearston K, Chatelais M, Guilbaud F, Meilhac O, Lambert G. The promises of PCSK9 inhibition. Curr Opin Lipidol. 2013;24:307-12.

76. Cohen JC, Boerwinkle E, Mosley TH, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. New Engl J Med. 2006;354:1264-72.

77. Levy E, Ben Djoudi Ouadda A, Spahis S, et al. PCSK9 plays a significant role in cholesterol homeostasis and lipid transport in intestinal epithelial cells. Atherosclerosis. 2013;227:297-306.

78. Salen G, Ahrens EH, Grundy SM. Metabolism of beta-sitosterol in man. J Clin Invest. 1970;49:952-67.

79. O'Neill FH, Sanders TAB, Thompson GR. Comparison of efficacy of plant stanol ester and sterol ester: short-term and longer-term studies. Am J Cardiol. 2005;96:29D-36D.

80. McKenney JM, Davidson MH, Jacobson TA, Guyton JR. Final conclusions and recommendations of the National Lipid Association Statin Safety Assessment Task Force. Am J Cardiol. 2006;97:89C-94C.

81. Jones PJ, Raeini-Sarjaz M, Ntanios FY, Vanstone CA, Feng JY, Parsons WE. Modulation of plasma lipid levels and cholesterol kinetics by phytosterol versus phytostanol esters. J Lipid Res. 2000;41:697-705.

82. Calpe-Berdiel L, Escolà-Gil JC, Blanco-Vaca F. New insights into the molecular actions of plant sterols and stanols in cholesterol metabolism. Atherosclerosis. 2009;203:18-31.

83. Racette SB, Lin X, Lefevre M, et al. Dose effects of dietary phytosterols on cholesterol metabolism: a controlled feeding study. Am J Clin Nutr. 2010;91:32-8.

84. Kaneko E, Matsuda M, Yamada Y, Tachibana Y, Shimomura I, Makishima M. Induction of intestinal ATP-binding cassette transporters by a phytosterol-derived liver X receptor agonist. J Biol Chem. 2003;278:36091-8.

85. Plat J, Mensink RP. Increased intestinal ABCA1 expression contributes to the decrease in cholesterol absorption after plant stanol consumption. FASEB J. 2002;16:1248-53.

86. Calpe-Berdiel L, Escolà-Gil JC, Julve J, Zapico-Muñiz E, Canals F, Blanco-Vaca F. Differential intestinal mucosal protein expression in hypercholesterolemic mice fed a phytosterol-enriched diet. Proteomics. 2007;7:2659-66.

Netherlands
The Journal of Medicine

REVIEW

Monitoring of unfractionated heparin in critically ill patients

R. Aarab[1*], J. van Es[1], A.C.J.M. de Pont[2], M.B. Vroom[2], S. Middeldorp[1]

Department of [1]Vascular Medicine and [2]Intensive Care, Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands, *corresponding author: tel.: +31 (0)20 5665976, fax: +31 (0)20 6968833, e-mail: r.aarab@amc.uva.nl

ABSTRACT

Background: In critically ill patients, dosing of unfractionated heparin (UFH) is difficult due to unpredictable pharmacokinetics, which has an impact on the time to reach therapeutic anticoagulation. We evaluated the quality of UFH therapy in critically ill patients in terms of activated partial thromboplastin time (APTT) test values and time to therapeutic range.

Methods: Patients admitted to the Intensive Care Unit (ICU) and Medium Care Unit (MCU) were screened for intravenous UFH administration. Time to therapeutic range was categorised into 0-12, 13-24 and >24 hours. APTT results were classified into categories of subtherapeutic, supratherapeutic and therapeutic tests. We identified to what extent the sub- and supratherapeutic values were aberrant of the limit of the therapeutic range (<5%, 5-15% and >15%).

Results: In 101 patients admitted to the ICU and MCU, time to therapeutic range was 24 hours in 56% of the population, whereas in 10% of the patients no therapeutic APTT was achieved during UFH treatment. Among the APTT levels, 29% of all test results measured in 24 hours were within the therapeutic range. Subtherapeutic values were found in 53% of the test results, of which 160/203 were more than 15% under the lower limit, whereas 18% of the test results were supratherapeutic, of which 40/69 more than 15% above the upper limit.

Conclusion: In this cohort of critically ill patients, therapeutic APTT values were reached within 24 hours in 56% of the patients. We conclude that intravenous UFH therapy can be improved in critically ill patients.

KEYWORDS

Activated partial thromboplastin time, unfractionated heparin, venous thromboembolism

INTRODUCTION

Anticoagulants are the cornerstone of treatment and secondary prevention of arterial and venous thromboembolism (VTE). Heparin was one of the first anticoagulants and is to date still extensively used for indications other than VTE, such as acute coronary syndrome (ACS), continuous dialysis techniques and various surgical procedures. Heparin catalyses the ability of the plasma protein antithrombin to inhibit the activity of thrombin, factor Xa, and factor IXa about 1000-fold.[1] There are two types of heparin with distinguishable pharmacological profiles that determine their use in clinical practice. Unfractionated heparin (UFH) can be administered by continuous intravenous infusion or – less commonly – by subcutaneous injection. The pharmacokinetics of UFH differ highly between individuals as well as within individuals over time, due to binding to various plasma proteins.[2] To establish a safe and optimal anticoagulant effect within a defined therapeutic range, close monitoring of UFH therapy is crucial. The activated partial thromboplastin time (APTT) is generally used for this purpose and its levels should be maintained between a predefined interval. An APTT level ranging from 1.5-2.5 times the baseline value is considered to be the optimal therapeutic range and has gained wide clinical acceptance. Low-molecular-weight heparin (LMWH) is a derivate of UFH, but has different and more favourable pharmacokinetics. Its superiority over UFH is documented by several trials for the treatment of VTE as well as for patients across the ACS spectrum in terms of fewer recurrent thrombotic events and myocardial infarction, respectively.[3-5] However, the magnitude of clinical benefit from LMWH is lower than estimated, as documented by cumulative evidence of 14 systematic reviews.[6] Twelve other studies involving 4971 patients treated with LMWH have shown an increased risk of major bleeding for those with a creatinine clearance <30 ml/min (OR 2.25, 95% CI

1.19-4.27).[7] Because patients with severe obesity or severe renal failure are often excluded from clinical trials, there are less data available for these populations. Consequently, because of the lack of evidence, UFH rather than LMWH is still often preferred in patients with an increased risk of bleeding, renal impairment or extreme obesity.

Too high and too low anticoagulant effects can be a serious threat in terms of either haemorrhage or ongoing thrombosis, respectively. Because of their severity of disease and altered metabolism, critically ill patients are particularly vulnerable for complications. The risk of overdosing in such cases may be intuitively lower with UFH compared with LMWH, and there is a perceived superior possibility to immediately reverse the anticoagulant effect.

In this pilot study we hypothesised that a stable and therapeutic APTT can be achieved within 24 hours with intravenous UFH in patients admitted to the Intensive Care Unit (ICU) and Medium Care Unit (MCU). An assessment of the quality of UFH treatment is made in terms of number of subtherapeutic, supratherapeutic and therapeutic APTT values.

MATERIAL AND METHODS

Study design and population

This is a retrospective cohort study involving data from the electronic medical records of patients admitted to the ICU and MCU. Due to the feasibility of data collection and analysis within the time frame that was allowed for this project, we aimed to include 100 patients.

We identified patients by performing a query in the laboratory database of patients in whom an APTT test was performed between January 2010 and December 2010. Inclusion was based on documentation of intravenous UFH therapy in the electronic general chart database (AZD). Further identification and exclusion went through the ICU patient data management system. As listed in the flowchart, exclusion criteria were non-therapeutic indications for UFH therapy, patients <18 years, UFH infusion for less than 24 hours or an interruption of infusion for 36 hours or more.

Data collection

We collected data from the electronic medical records including age, gender, weight and indication for anticoagulant therapy with UFH. Deep venous thrombosis (DVT) and pulmonary embolism (PE) were considered to be different manifestations of the same condition – venous thromboembolism (VTE) – and all other thrombotic events, such as sinus or abdominal thrombosis in either arterial or venous vessels were defined as 'other thrombotic events'. Patients receiving continuous veno-venous haemofiltration (CVVH) were reported.

For the current analysis, data are truncated at intervals of 0-12, 13-24 and >24 hours. The APTT results were classified into categories of subtherapeutic, supratherapeutic and therapeutic tests based on the individual therapeutic range. Furthermore, we identified to what extent the sub- and supratherapeutic values were aberrant of the lower or upper limit of the therapeutic range, respectively (<5%, 5-15% and >15%). The therapeutic APTT range in seconds was defined as set in the medical record by the treating physician before the start of infusion. When no individual therapeutic range was documented, we considered an APTT range of 1.5-2.0 times the upper normal value of 30 seconds as therapeutic (according to the local protocol of the AMC, Amsterdam, the Netherlands).

We also measured the number of patients in whom anticoagulation had to be discontinued, as well as the number of dose adjustments. We used the 'Dose adjustments/non-Therapeutic Test ratio' (DnTT ratio), defined as the number of dose adjustments in proportion to the number of non-therapeutic APTT levels during time of UFH infusion. The results are presented in the categories <1 and ≥1.

Finally, information on events such as (recurrent) thromboembolism and bleeding during UFH administration were collected. A bleeding event was defined as clinically overt bleeding in a critical location (intracranial, retroperitoneal, or pericardial), or bleeding leading to a decrease in haemoglobin level of 1 mmol/l or more, or leading to transfusion of two or more units of packed red blood cells.

Dosing regimen

The treating physician was allowed to determine the dosing regimen based on patient characteristics and clinical presentation. The target APTT level for each patient had to be set and documented in the electronic record. After an initial standard dose at a rate of 1000 units/hour, the treating physician had to evaluate whether a bolus of 1000-5000 units was indicated. Consensus was to measure the APTT four hours after the start of infusion; monitoring tests had to be performed at a six-hour interval, even when the APTT test value was within the therapeutic range.

Outcomes

The primary outcome was the time to achieve a therapeutic APTT level per patient (<12, <24 or >24 hours). The main secondary outcome was the percentage of subtherapeutic, supratherapeutic and therapeutic range of all APTT tests performed after UFH administration in time ranges as mentioned.

RESULTS

A total of 1906 patients in whom an APTT test was performed were screened for intravenous UFH administration. A total of 249 patients were eligible for the current analysis of whom 101 consecutive patients were included. The mean age was 66 years (range 23-88) and 40% were female. Main indications for UFH therapy were VTE (24%) and presence of a mechanical heart valve (23%). Clinical characteristics are listed in *table 1*.

The time to reach at least one therapeutic APTT test result is shown in *figure 1*. Overall, 56% of the patients achieved a therapeutic APTT within 24 hours and 24% were therapeutic in both 0-12 and 13-24 hours.

Table 1. *Baseline patient characteristics of the study population (n=101)*

Characteristic	
Age in years, median (range)	69 (23-88)
Female (%)	40
Weight in kg, median (range)	80 (35-120)
Receiving CVVH (%)	24
Indication for anticoagulant therapy, %	
VTE (DVT and/or PE)	24
Other locations thrombotic event (i.e. sinus/ abdominal)	11
Mechanical heart valve	23
Atrial fibrillation	16
ACS or cardiac ischaemia	13
IABP or Impella	5
Cardiac surgery	4
Other	5
Time of UFH infusion in hours, median (range)	86 (24-695)

kg= kilogram; CVVH= continuous veno-venous haemofiltration; VTE=venous thromboembolism; DVT = deep venous thrombosis; PE= pulmonary embolism; ACS=acute coronary syndrome; IABP=intra-aortic balloon pump.

Other outcomes are listed in *table 2*. Within 12 hours of UFH infusion, 20% of the APTT measurements were within the therapeutic range. Additionally, 58% and 22% consisted of sub- and supratherapeutic test results, respectively. Of the subtherapeutic APTT levels, 105/121 were more than 15% below the lower limit of the target;

Table 2. *Primary and secondary outcomes concerning (non) therapeutic APTT level*

Outcome	0-12 h	0-24 h	>24 h
Number of APTT tests performed after start of UFH infusion per patient, median (range)	2.0 (1-5)	4.0 (2-7)	9.0 (0-111)
Tests within time range, n (%)			
Total subtherapeutic	**121 (58)**	**203 (53)**	551(32)
Of which aberrant from the lower limit, n (%)			
≤5%	9 (7)	16 (8)	
5-15%	7 (6)	27 (13)	
>15%	105 (87)	160 (79)	
Therapeutic	**41 (20)**	**110 (29)**	
Total supratherapeutic	**45 (22)**	**69 (18)**	
Of which aberrant from the upper limit, n (%)			
≤5%	6 (13)	12 (17)	1008 (58)
5-15%	7 (16)	17 (25)	170 (10)
>15%	32 (71)	40 (58)	
Dose adjustments during time of UFH infusion, median (range)	**5 (0-34)**		
DnTT ratio <1, %	18		
DnTT ratio ≥1, %	78		
No dose adjustments, %	4		

APTT= activated partial thromboplastin time; UFH= unfractionated heparin; h=hours; n = number; DnTT= dose adjustments/non-therapeutic test ratio.

Figure 1. *Flowchart of study population*

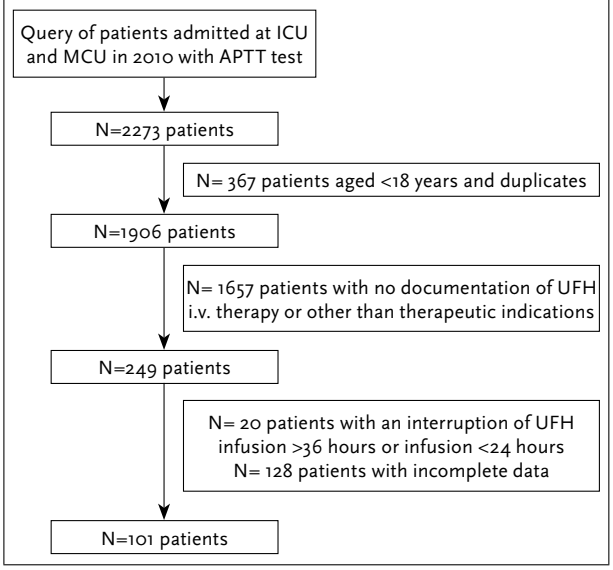
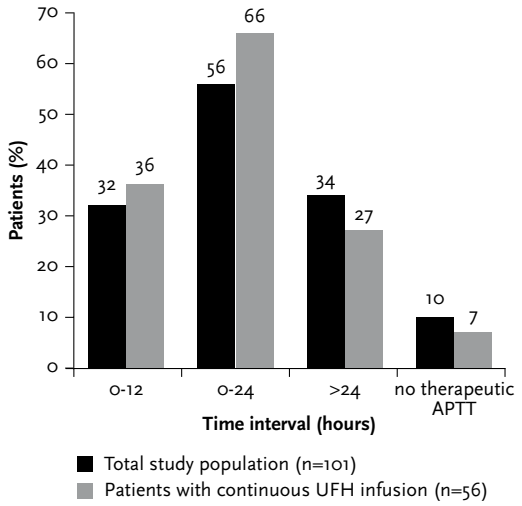


Figure 2. *Time to therapeutic range*



of the supratherapeutic levels, 32/45 were more than 15% above the upper limit of the target. Among the APTT levels measured at 24 hours, a therapeutic range was observed in 29% of the test results. Subtherapeutic values were found in 53% of the test results, of which 160/203 were more than 15% under the lower limit, whereas 18% of the test results were supratherapeutic, of which 40/69 more than 15% above the upper limit.

During the course of UFH therapy, ten patients had a bleeding event and six patients experienced a thrombotic event. The DnTT ratio was ≥1 in 78% of the study population.

In order to perform a medical procedure, UFH infusion was electively stopped in 44% of the cases. *Figure 1* shows that in the subpopulation (n=56) in which infusion remained continuous, 66% of the patients were therapeutically anticoagulated in 24 hours; the subtherapeutic, therapeutic and supratherapeutic rates were 51%, 33% and 15% respectively.

DISCUSSION

This cohort study demonstrates that among patients admitted to the ICU and MCU, achieving therapeutic heparinisation with UFH is challenging. Based on the time to therapeutic range being 24 hours for 56% of the study population and high percentages of subtherapeutic APTT results, we would expect a higher number of recurrent thrombotic events instead of bleeding. We identified ten patients in total in whom a bleeding event occurred; 4/10 patients had at least one supratherapeutic APTT level in 24 hours, and a small minority (12%) of all performed APTT tests were above the therapeutic range. We assume that other factors contributed to the bleeding events, independent of UFH therapy. Although an association between the incidence of bleeding and APTT values has been reported by subgroup analysis of large randomised trials, other investigators could not confirm similar results.[8-12]

However, there is evidence that failure to achieve therapeutic values within the first 24 hours is predictive of future recurrent VTE.[13] Research on patients with acute PE has also shown that reaching therapeutic anticoagulation within 24 hours lowers 30-day mortality and in-hospital mortality, which highlights the importance of rapid therapeutic anticoagulation.[14] To what extent rapid anticoagulation is achieved differs among trials which demonstrate therapeutic rates as low as 22%, while subanalysis of several studies found a significantly larger proportion of the patients reaching a therapeutic APTT level within 24 hours.[15-19] Whether these results apply for critically ill patients is uncertain, since these trials are not carried out in that specific patient category. We observed that in 56% of our study population, time to therapeutic range was 24 hours. It should be mentioned that the low therapeutic rates in our cohort could be due to the high percentage of patients who had a discontinuation of UFH infusion. We could not confirm whether an APTT was measured when the infusion was stopped, thus making the measured APTT not representative for UFH therapy. A subanalysis was therefore conducted which showed that in the subpopulation (n=56) treated with continuous UFH infusion, 66% achieved a therapeutic APTT within 24 hours, against 56% of the whole population (n=101). In terms of APTT test results in 24 hours, we noticed a small increase in therapeutic rates from 29% in the whole population to 33% in the subpopulation (n=56). Therefore, we conclude that APTT tests performed during discontinuation of UFH infusion are only to a small extent responsible for the low therapeutic rates in our cohort.

Conflicting observations between studies may have arisen from differences in protocols (e.g. fixed-dose versus weight-based regimen) or using different analytical techniques for determining UFH. Which approach concerning UFH therapy is superior is still inconclusive. For instance, there is considerable evidence that using weight combined with height and gender is more efficient for dosing UFH than weight alone.[15,20] But not all weight-based nomograms are consistent and they require a considerable amount of time for training healthcare professionals in their use in order to limit the potential for medication errors.[21] The American College of Chest Physicians (ACCP) therefore recommends – based on three available RCTs – a fixed-dose or a weight-adjusted regimen.[22]

Additionally, the APTT test itself is associated with significant intra- and inter-patient variability that is not related to circulating blood heparin activity or patient variables.[23-25] There is a lack of standardisation of methods since different reagents and instruments are used to perform an APTT test. To what extent the efficacy of UFH in general is dependent on (early) APTT test results is also uncertain.[26,27] An alternative for APTT would be monitoring the anti-Xa activity; its advantages have been discussed by several studies.[17,23] For example, a weight-based protocol in combination with the use of anti-Xa as monitoring technique results in a therapeutic heparinisation rate of 90% within 24 hours.[17,28] Despite the advantages, the anti-Xa assay is not widely incorporated in clinical use because of high costs and limited availability, whereas inter-laboratory variation still remains.[7,23,29]

Besides dosing regimens and laboratory instruments, the interpretation of the therapeutic range of the APTT levels might also play an important role in dosing UFH. A review performed by Raschke and colleagues included studies where different APTT reagents were used, so UFH

concentrations that were associated with a target APTT ratio of 1.5-2.5 times the control value, differed noticeably between trials.[30] Also, the clinical relevance of the fixed therapeutic range of 1.5-2.0 times the control APTT is uncertain and has not been confirmed by randomised trials.[7,31] Because of that and other methodological concerns, the ACCP recommends that the therapeutic APTT range at a particular laboratory should be adapted to responsiveness of the reagent and coagulometer used.[7]

Our study has several limitations. This analysis was designed as a pilot study, therefore the study sample was small. However, the included patients and clinical setting reflect real-life practice. Since the purpose of this survey was only to register the number of subtherapeutic, supratherapeutic and therapeutic APTT levels, we did not register the administered dose or infusion rate of UFH. The DnTT ratio was based on the total amount of dose adjustments per patient during time of infusion. Whether the dose is consecutively adjusted after a non-therapeutic APTT level is not represented. However, the DnTT ratio gives some indication of adherence to the dosing regimen; based on the ratio being ≥1 in 78% of the study population, we believe that the low therapeutic APTT rates are not the result of inadequate dose adjustment.

To conclude, time to therapeutic range was 24 hours in 56% of in total 101 patients admitted to the ICU and MCU. Of all obtained APTT results in 24 hours, 71% were sub- or supratherapeutic of which 200/272 were >15% aberrant from the therapeutic range. Therefore, intravenous UFH therapy can be improved in critically ill patients. More clinical trials are needed to examine optimal dosage regimens and to investigate the performance of other alternative anticoagulant therapies in critically ill patients.

ACKNOWLEDGEMENTS

The authors would like to thank Erik-Jan van den Dool of the clinical chemical laboratory of the Academic Medical Centre, Amsterdam, the Netherlands, for his expertise and help in offering the resources for this study.

REFERENCES

1. Middeldorp S. Heparin: from animal organ extract to designer drug. Thromb Res. 2008;122:753-62.
2. Hylek EM, Regan S, Henault LE, et al. Challenges to the effective use of unfractionated heparin in the hospitalized management of acute thrombosis. Arch Intern Med. 2003;163:621-7.
3. Murphy SA, Gibson CM, Morrow DA, et al. Efficacy and safety of the low-molecular weight heparin enoxaparin compared with unfractionated heparin across the acute coronary syndrome spectrum: a meta-analysis. Eur Heart J. 2007;28:2077-86.
4. Quinlan DJ, McQuillan A, Eikelboom JW. Low-molecular-weight heparin compared with intravenous unfractionated heparin for treatment of pulmonary embolism: a meta-analysis of randomized, controlled trials. Ann Intern Med. 2004;140:175-83.
5. van Dongen CJ, van den Belt AG, Prins MH, Lensing AW. Fixed dose subcutaneous low molecular weight heparins versus adjusted dose unfractionated heparin for venous thromboembolism. Cochrane Database Syst Rev. 2004;(4):CD001100.
6. Krishnan JA, Segal JB, Streiff MB, et al. Treatment of venous thromboembolism with low-molecular-weight heparin: a synthesis of the evidence published in systematic literature reviews. Respir Med. 2004;98:376-86.
7. Garcia DA, Baglin TP, Weitz JI, Samama MM. Parenteral anticoagulants: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012 ;141(Suppl 2):e24S-e43S.
8. Hull RD, Raskob GE, Brant RF, Pineo GF, Valentine KA. Relation between the time to achieve the lower limit of the APTT therapeutic range and recurrent venous thromboembolism during heparin treatment for deep vein thrombosis. Arch Intern Med. 1997;157:2562-8.
9. Raschke RA, Reilly BM, Guidry JR, Fontana JR, Srinivas S. The weight-based heparin dosing nomogram compared with a "standard care" nomogram. A randomized controlled trial. Ann Intern Med. 1993;119:874-81.
10. Cossette B, Pelletier ME, Carrier N, et al. Evaluation of bleeding risk in patients exposed to therapeutic unfractionated or low-molecular-weight heparin: a cohort study in the context of a quality improvement initiative. Ann Pharmacother. 2010 ;44:994-1002.
11. Bauer SR, Ou NN, Dreesman BJ, Armon JJ, Anderson JA, Cha SS, et al. Effect of body mass index on bleeding frequency and activated partial thromboplastin time in weight-based dosing of unfractionated heparin: a retrospective cohort study. Mayo Clin Proc. 2009;84:1073-8.
12. Thomas MP, Mahaffey KW, Chiswell K, et al. Activated partial thromboplastin time measurement is not associated with clinical outcomes in patients with high-risk non-ST-segment elevation acute coronary syndromes treated with unfractionated heparin. J Thromb Thrombolysis. 2012;34:114-9.
13. Hull RD, Pineo GF. Heparin and low-molecular-weight heparin therapy for venous thromboembolism: will unfractionated heparin survive? Semin Thromb Hemost. 2004 ;30(Suppl 1):11-23.
14. Smith SB, Geske JB, Maguire JM, Zane NA, Carter RE, Morgenthaler TI. Early anticoagulation is associated with reduced mortality for acute pulmonary embolism. Chest. 2010;137:1382-90.
15. Linke LC, Katthagen BD. Weight-based heparin dosing is more effective in the treatment of postoperative deep vein thrombosis. Arch Orthop Trauma Surg. 1999;119:208-11.
16. Lee HN, Cook DJ, Sarabia A, et al. Inadequacy of intravenous heparin therapy in the initial management of venous thromboembolism. J Gen Intern Med. 1995;10:342-5.
17. Guervil DJ, Rosenberg AF, Winterstein AG, Harris NS, Johns TE, Zumberg MS. Activated partial thromboplastin time versus antifactor Xa heparin assay in monitoring unfractionated heparin by continuous intravenous infusion. Ann Pharmacother. 2011;45:861-8.
18. Cheng S, Morrow DA, Sloan S, Antman EM, Sabatine MS. Predictors of initial nontherapeutic anticoagulation with unfractionated heparin in ST-segment elevation myocardial infarction. Circulation. 2009;119:1195-202.
19. Bernardi E, Piccioli A, Oliboni G, Zuin R, Girolami A, Prandoni P. Nomograms for the administration of unfractionated heparin in the initial treatment of acute thromboembolism--an overview. Thromb Haemost. 2000;84:22-6.

20. Rosborough TK, Shepherd MF. Achieving target antifactor Xa activity with a heparin protocol based on sex, age, height, and weight. Pharmacotherapy. 2004;24:713-9.

21. Dobesh PP. Unfractionated heparin dosing nomograms: road maps to where? Pharmacotherapy. 2004;24(8 Pt 2):142S-5S.

22. Holbrook A, Schulman S, Witt DM, Vandvik PO, Fish J, Kovacs MJ, et al. Evidence-based management of anticoagulant therapy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012;141(2 Suppl):e152S-e184S.

23. Rosenberg AF, Zumberg M, Taylor L, LeClaire A, Harris N. The use of anti-Xa assay to monitor intravenous unfractionated heparin therapy. J Pharm Pract. 2010;23:210-6.

24. Manzato F, Mengoni A, Grilenzoni A, Lippi G. Evaluation of the activated partial thromboplastin time (APTT) sensitivity to heparin using five commercial reagents: implications for therapeutic monitoring. Clin Chem Lab Med. 1998;36:975-80.

25. Zehnder J, Price E, Jin J. Controversies in heparin monitoring. Am J Hematol 2012;87(Suppl 1):S137-S140.

26. Anand S, Ginsberg JS, Kearon C, Gent M, Hirsh J. The relation between the activated partial thromboplastin time response and recurrence in patients with venous thrombosis treated with continuous intravenous heparin. Arch Intern Med. 1996;156:1677-81.

27. Anand SS, Bates S, Ginsberg JS, et al. Recurrent venous thrombosis and heparin therapy: an evaluation of the importance of early activated partial thromboplastin times. Arch Intern Med. 1999;159:2029-32.

28. Smith SB, Geske JB, Maguire JM, Zane NA, Carter RE, Morgenthaler TI. Early anticoagulation is associated with reduced mortality for acute pulmonary embolism. Chest. 2010;137:1382-90.

29. Cuker A, Raby A, Moffat KA, Flynn G, Crowther MA. Interlaboratory variation in heparin monitoring: Lessons from the Quality Management Program of Ontario coagulation surveys. Thromb Haemost. 2010;104:837-44.

30. Raschke R, Hirsh J, Guidry JR. Suboptimal monitoring and dosing of unfractionated heparin in comparative studies with low-molecular-weight heparin. Ann Intern Med. 2003;138:720-3.

31. Eikelboom JW, Hirsh J. Monitoring unfractionated heparin with the aPTT: time for a fresh look. Thromb Haemost. 2006;96:547-52.

Netherlands
The Journal of Medicine

ORIGINAL ARTICLE

Influenza vaccination coverage in patients treated with chemotherapy: current clinical practice

M.L. Wumkes[1]*, A.M.T. van der Velden[1], A.W.G. van der Velden[2], J.M.L. Stouthard[3], M.R. Nijziel[4], M. Westerman[5], A. Beeker[6], A. Meerveld-Eggink[7], G.T. Rijkers[8] D.H. Biesma[7]

[1]Department of Internal Medicine, Tergooi Hospitals Hilversum, the Netherlands, [2]Department of Internal Medicine, Martini Hospital Groningen, the Netherlands, [3]Department of Internal Medicine, Maasstad Hospital Rotterdam, the Netherlands, [4]Department of Internal Medicine, Maxima Medical Centre Eindhoven, the Netherlands, [5]Department of Internal Medicine, Medical Centre Alkmaar, the Netherlands, [6]Department of Internal Medicine, Spaarne Hospital Hoofddorp, the Netherlands, [7]Department of Internal Medicine, St. Antonius Hospital Nieuwegein, the Netherlands, [8]Department of Medical Microbiology and Immunology, St. Antonius Hospital Nieuwegein, the Netherlands, *corresponding author: tel.: +31 (0)88 7531753, fax: +31 (0)35 5391235, e-mail: Mwumkes@tergooi.nl

ABSTRACT

Background: Influenza virus vaccination is recommended for patients treated with chemotherapy. Little is known about vaccination coverage in these patients.

Methods: Vaccination coverage in the Netherlands was analysed by questionnaires completed by general practitioners, within a catchment area of 1.3 million people, in the period 2010-2011.

Results: Of 433 eligible adult patients treated with chemotherapy for breast or colorectal cancer, 144 patients gave permission for us to approach their general practitioner with a questionnaire. General practitioners were asked about vaccination coverage, awareness of recommendations and their opinion about the responsibility for vaccination. We received 114 (79%) completed questionnaires. Sixty-seven out of 114 patients (59%) were vaccinated against influenza. Forty-four (66%) of these patients also had an indication for vaccination based on age (age ≥60 years). According to 48% of the general practitioners, the responsibility for vaccination belongs to the competence of the treating medical oncologist.

Conclusion: Influenza vaccination coverage is limited to 59% of patients treated with chemotherapy. Guidelines for responsibility (general practitioner or medical oncologist) may increase the vaccination rate of cancer patients.

KEYWORDS

Chemotherapy, influenza virus vaccination, breast cancer, colorectal cancer, vaccination coverage

INTRODUCTION

Influenza viruses cause important epidemic infections with a potential risk of developing acute respiratory disease. Infection rates are highest in children, but morbidity and mortality are highest in various high-risk groups.[1] Among these groups are patients aged ≥60 years, patients with heart or lung diseases and patients with diminished immune function due to treatment with chemotherapy or other immunosuppressive drugs.[2] Patients receiving chemotherapy or immunosuppressive drugs have an impaired immune response to bacterial and viral infections and are therefore at risk of serious post-influenza complications.[3] In the United States yearly 441 per 100,000 oncology patients are hospitalised due to influenza. This is 3-5 times higher than in the general population. The mortality rate is 9% in cancer patients with a relative risk of 4.0 in comparison with the general population.[4]

Therefore, yearly influenza vaccination is strongly recommended for patients with reduced function of the immune system due to treatment with chemotherapy or immunosuppressive drugs.[2]

Various factors influence the immune status and the response to immunisation in patients with cancer. First, the disease state can be directly immunosuppressive, in particular in patients with haematological malignancies. Second, treatment modalities for cancer can have (severe) immunosuppressive effects. Chemotherapy has its cytotoxic effects particularly on rapidly growing tumour cells, but the full functional capacity of the immune system also depends on rapid proliferation of cells and is therefore adversely affected.[5]

This negative effect of chemotherapy on immune function is, however, variable, depending on the chemotherapy regimen. Influenza virus vaccination in this group of patients is important to reduce the risk of serious complications of an influenza infection as well as to avoid potential risk of postponing the chemotherapy treatment due to infection. However, the immune response to the vaccine may be suboptimal because the protection conferred by immunisation is lower in immunosuppressed cancer patients.[5]

Despite the recommendations, vaccination coverage in high-risk patients can be improved. In the Netherlands, seasonal influenza vaccination is offered by the government free of charge to elderly and other risk groups and distributed through general practitioners (GPs).[6] Influenza vaccine coverage in 2010-2011 was about 75% for adults aged ≥60 years as well as in patients with reduced immune function (patients with liver cirrhosis, asplenia, autoimmune diseases and patients treated with chemotherapy or other immunosuppressive drugs). Vaccine coverage of patients treated with chemotherapy as a separate risk group is unknown.[7] Reasons given by physicians not to vaccinate their patients during chemotherapy are lack of awareness of the recommendations and concern about the efficacy of the influenza vaccination in patients with solid tumours treated with chemotherapy.[3]

The aim of this study was to evaluate GPs current practice with regard to influenza vaccination in patients treated with chemotherapy for breast cancer or colorectal cancer in the Netherlands.

PATIENTS AND METHODS

This study was conducted in patients treated with chemotherapy for breast cancer or colorectal cancer during the influenza period 2010-2011. Defining this patient group was considered to reflect a comprehensive picture of current practice, because breast cancer as well as colorectal cancer have high incidence and occur in patients with a relatively wide age range.

To identify patients the following strategy was used. Six non-academic teaching hospitals participated in this study (catchment area 1.3 million people). The principal investigators of each participating hospital were asked to register all adult patients treated with adjuvant or palliative chemotherapy for breast cancer or colorectal cancer during the influenza period of 2010-2011. Patients were then approached by letter and asked for written informed consent to contact their GP for completion of a questionnaire. The questionnaire consisted of questions concerning vaccination coverage of patients and their informal carers, timing of vaccination and influence of chemotherapy regimen as well as the GP's opinion regarding the responsibility of the medical oncologist for instructions on influenza vaccination during chemotherapy. The questionnaire is shown in *appendix A*. A descriptive analysis has been carried out.

RESULTS

In the six participating hospitals, 433 patients were identified with breast cancer or colorectal cancer receiving treatment with chemotherapy in the influenza period of 2010-2011 (*figure 1*). A total of 418 patients were approached for informed consent, 35% responded. This percentage was approximately the same in each participating centre. Reasons for not participating were not obtained. Informed consent was given by 98% of the patients. A total of 141 GPs were approached and asked to complete the questionnaire of which 79% returned the questionnaire. Due to the fact that some patients had the same GP, a total of 107 different GPs participated in this study.

Vaccination

Of the 114 patients, 67 patients (59%) were vaccinated against influenza (*figure 2*). Of these patients, 66% already had an indication for vaccination based on age (age ≥60 years). Thirty-four patients' informal carers (30%) were vaccinated, two of them had no indication for influenza other than their partners' treatment with chemotherapy (*figure 3*).

Figure 1. *Overview of inclusion and response*

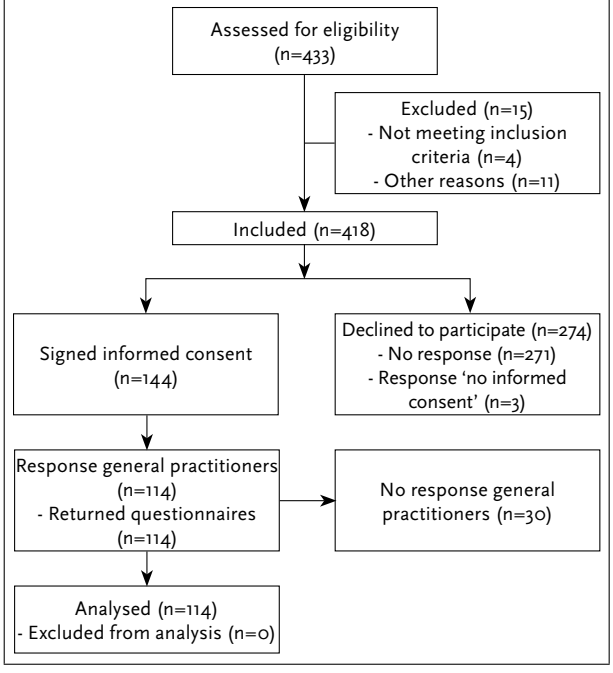


Figure 2. *Vaccination status of patients treated with chemotherapy in proportions*

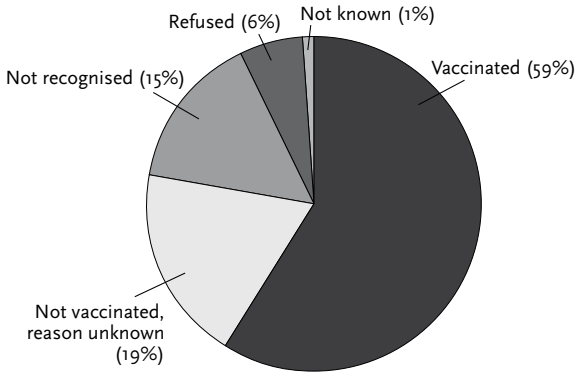
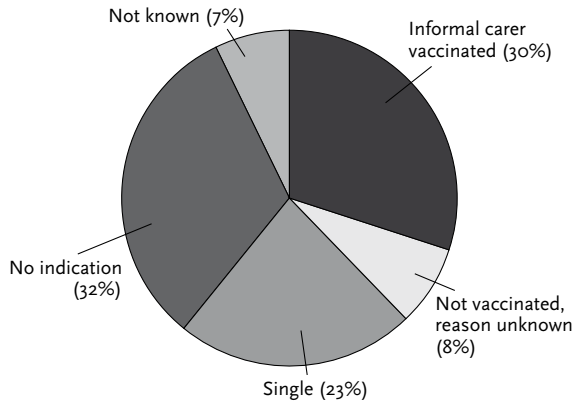


Figure 3. *Vaccination status of informal carers in proportions*



Information

During the influenza period of 2010-2011, 60% of the participating GPs were approached 1-5 times by a patient treated with chemotherapy for advice concerning influenza vaccination; 8% were consulted 6-10 times. This consultation was independent of the chemotherapy regimen or underlying malignancy. Of the GPs, 32% were not contacted at all.

Awareness of vaccination recommendations

Forty-nine of the participating GPs were not aware of the fact that yearly influenza vaccination is recommended in the national guidelines for all patients treated with chemotherapy or other immunosuppressive drugs.

Of the GPs, 48% would not approach patients treated with chemotherapy and stated that vaccination against influenza is the responsibility of the treating medical oncologist.

One-third of the GPs actively approached patients treated with chemotherapy to provide an influenza vaccination, 18% approached their patients occasionally when they remembered to do so.

Fifty-two percent of the GPs indicated that their decision to provide an influenza vaccination to a patient treated with chemotherapy was not influenced by the chemotherapy regimen itself. On the contrary, 43% of the GPs stated that their decision was influenced by the chemotherapy and the degree of myelosuppression it causes. Only a small percentage (3%) consulted the treating medical oncologist.

Optimal timing of vaccination

The results regarding the optimal timing of influenza vaccination during chemotherapy are presented in *table 1*. One-third of the GPs would consult the treating medical oncologist or did not provide an answer to the question on what is known about the optimal timing of vaccination.

The majority (94%) of the GPs are in need of more information about the efficacy and optimal timing in patients treated with chemotherapy.

DISCUSSION

We studied the current practice of GPs with regard to influenza vaccination in patients treated with chemotherapy for breast cancer or colorectal cancer in different parts of the Netherlands. It should be emphasised that this issue is not only relevant in the Netherlands but worldwide.

Limitations of the study

The main limitation of the study is the low response rate of patients. Two-thirds of the identified patients did not respond for reasons that are unknown. Patients were not approached by a clinician but by letter which could have influenced the response in a negative way. The low response rate might have led to a representation bias: patients who were not interested in participating in this study might also not be aware of the risks associated with their immunocompromised state. It is also possible that patients not responding were already vaccinated and

Table 1. *GPs opinion on optimal timing of vaccination during chemotherapy (n=GPs)*

Optimal timing of vaccination	Frequency (n)	(n) %
Before the next dose of chemotherapy	25	23
Two weeks after the last regimen	26	24
Not useful to vaccinate, no serological response	17	16
Other	39	37
Total	107	100

informed about the risks and therefore did not feel the need to participate.
Furthermore, 21% of GPs did not return the questionnaire. Potentially their proportion of vaccination coverage is lower than those who actually responded.

In the Netherlands, vaccine coverage in 2010-2011 was 75% for patients with a reduced immune function. No distinction was made between the different groups of patients with a reduced immune state, therefore the vaccine coverage in patients treated with chemotherapy is not known.[7] In this study of 114 Dutch patients vaccination coverage was 59%. Since two-thirds of these patients already had an indication for vaccination based on age (age ≥60 years), it is difficult to determine how many patients were vaccinated because they were receiving chemotherapy.

Awareness of the recommendations
Almost 50% of the participating GPs were not aware of the fact that yearly influenza vaccination is recommended in the national guidelines for all patients treated with chemotherapy or other immunosuppressive drugs independently of the chemotherapy regimen or the degree of immunosuppression it causes.[2] Scarce available data about the efficacy and optimal timing of influenza vaccination in this high-risk group might be an explanation. Despite the guidelines, 40% did not receive the vaccination; of these patients 37% were not identified as eligible for influenza vaccination by a computerised search on ICPC (International Classification of Primary Care) codes. Since there is no ICPC code for treatment with chemotherapy, these patients are not automatically selected. The other 60% were identified as eligible for influenza vaccination by an ICPC code based on age or other comorbidity.
The development of an ICPC code for treatment with chemotherapy could lead to identification of this high-risk group and therefore improve vaccination coverage. However, patient records need to be accurately documented by the GPs to succeed.

Regarding the relatively low vaccination coverage of the patients' carers, routine vaccination of informal carers of a patient treated with chemotherapy is not recommended in the Netherlands. A GP may decide to vaccinate informal carers if considered necessary, but the vaccine will not be covered by health insurance.[2] Therefore, it is not surprising that informal carers without an indication of their own are not vaccinated.

Optimal timing of vaccination
Limited recent data are available on the optimal timing and efficacy of influenza vaccination while receiving ongoing chemotherapy treatment.
Vilar-Compte *et al.* showed that during chemotherapy, 183 patients with breast cancer indeed had a lower response to vaccination than patients who did not receive chemotherapy.[8] Brydak *et al.* found that immune response to influenza vaccination in breast cancer patients with or without chemotherapy was as effective as vaccination of healthy adults. The patient group, however, was very small (n=9) and heterogeneous.[3]
In our study, the GPs have various opinions on the optimal timing of vaccination. This is expected because of the absence of specific guidelines and the limited recent data available on the optimal timing of vaccination. In general it is advised to vaccinate patients before starting chemotherapy.[9,10] Because the seasonal influenza vaccination campaign is restricted to the months October and November, it is not always possible to administer the vaccine before the start of the chemotherapy. Ortbals *et al.* vaccinated oncology patients on the day of chemotherapy or at the time of their white blood cell count nadir. Only 50% of the patients achieved seroconversion on the day of chemotherapy, whereas 93% of the patients vaccinated in their nadir showed an adequate antibody response. This study, which was conducted in 1977, still forms the basis of current recommendations. It should be noted that the Ortbals study only included a very limited number of patients with breast cancer (n=11),[11] (reviewed by Pollyea et al). Most of the other influenza vaccination studies in cancer patients were performed in the 1970s and 1980; over the years considerable changes have been made in vaccine formulations and chemotherapy regimens.

During the influenza vaccination period in 2009, we conducted a pilot study in patients with breast cancer (Meerveld-Eggink *et al.*) Breast cancer patients received influenza vaccination during 5-fluorouracil, epirubicin and cyclophosphamide (FEC)-containing chemotherapy regimens. Thirty-eight patients were randomised for early (day 4) or late (day 16) vaccination during the chemotherapy cycle.
In this pilot study, patients vaccinated at day 4 tended to reach higher antibody levels compared with patients vaccinated at day 16.[13] Studies with larger patient numbers have to be conducted to confirm this effect.

Responsibility
Of the GPs, 48% did not approach patients receiving chemotherapy because in their perspective it is the responsibility of the treating medical oncologist that their patient is vaccinated against influenza. In the Netherlands, the influenza vaccine is issued to GPs by the government and the GPs are primarilly responsible for administering a vaccine to every eligible patient. However, in our study GPs considered it the medical oncologist's responsibility to vaccinate patients undergoing chemotherapy treatment.

Since the awareness of the indication for vaccination, in combination with specific knowledge on the immune suppressive effects of the anti-cancer treatment, might be greater among medical oncologists, we believe that medical oncologists should identify patients eligible for vaccination.

The high response rate of the GPs (79%), the high percentage of GPs in need of more information about the efficacy, optimal timing and recommendations, as well as the high number of patients consulting the GP for advice indicate that influenza vaccination is a timely topic and that further studies have to be conducted to achieve more information in order to establish (inter)national guidelines.

In conclusion, vaccination coverage in patients treated with chemotherapy in the Netherlands can be improved. Different strategies towards improvement of vaccination coverage could be developed. We suggest better education and information for GPs.
Further studies have to be conducted to assess the optimal timing of vaccination during chemotherapy. Medical oncologists should be encouraged to actively inform the GP about the need for influenza vaccination of their individual patients. A national campaign might improve the vaccination coverage in this high-risk group.

ACKNOWLEDGEMENTS

We thank all patients and general practitioners for their participation in this study.
Grant support: not applicable. There is no conflict of interest.

REFERENCES

1. Fiore A, Shay D, Haber P, et al. Prevention and control of influenza. Recommendation of the Advisory Committee on Immunization Practices (ACIP) 2008. MMWR Recomm Rep. 2008; 57:1-60.
2. Health Council of the Netherlands. Influenza vaccination: revision of the indication. The Hague: Health Council of the Netherlands, 2007; publication no.2007/09E. ISBN 978-90-5549-712-6.
3. Brydak L, Guzy J, Starzyk J, Machala M, Góźdź S. Humoral immune response after vaccination against influenza in patients with breast cancer. Support Care Cancer. 2001;9:65-8.
4. Cooksley C, Avritscher E, Bekele B, Rolston K, Geraci J, Elting L. Epidemiology and outcomes of serious influenza-related infections in the cancer population. Cancer. 2005;104:618-28.
5. Arrowood J, Hayney M. Immunization recommendations for adults with cancer. Ann Pharmacother. 2002; 36:1219-29.
6. Van Essen G, Bueving H, Voordouw A, et al. NHG-Standaard Influenza en influenzavaccinatie, Eerste herziening. Huisarts Wet. 2008;51:1-12 (http://nhg.artsennet.nl/).
7. Jansen B, Tacken M, Mulder J, Visscher S, Tiersma W, Brasspenning J. Scientific institute for quality of health care. Monitoring vaccine coverage national program flu prevention 2011. ISBN:978-90-76316-41-3.
8. Vilar-Compte D, Cornejo P, Valle-Salinas A, et al. Influenza vaccination in patients with breast cancer: a case-series analysis. Med Sci Monit. 2006;12:CR332-CR336
9. Sommer A, Wachel B, Smith J. Evaluation of vaccine dosing in patients with solid tumors receiving myelosuppressive chemotherapy. J Oncol Pharm Pract. 2006;12:143-54.
10. Melcher L. Recommendations for influenza and pneumococcal vaccinations in people receiving chemotherapy. Clin Oncol (R Coll Radiol). 2005;17:12-5.
11. Ortbals D, Liebhaber H, Presant C, Van Amburg III A, Lee J . Influenza immunization of adult patients with malignant diseases. Ann Intern Med. 1977;87:552-7.
12. Pollyea D, Brown J, Horning S. Utility of Influenza Vaccination for Oncology Patients. J Clin Oncol. 2010; 28:2481-90.
13. Meerveld-Eggink A, de Weerdt O, van der Velden AMT, et al. Response to influenza virus vaccination during chemotherapy in patients with breast cancer. Ann Oncol. 2011;22:2031-35.

APPENDIX A – QUESTIONNAIRE

1. During last influenza season (2010-2011), was the influenza vaccine administered to the patient mentioned above?
   a. Yes
   b. No, reason: .....

2. Did the informal carers of the patient mentioned above get vaccinated with the influenza vaccine?
   a. Yes
   b. No, reason: .....

3. Do you (actively) approach patients who are treated with chemotherapy during the influenza season to get vaccinated against influenza?
   a. Yes
   b. No, responsibility of oncologist
   c. Sometimes, reason: .....

4. Does the chemotherapy regimen and the degree of myelosuppression it causes influence your decision to administer the influenza vaccine?
   a. Yes
   b. No

5. During last influenza season (2010-2011), were you consulted frequently by patients with a malignancy for advice regarding influenza vaccination ?
   a. Yes, approximately: .....times
   b. No, approximately:.....times

6. In your opinion, what is the optimal timing of influenza vaccination in patients on chemotherapy?
   a. Just before the next chemotherapy cycle
   b. Two weeks after the last gift of chemotherapy
   c. Do not administer the vaccine when on treatment with chemotherapy, there will not be an adequate (serological) response
   d. Differently, explanation: .....

7. Would you like more information about the optimal timing of vaccination in this patient category?
   a. Yes
   b. No

8. In your opinion, is this subject relevant?
   a. Yes
   b. No, reason: .....

Netherlands
The Journal of Medicine

CASE REPORT

Fasciola hepatica as a cause of jaundice after chewing khat: a case report

L.C.J. de Bree*, A.G.L. Bodelier, G.P. Verburg

Department of Internal Medicine and Gastroenterology, Amphia Hospital, Breda, the Netherlands,
*corresponding author: tel: +31 (0)76 5955000, fax: +31(0)76 5952410, e-mail: cdebree@amphia.nl

ABSTRACT

Fasciola hepatica is a worldwide distributed zoonotic trematode incidentally infecting humans. Although often symptomatic, fascioliasis can cause a wide spectrum of disease. The diagnosis can be established by stool examination detecting ova of the parasite, although serological testing has a higher sensitivity and specificity in the acute phase of disease. This case presents a 24-year-old Somalian man admitted with jaundice and abdominal discomfort due to fascioliasis after chewing khat. The patient was treated successfully with a single dose of triclabendazole.

KEYWORDS

Fasciola hepatica, fascioliasis, jaundice, parasitosis, khat

CASE

A 24-year-old Somalian man was admitted because of jaundice. He had a one-month history of pruritus and longer existing intermittent upper abdominal discomfort. He was known for a bilateral panuveitis caused by tuberculosis for which he had completed tuberculostatic treatment. He lived in the Netherlands and his last visit to Somalia was four years ago. Physical examination revealed jaundice and right hypogastric tenderness. The liver and spleen were not enlarged. Laboratory findings revealed a total white blood count of 10.0 x 10^9/l (normal values 5.0-10.0 x 10^9/l) with marked eosinophilia of 5.0 x 10^9/l (0-0.5 x 10^9/l), as well as elevated liver tests with a total and conjugated bilirubin of 157 µmol/l (<20 µmol/l), alanine aminotransferase 68 U/l (<35 U/l), alkaline phosphatase 394 U/l (<115 U/l), gamma glutamyltranspeptidase 46 U/l (<55 U/l) and lactate dehydrogenase 394 U/l (<248 U/l). Abdominal ultrasound was completely normal. Serological tests for hepatitis A, B and C, Epstein-Barr virus, cytomegalovirus, human immunodeficiency virus, strongyloides and schistosoma infection were negative, as well as markers for autoimmune hepatitis. Stool samples sent for microscopic examination did not show any parasites. MRCP revealed a dilated common bile duct with distal tapering (*figure 1*). Serum Fas2 ELISA, detecting IgG-antibodies of the liver fluke *Fasciola hepatica,* was positive at a titre of 1: 350 (negative <1:32). In new stool samples, ova of the liver fluke were detected (*figure 2*). The patient admitted chewing freshly imported khat leafs, a psychoactive drug traditionally used and cultivated in the Horn of Africa, which was most likely the source of infection. Patient was treated successfully with a single dose of triclabendazole 950 mg orally.

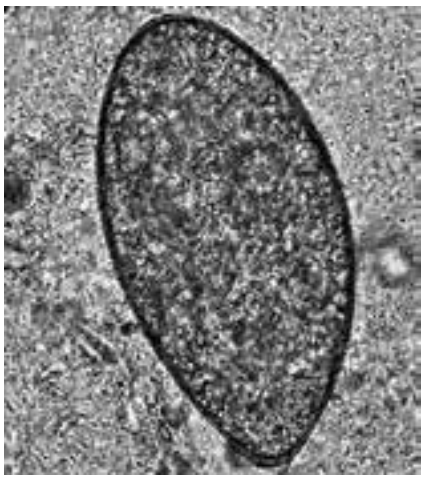
DISCUSSION

Fascioliasis is a zoonotic foodborne disease caused by the trematode *Fasciola hepatica*. This parasite, causing liver rot in sheep and cattle, incidentally infects humans after ingestion of contaminated water, fish or uncooked aquatic vegetation. Although the source of fascioliasis in this case could not be proven, it is very likely attributed to chewing freshly imported khat leaves. Anecdotal evidence of fascioliasis after chewing khat has been reported before.[1,2] As one of the 'neglected' tropical diseases, *F. hepatica* has a substantial impact on global public health, with an estimated 2.4-17 million people infected worldwide.[3] Nowadays *F. hepatica* has a worldwide distribution including Europe, where a watercress-related outbreak in France was reported.[4] However, highest prevalences are reported in Andean countries, Northern Africa and parts of the Middle East.[5,6]

After ingestion, metacercariae excyst and migrate in 4-6 weeks through the intestinal wall and Glisson's capsule to the liver where they cause parenchymatous destruction, and migrate to the biliary ducts. Matured liver flukes release eggs via the biliary system into the small bowel.

Figure 1. *Magnetic resonance cholangiopancreatography showing dilated bile ducts with distal tapering of the common bile duct*

Figure 2. *Stool examination showing an ovum of the trematode Fasciola hepatica*



Subsequently the eggs become embryonated and upon reaching water they release miracidia, which are capable of invading freshwater snails as their intermediate hosts. Subsequently cercariae are released and transformed to metacercariae on aquatic vegetation.

Although often asymptomatic, *F. hepatica* can cause a wide spectrum of disease depending on the degree of fluke burden and disease stage. The acute hepatic phase is usually mild and is sometimes accompanied by fever, abdominal pain and hepatomegaly. Biliary colics, epigastric pain and jaundice can be seen in the chronic biliary phase, which can last from a matter of months to up to ten years. Complications such as cholangitis, pancreatitis and cirrhosis can occur. Marked eosinophilia is the most common laboratory finding.

Detection of eggs in faeces is considered to be the gold standard for diagnosing fascioliasis, although eggs are often undetectable during the acute phase of the disease. The diagnosis can also be established serologically by Fas2 ELISA, which is able to detect IgG-antibodies against Fas2 antigen as early as ten days after infection.[7] Therefore it can be used in the initial phase of disease, having a sensitivity of 92.5% and a specificity of 70.8-92.9%.[8] In the acute phase, characteristic findings on imaging studies are multiple subcapsular small nodular or branching lesions in the liver. In the chronic phase, bile duct dilatation, gallbladder or bile duct oedema and even parasites can be seen.[9]

In any phase of disease, successful treatment can be achieved with triclabendazole, in a single dose of 10 mg/kg. As in many other countries, in the Netherlands triclabendazole is only available via veterinary pharmacies. Cure rates of more than 90% are reported.[10]

CONCLUSION

As illustrated in this case, diagnosing fascioliasis can be challenging in non-highly endemic countries. However, *F. hepatica* infection should be considered in patients with abdominal pain, jaundice and eosinophilia. We recommend serological testing above stool sample microscopy in early stages. Special attention should be given to food, drugs and travel history.

REFERENCES

1. Doherty JF, Price N, Moody AH, Wright SG, Glynn MJ. Fascioliasis due to imported khat. Lancet. 1995; 345:462.
2. Cats A, Scholten P, Meuwissen SG, Kuipers EJ. Acute Fasciolia hepatica infection attributed to chewing khat. Gut. 2000;47:584-5.
3. Mas-Coma S, Valero MA, Bargues MD. Chapter 2. Fasciola, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. Adv Parasitol. 2009:69:41.
4. Mailles A, Capek I, Ajana F, Schepens C, Ilef D, Vaillant V. Commercial watercress as an emerging source of fascioliasis in Northern France in 2002: results from an outbreak investigation. Epidemiol Infect. 2006;134:942-5.
5. Parkinson M, O'Neill SM, Dalton JP. Endemic human fasciolosis in the Bolivian Altiplano. Epidemiol Infect. 2007;135:669-74.
6. Haseeb AN, el-Shazly AM, Arafa MA, Morsy AT. A review on fascioliasis in Egypt. J Egypt Soc Parasitol. 2002;32:317-54.
7. Timoteo O. Maco V, Neyra V, Yi PJ, Leguia G, Espinoza JR. Characterization of the humoral immune response in alpacas (Lama pacos) experimentally infected with Fasciola hepatica against cysteine proteinases Fas2 and Fas2 and histopathological findings. Vet Immunol Immunopath. 2005;106:77-86.
8. Espinoza JR, Maco V, Marcos L, et al. Evaluation of Fas2-ELISA for the serological detection of Fasciola hepatica infection in humans. Am J Trop Med Hyg. 2007;76:977-82.
9. Kabaalioglu A, Ceken K, Alimoglu E, et al. Hepatobiliary fascioliasis: sonographic and CT findings in 87 patients during the initial phase and long-term follow-up. AJR Am J Roentgenol. 2007;189:824-8.
10. Marcos LA, Tagle M, Terashima A, et al. Natural history, clinicoradiologic correlates, and response to triclabendazole in acute massive fascioliasis. Am J Trop Med Hyg. 2008;78:222.

Netherlands
The Journal of Medicine

PHOTO QUIZ

A male with extensive renal vein thrombosis

L. Tonneijck*, M. Schouten, M.C. Weijmer, C.E.H. Siegert

Department of Nephrology, St. Lucas Andreas Hospital, Amsterdam, the Netherlands, tel.:
*corresponding author: tel.: +31(0)20 5108770, fax: +31(0)20 6838771, e-mail: l.tonneijck@gmail.com

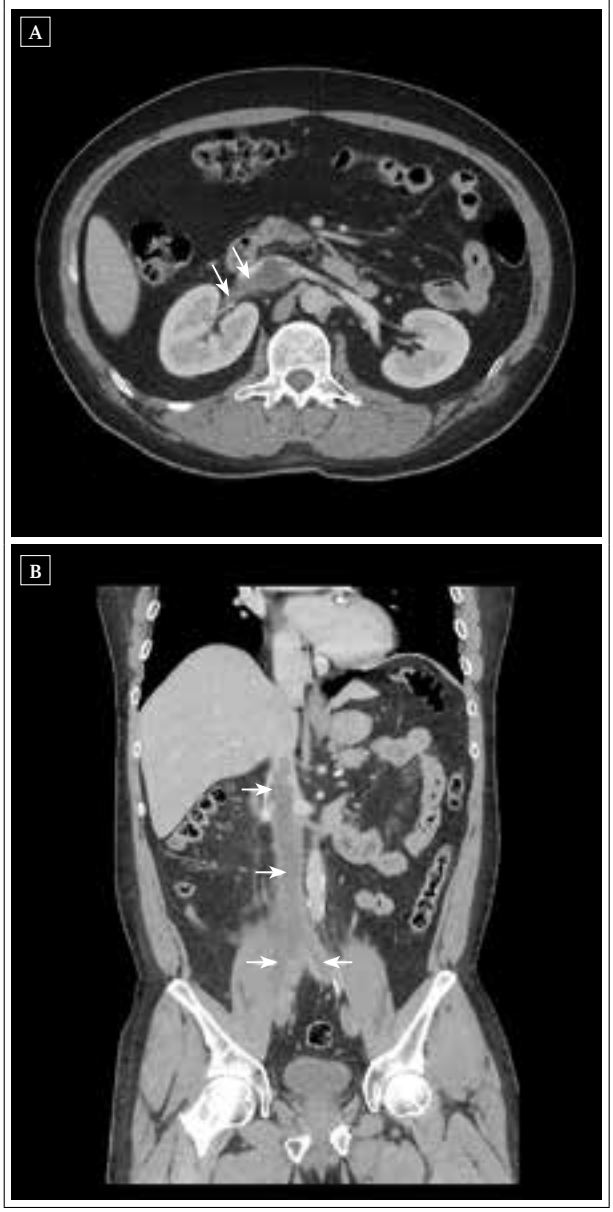
CASE REPORT

A 48-year-old male presented with right-sided flank pain of three weeks duration, which had radiated to the abdomen and groins during the last 24 hours. He reported no haematuria. His medical history included myocardial infarction (2010) and urolithiasis. Physical examination demonstrated mild pretibial oedema. His blood pressure was 111/61 mmHg. Laboratory investigation showed leucocytes 12.9 (4-10) x 10^9, C-reactive protein 167 (<10) mg/l, albumin 26 (38-52) g/l and creatinine 111 (60-110) µmol/l with an estimated glomerular filtration rate (eGFR) of 62 (60-125) ml/min. Ultrasound imaging did not show any signs of urolithiasis, appendicitis or diverticulitis. Additional computed tomography (CT) scanning demonstrated a thrombus in the right renal vein extending into the inferior vena cava and both iliac veins (*figures 1A* and *1B*), which was confirmed by Doppler ultrasonography. Anticoagulant therapy was started instantly. Additional urinalysis revealed erythrocyturia (189/µl) without dysmorphia and 14.5 grams protein in 24 hours. Clotting assays showed no abnormalities and autoimmune serology and screening for malignancies and chronic infections were negative.

WHAT IS YOUR DIAGNOSIS?

See page 484 for the answer to this photo quiz.

Figure 1. *(A) Contrasted CT scan showing a hypodense mass in the right renal vein suggestive for thrombus. (B) Thrombus extending into the inferior vena cava and both iliac veins*



Netherlands
The Journal of Medicine

PHOTO QUIZ

Skin lesions in a patient with head and neck cancer

C.M.L. Driessen[1]*, M.M. van Rossum[2], W.A.M. Blokx[3], C.M.L. van Herpen[1]

Department of [1]Medical Oncology, [2]Dermatology, and [3]Pathology, Radboud university medical center, Nijmegen, the Netherlands, *corresponding author: tel.: +31 (0)24 3667264, fax: +31 (0)24 3540788, e-mail: chantal.driessen@radboudumc.nl

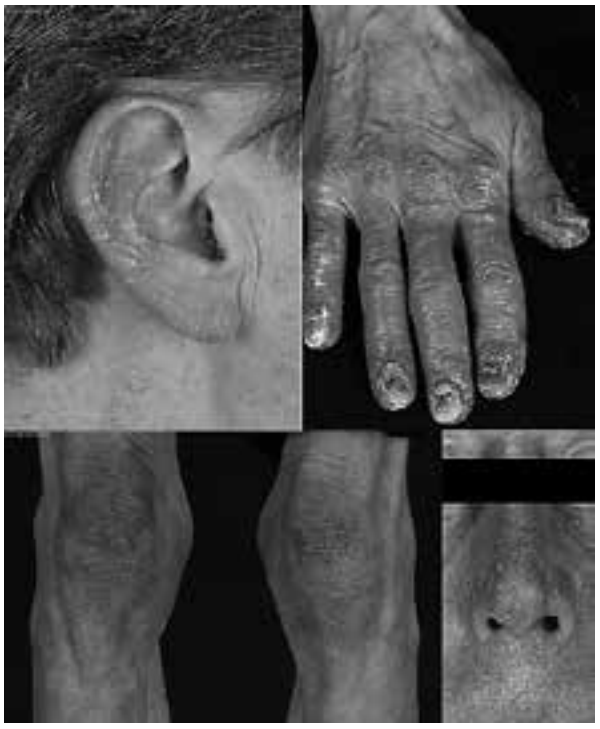
CASE REPORT

A 61-year-old man presented with a six-month history of skin lesions. It started with blisters on both hands and pruritis. Shortly thereafter, his feet were also involved and they became swollen. After four months, skin lesions evolved on his nose and ears. The patient reported weight loss, but no further symptoms. Before presentation in our clinic, he was treated with class III corticosteroid ointment oral antibiotics and zinc ointment, without effect.

His medical history revealed a tumour resection followed by postoperative radiotherapy for a $pT_4N_{2b}M_0$ squamous cell carcinoma of the hypopharynx 13 years before presentation with the skin lesions.

On dermatological examination in our clinic, livid erythematous squamous plaques on the hands, feet, knees and nose were seen. There was a subungual hyperkeratosis with onycholysis on both the hands and feet (*figure 1*).

Figure 1. *Our patient at presentation*



WHAT IS YOUR DIAGNOSIS?

See page 485 for the answer to this photo quiz.

Netherlands
The Journal of Medicine

PHOTO QUIZ

Bilateral abdominal mass

T.M. Anoop*, N. Jain, S.G Nair, N. Geetha

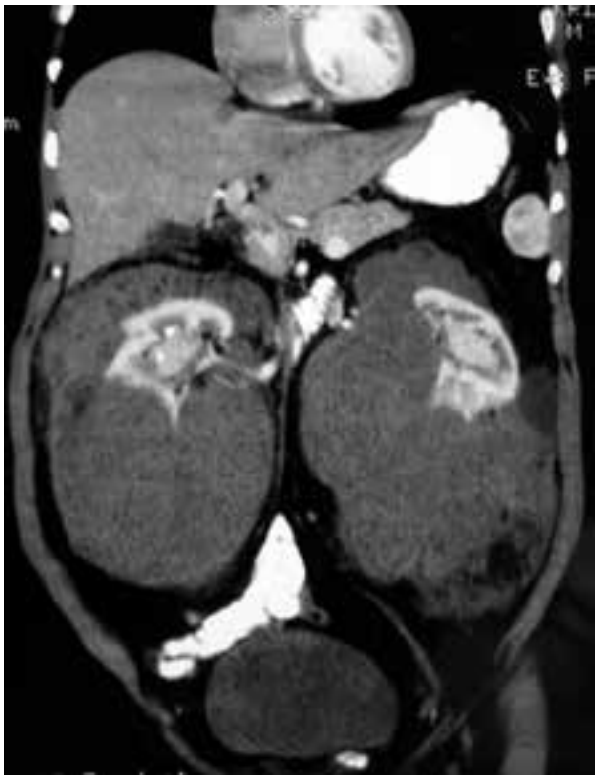
Department of Medical Oncology, Regional Cancer Centre, Thiruvananthapuram, Kerala, South India,
*corresponding author: e-mail: dranooptm@yahoo.co.in

A 58-year-old man presented with abdominal distension of four months duration. On examination, a ballotable abdominal mass was palpable on both sides of the abdomen. Computer tomography (CT) of the abdomen and pelvis revealed deformed kidneys almost encased by a bulky soft-tissue dense mass bilaterally (*figure 1*). CT of the chest was normal. Renal function tests were normal. CT-guided biopsy from the mass showed collagen-forming spindle cells.

WHAT IS YOUR DIAGNOSIS?

See page 486 for the answer to this photo quiz.

Figure 1A. *Computer tomography (CT) of abdomen and pelvis showing deformed kidneys almost encased by a bulky soft-tissue dense mass bilaterally*



Netherlands
The Journal of Medicine

PHOTO QUIZ

A diagnosis on the basis of a blood smear

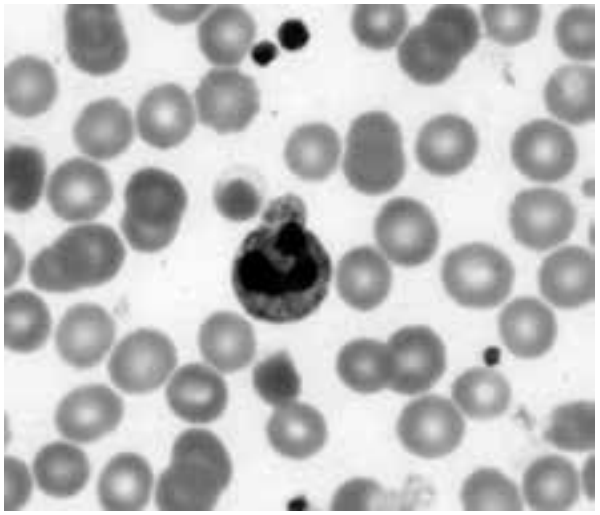
A.P.M. Kerckhoffs[1]*, A.H.E. Herbers[1], N.C.V. Pequeriaux[2]

Department of [1]Internal Medicine and [2]Clinical Chemistry and Haematology, Jeroen Bosch Hospital, Den Bosch, the Netherlands, *corresponding author: angelekerckhoffs@hotmail.com

CASE

A 64-year-old women of mixed German and Scandinavian descent presented to the emergency department with a two-day history of abdominal pain, diarrhoea and dark urine. There was no fever present. One month before, she had travelled to Brazil for which no malaria prophylaxis was recommended. Physical examination revealed an icteric women with a body temperature of 36.4°Celsius. Palpation of the upper abdomen was slightly painful. Hepatosplenomegaly was absent.
Laboratory results showed a Coombs-negative haemolytic anaemia (haemoglobin 6.4 mmol/l, reticulocytes 206 x 10^9/l, haptoglobin <0.08 g/l and lactate dehydrogenase 744 U/l) with a thrombocyte count of 203 x 10^9/l and a leukocyte count of 13.4 x 10^9/l. C-reactive protein was 25 mg/l. In *figure 1* the blood smear is shown.

Figure 1. *Blood smear*



WHAT IS YOUR DIAGNOSIS?

See page 487 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 480)

A MALE WITH EXTENSIVE RENAL VEIN THROMBOSIS

DIAGNOSIS

The patient was diagnosed with nephrotic syndrome complicated by renal vein thrombosis (RVT). He commenced prednisolone 60 mg once daily for a presumed diagnosis of idiopathic membranous nephropathy (IMN) or minimal change disease (MCD). Sodium restriction and subsequent angiotensin converting enzyme inhibitor (ACE-I) treatment were installed consecutively. After ten weeks of therapy, control of proteinuria was inadequate and tacrolimus 5 mg once daily was added, since both IMN and MCD have shown favourable clinical responses to this treatment.[1,2] This led to a significant reduction in the proteinuria (0.46 grams in 24 hours). After three months, CT scanning showed almost complete resolution of thrombus, after which anticoagulant treatment was stopped and a kidney biopsy was performed, whereupon the diagnosis of IMN was confirmed.

The nephrotic syndrome is defined by a urinary protein level exceeding 3.5 grams in 24 hours and is associated with oedema, hypoalbuminaemia, hyperlipidaemia, and infectious and thromboembolic complications.[3] Thromboembolic disease is a serious complication in patients with nephrotic syndrome and may arise from preferential loss of proteins which inhibit systemic haemostasis, increased synthesis of prothrombic factors and local activation of glomerular haemostasis.[4] Venous thromboembolic complications in nephrotic syndrome include deep venous thrombosis, pulmonary embolism and RVT. RVT may be associated with nephrotic syndrome of any aetiology but most commonly occurs in patients with membranous nephropathy, with a prevalence of 37%.[4] Symptoms of RVT can include acute flank pain, gross haematuria, and deterioration of renal function but is usually asymptomatic.[4-6] Doppler ultrasonography is the primary modality for detection of RVT, alternatives being contrast-enhanced CT and magnetic resonance imaging.[6] Anticoagulant therapy is the treatment of choice[4] in addition to treatment of the proteinuria (ACE inhibition, immunomodulation) and of the underlying disease if present.

ACKNOWLEDGEMENTS

No funding sources or conflicts of interest to report.
The authors thank H.M. Suliman, Department of Radiology, St. Lucas Andreas Hospital Amsterdam, the Netherlands, for interpreting the CT scan and providing the images.

REFERENCES

1. Yuan H, Liu N, Sun GD, et al. Effect of prolonged tacrolimus treatment in idiopathic membranous nephropathy with nephrotic syndrome. Pharmacology. 2013;91:259-66.
2. Li H, Shi X, Shen H, et al. Tacrolimus versus intravenous pulse cyclophosphamide therapy in Chinese adults with steroid-resistant idiopathic minimal change nephropathy: a multicenter, open-label, nonrandomized cohort trial. Clin Ther. 2012;34:1112-20.
3. Orth SR, Ritz E. The nephrotic syndrome. N Engl J Med. 1998;338:1202-11.
4. Singhal R, Brimble KS. Thromboembolic complications in the nephrotic syndrome: pathophysiology and clinical management. Thromb Res. 2006;118:397-407.
5. Llach F, Papper S, Massry SG. The clinical spectrum of renal vein thrombosis: acute and chronic. Am J Med. 1980;69:819-27.
6. Yang GF, Schoepf UJ, Zhu H, et al. Thromboembolic complications in nephrotic syndrome: imaging spectrum. Acta Radiol. 2012;53:1186-94.

ANSWER TO PHOTO QUIZ (PAGE 481)

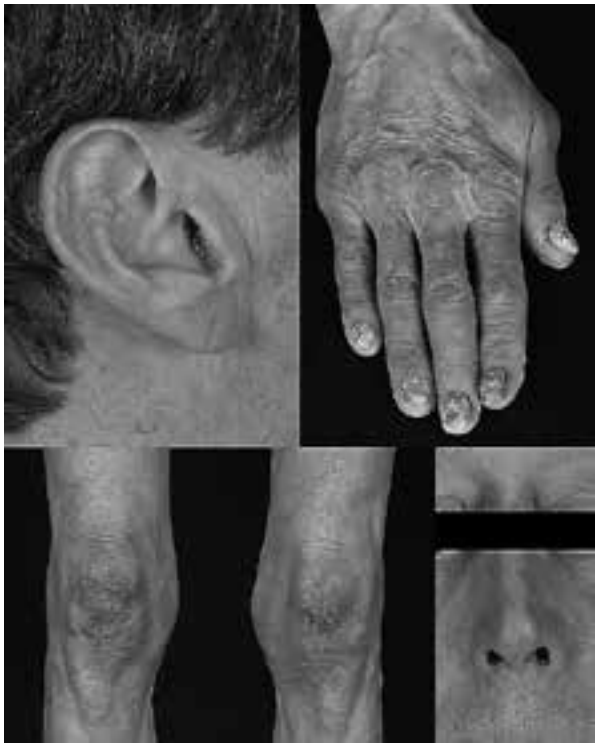
SKIN LESIONS IN A PATIENT WITH HEAD AND NECK CANCER

DIAGNOSIS

Histopathological examination of a lesion on his left hand revealed a slightly acanthotic epidermis with hyperparakeratosis. There was a perivascular, partially bandlike, mononuclear inflammatory infiltrate with spongiosis. Furthermore, an interface dermatitis was seen. Both the clinical and histopathological picture were compatible with the Bazex syndrome. By means of a fungal culture, an onychomycosis was diagnosed.

Physical examination by the ENT physician showed a second primary $T_2N_1M_1$ hypopharynx tumour with axillary lymph node metastasis. Because palliative chemotherapy was not an option due to his low WHO performance score, we started dexamethasone 10 mg once daily. Within one week an evident improvement of his skin lesions was observed (*figure 2*). The patient continued his treatment and tapered the dexamethasone gradually with good results until he died three months later.

Figure 2. *Our patient after one week treatment with dexamethasone*



DISCUSSION

The Bazex syndrome, also called acrokeratosis paraneoplastica, is a rare paraneoplastic acral erythematosquamous dermatosis associated with internal malignancy. This syndrome was first described in 1965 by Bazex *et al.* in a patient with a squamous cell carcinoma of the head and neck region in association with cutaneous lesions which resolved after the malignancy was treated.[1] No cases without underlying malignancy have been described. The most common associated neoplasms are squamous cell carcinomas of the upper aerodigestive tract and other malignancies with cervical or mediastinal lymph node metastases.[1,2] The syndrome is more common in men and the mean age is 60 years. In almost 70% of the cases the cutaneous symptoms precede the diagnosis of the malignancy by several months. Therefore diagnosis is often difficult.[2] The clinical features are violet to red psoriasiform plaques on acral sites. The nails are often involved with subungual hyperkeratosis, onycholysis, yellow discoloration and ridging.[3] Histopathological findings are often aspecific and clinical and histopathological features can mimic several other conditions such as psoriasis, eczematous dermatitis or lichen planus.[3] The pathophysiology of the syndrome is still unknown, but it is hypothesised that an underlying immunological mechanism or tumour-produced growth factor play a role.[2-4] The symptoms improve or resolve after treatment of the underlying malignancy.[2-4] Treatment with dexamethasone has not been described before, but showed to be effective in our patient with an improvement in his quality of life.

REFERENCES

1. Bazex A, Salvador R, Dupré A, et al. Syndrome paranéoplasique á type d'hyperkératose des extrémités. Guérison après le traitement de l'épithélioma laryngé. Bull Soc Fr Dermatol Syphiligr. 1965;72:182.
2. Bolognia JL, Brewer YP, Cooper DL. Bazex síndrome (Acrokeratosis Paraneoplastica). An analytic review. Medicine. 1991;70:269-80.
3. Bolognia JL. Bazex' syndrome. Clin Dermatol. 1993;11:37-42.
4. Pecora AL, Landsman L, Imgrund SP, et al. Acrokeratosis paraneoplastica (Bazex's syndrome). Arch Dermatol. 1983;199:820-6.

ANSWER TO PHOTO QUIZ (PAGE 482)

BILATERAL ABDOMINAL MASS

DIAGNOSIS

CT-guided biopsy from the mass showed a collagen-forming spindle-cell lesion suggestive of fibromatosis. A diagnosis of fibromatosis involving both kidneys was made. Fibromatosis or desmoid tumour covers a broad spectrum of benign fibrous tissue proliferations. Fibromatosis is usually characterised by infiltrative growth and a tendency towards recurrence. It never develops metastasis.[1] It can occur in a variety of anatomic locations such as intra-abdominal, retroperitoneal or in the thoracic wall. However, solitary occurrence of fibromatosis involving both kidneys has not been reported.

Association with surgical trauma and familial adenomatous polyposis (Gardner syndrome) has been reported.[2]

Complete resection is the therapy of choice for fibromatosis.[3] Radiation therapy is accepted as an effective treatment after incomplete resection. Adjuvant therapy using non-steroidal anti-inflammatory drugs (NSAIDs), tamoxifen, interferon, antineoplastic agents is not promising.

Since our patient had only minimal symptoms with preserved renal function, no active treatment was given and he was put on follow-up. The patient has been asymptomatic for the past four years of follow-up.

REFERENCES

1. Weiss SW, Goldblum JR: Fibromatosis. In Soft tissue tumors. Weiss SW, Goldblum JR (editors). St. Louis, Mosby; 2001:309-46.
2. Rodriguez-Bigas MA, Mahoney MC, Karakousis CP, Petrelli NJ. Desmoid tumors in patients with familial adenomatous polyposis. Cancer. 1994;74:1270-4.
3. Overhaus M, Decker P, Fischer HP, Textor HJ, Hirner A. Desmoid tumors of the abdominal wall: A case report. [http://www.wjso.com/content/1/1/11] World J Surg Oncol. 2003;1:11.

ANSWER TO PHOTO QUIZ (PAGE 483)

A DIAGNOSIS ON THE BASIS OF A BLOOD SMEAR

Prompt blood smear examination showed mainly blister cells. Blister cells are characteristic of acute haemolysis induced by oxidative stress. Because of the oxidative denaturation of the haemoglobin, it is piled at one side of the cell.[1,2] These blister cells raised a strong suspicion on the presence of a glucose-6-phosphate dehydrogenase (G6PD) deficiency. G6PD deficiency was confirmed by decreased erythrocytic G6PD activity (5 U/gram Hb).

This X-linked hereditary disorder mainly affects people from Mediterranean, African, Middle Eastern and South Asian descent.[3] Reduced G6PD concentrations render erythrocytes susceptible to haemolysis under oxidative conditions induced by oxidant drugs, infection, chemicals such as naphthalene or ingestion of fava beans.

Our patient had a urinary tract infection and had eaten fresh fava beans two days before presentation. During the trip to Brazil, she had put mothballs in her pocket. Mothballs with naphthalene are not produced anymore. This case is remarkable for the German/Scandinavian descent and the age of first symptoms. First presentation at an older age can be due to X-chromosome inactivation.[4]

REFERENCES

1. Fischer TM, Meloni T, Pescarmona GP, Arese P. Membrane cross bonding in red cells in favic crisis: a missing link in the mechanism of extravascular haemolysis. Br J Haemotol. 1985;59:159.
2. Bain BJ. Diagnosis from the blood smear. New Engl J Med. 2005;353:498-507.
3. Cappellini. Glucose-6-phosphate dehydrogenase deficiency. Lancet. 2008;371:64-74.
4. Au WY, Lam V, Pang A, et al. Glucose-6-phosphate dehydrogenase deficiency in female octogenarians, nanogenarians and centenarians. J Gerontol A Biol Sci Med Sci. 2006;61:1086.

Netherlands
The Journal of Medicine

SPECIAL ARTICLE

Red cell distribution width as predictor for mortality in critically ill patients

I.A. Meynaar[1,2], A.H.M. Knook[1], S. Coolen[3], H. Le[3], M.M.E.M. Bos[4], F. van der Dijs[3], M. von Lindern[5], E.W. Steyerberg[6]

[1]Intensive Care Unit, [3]Department of Clinical Chemistry and [4]Department of Medical Oncology, Reinier de Graaf Hospital, Delft, the Netherlands, [2]Intensive Care Unit, Haga Hospital, The Hague, the Netherlands, [5]Sanquin Research and Landsteiner Laboratory Amsterdam Medical Centre, Amsterdam University, Amsterdam, the Netherlands, [6]Department of Public Health, Erasmus MC – University Medical Centre Rotterdam, Rotterdam, the Netherlands, *corresponding author: e-mail: iwanmeynaar@gmail.com

ABSTRACT

Background: The objective of this study was to evaluate whether the red cell distribution width (RDW) is a significant risk factor for hospital mortality in critically ill patients and to investigate whether RDW is a parameter indicating inflammation, or a risk factor independent of inflammation.

Methods: We studied all patients admitted to a ten-bed mixed intensive care unit in the Netherlands between May 2005 and December 2011 for whom RDW was available, and who had not received a blood transfusion in the preceding three months. Inflammation was measured by C-reactive protein and leucocyte count. Analyses included correlation, logistic regression analysis, and receiver-operating characteristic (ROC) curves.

Results: We included 2915 patients, of whom 387 (13.3%) did not survive to hospital discharge. In univariate analysis higher RDW values were associated with increased hospital mortality. In multivariate analysis RDW remained an independent risk factor for mortality after correction for APACHE II score, age, admission type and mechanical ventilation (odds ratio 1.04, 95% confidence interval 1.02-1.06, for each femtolitre of RDW). Adding RDW to APACHE II, however, increased the area under the ROC curve marginally (from 0.845 to 0.849, p<0.001). RDW was not correlated with C-reactive protein and leucocyte count, refuting the hypothesis that the association between RDW and outcome is mediated through inflammation.

Conclusion: In critically ill patients, the RDW on ICU admission was an independent predictor of mortality. Since RDW was not correlated with inflammation, the underlying mechanism of this association warrants further investigation.

KEYWORDS

Anaemia, critical care, CRP, inflammation, outcome, RDW

INTRODUCTION

Erythrocytes differ in size, getting gradually smaller during ageing. Their mean volume is quantified by the mean corpuscular volume (MCV) and the variation in size by the red cell distribution width (RDW). RDW is calculated automatically when a full blood count is requested, but the clinical usefulness of RDW is limited to the differential diagnosis of anaemia. Recent studies found associations of an increased variation in the size of the erythrocytes as measured by RDW with mortality irrespective of mean cellular volume (MCV) and haemoglobin levels. Associations were reported for patients with heart failure,[1-3] acute myocardial infarction,[1,4] community-acquired pneumonia,[5] pulmonary hypertension[6] and in the general population.[7-9] In three studies involving critically ill patients, an increased RDW was independently associated with increased mortality.[10-12] In patients with pulmonary hypertension, RDW was superior to N-terminal-pro-B-type natriuretic peptide (NT-pro-BNP) in predicting outcome.[6] In patients with heart failure, RDW was as good as NT-pro-BNP and superior to New York Heart Association class, renal function and even ejection fraction in predicting outcome.[3,13] While the association between increased RDW and increased mortality seems to be an almost universal finding, the reason for this remains to be elucidated. One of the possible explanations, suggested by several authors, is that an increased RDW is caused by a state of

inflammation.[4] Lippi *et al*. found a correlation between RDW and erythrocyte sedimentation rate in their analysis of routinely acquired haematological data of outpatients.[14] Perlstein *et al*. found an association between RDW and C-reactive protein in a community-based cohort, but since no explanation for this association was given it is conceivable that the association was found by chance and for instance based on confounding.[9] Vitamin and nutritional deficiency, especially deficiencies in iron, folate and vitamin B12, can cause an increased RDW, but Perlstein *et al*. found RDW to be an independent predictor of mortality even after correction for vitamin deficiencies.[9] Bone marrow dysfunction, haemodilution, renal insufficiency and abnormalities of erythropoietin response have also been mentioned as possible explanations.[2,3]
The objective of this study was to evaluate the prognostic importance of increased red cell distribution width (RDW) with hospital mortality in critically ill patients. Specifically, we aimed to test the hypothesis that inflammation explains the association between RDW and mortality.

MATERIALS AND METHODS

Setting

The study was conducted in the single mixed medical and surgical adult intensive care unit (ICU) of the Reinier de Graaf Hospital in Delft, the Netherlands. The hospital is a 500-bed teaching hospital covering all specialities except cardiac surgery and neurosurgery. The closed format ICU has ten beds and is intensivist-run with 24/7 availability. The intensivists decide on admission to and discharge from the ICU. The nurse to patient ratio is 1:2. During the study period APACHE II standardised mortality rate (SMR), the ratio between observed hospital mortality and expected hospital mortality according to APACHE II, was 0.73. The hospital has an ICU-based medical emergency team.

Patient selection

All consecutive patients admitted to the ICU between 1 May 2005 and 31 December 2011, for whom a full blood count with RDW was available in the laboratory database, were entered into the study. If a patient was readmitted to the ICU during the study period, only the first admission was used. The need for ethical approval and informed consent was waived by the local medical ethics committee (METZ Zuid West Holland).

Patient data

Demographic data, APACHE II scores, expected mortality and outcome data (ICU and hospital mortality) were collected on admission as part of the routine and mandatory data collection for the Dutch Intensive Care Registry (National Intensive Care Evaluation, NICE). Physiological data and data about patient history were collected on paper by the intensivist on call, demographic data and laboratory data were collected electronically. All data were entered into a dedicated ICU database (Mediscore, Itemedical, Tiel, the Netherlands). Data quality was checked regularly by external officials from NICE. In accordance with APACHE II and NICE definitions, patients were defined as medical patients if they had not undergone surgery in the preceding seven days and were not admitted to prepare them for surgery. Planned or elective surgery was defined as surgery according to the schedule, emergency or urgent surgery was defined as surgery that had not been planned beforehand. Patients fulfil APACHE II criteria when they have been treated in the ICU for at least eight hours, when they are 16 years or older and when they have not been admitted to the ICU before during this hospital admission.

Laboratory investigations

The starting date of May 2005 was chosen because from that moment on RDW was measured automatically whenever haemoglobin (Hb) was requested. For all requested Hb values RDW, haematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were available in the laboratory database. Leucocyte count and CRP were not completely available. For the study we only included laboratory results from blood samples that were taken within 24 hours before until 24 hours after the time of admission to the ICU. RDW is the standard deviation of MCV and can be given in femtolitre (fl) as is done in this study. Some authors present RDW as the coefficient of variation which is calculated as 100 x (RDW/MCV). Normal values for Hb were 7.3-9.8 mmol/l (adult women) and 8.4-10.9 mmol/l (adult men), for Ht 0.36-0.48 l/l and 0.42-0.51 l/l respectively, for MCV 83-98 fl, for RDW 37.0-48.0 fl, for leucocyte count 3.5-11.0 x 10^9/l, for MCH 1.60-2.06 mmol/cell, for MCHC 19.5-22.5 mmol/l and for CRP 0-10 mg/l. Haematological parameters were determined using XE-5000 analysers (Sysmex Corp., Tokyo, Japan). This analyser calculates the MCV, MCH and MCHC based on measurement of Hb and Ht. CRP was determined by a latex immunoassay using the Architect C16000(Abbott, Illinois, USA).

Statistical analysis

The primary study endpoint was hospital mortality. We studied the association between hospital mortality and available potential risk factors for mortality, namely APACHE II score, age, mechanical ventilation during ICU treatment, sepsis on admission to the ICU, admission type, and also the laboratory parameters Hb, Ht, RDW, MCV, MCH, MCHC, leucocyte count and CRP. We only used

the laboratory results taken within 24 hours before to 24 hours after the time of admission to the ICU. Continuous data are reported as mean and standard deviation (SD) when normally distributed or otherwise as median and interquartile range (IQR) and compared using Student's t-test or Mann Whitney U test as appropriate. Normality was checked using histograms. Categorical data were compared using the Chi square test and by calculating odds ratios and 95% confidence intervals (CI). Univariate association between potential risk factors and mortality was done by stratified analysis using quartiles. Logistic regression analysis was used to study risk factors for hospital mortality. For logistic regression analysis APACHE II score, age, RDW, Ht, Hb, MCV, MCH, MCHC and CRP were entered as continuous variables. Sepsis on admission to the ICU (yes/no), mechanical ventilation during ICU treatment (yes/no), admission type (medical, planned surgery or emergency surgery) and leucocyte count (using quartiles) were entered as categorical variables. Improvement in model fit was statistically tested by likelihood ratio statistics and further quantified by the area under the receiver operating characteristic (AUC-ROC) curve. To avoid multicollinearity correlation between parameters this was checked using Pearson's R for normally distributed parameters or otherwise with Spearman's rho. To test the hypothesis that RDW is associated with increased mortality because RDW is associated with inflammation, the correlation between RDW and leucocyte count and correlation between RDW and CRP was quantified using Spearman's rho. Two-sided comparisons with 95% confidence intervals (95% CI) were used and p values of less than 0.05 were considered statistically significant. Data were analysed with SPSS 18.0 (SPSS, Chicago, IL, USA).

RESULTS

Between May 2005 and December 2011 there were 4568 ICU admissions of 3954 individual patients. RDW was available for 3345 patients, and absent for 609 patients. We excluded the 330 patients who had received a blood transfusion in the three months preceding ICU admission. This left us with 2915 patients fulfilling inclusion criteria to be analysed. Basic characteristics are shown in *table 1*. The top 5 primary medical diagnoses were sepsis (n=224, 19.3%), respiratory disease (n=124, 10.7%), pneumonia (n=119, 10.3%), drug overdose (n=98, 8.5%) and cardiac arrest (n=94, 8.1%). The top 5 primary surgical diagnoses were gastrointestinal surgery (n=635, 36.1%), vascular surgery (n=507, 28.9%), sepsis (n=169, 9.6%), thoracic surgery for cancer (n=116, 6.6%) and renal/bladder surgery (n=115, 6.5%). Univariate analysis, using quartiles, indicated that hospital mortality was associated with haemoglobin, haematocrit, RDW, MCHC, leucocytes and CRP, but not with MCV and MCH (*table 2*). The association was linear except for leucocytes where the association was curvilinear: mortality was lowest in the range of 7.94-10.88 x 10^9/l and higher below and above that range. High correlations were found between haemoglobin and haematocrit (R=0.975), between MCV and MCH (R=0.817), between MCH and MCHC (R=0.481) and between sepsis and CRP (R=0.478). We hypothesised that the increase in RDW would be a sign of inflammation and therefore there should be a correlation between RDW and CRP or leucocytes. However, the correlation between RDW and leucocytes was very low (Pearson R = 0.003) and the correlation between RDW and CRP (in 1743 patients with CRP available) was also low (Spearman's rho = 0.062), refuting this hypothesis.

Logistic regression analysis showed that RDW was the only haematological parameter that was an independent risk factor for mortality (*table 3*, Wald statistic 14, p<0.001). However, the APACHE II score (Wald statistic 176), age (Wald statistic 64), and mechanical ventilation (Wald statistic 31) were strong predictors. Addition of RDW to the APACHE II score only increased the AUC-ROC from 0.845 to 0.849 (p<0.001, likelihood ratio test). The interrelated parameters haemoglobin and haematocrit, MCV and MCHC, and leucocytes were not independently related to mortality in a model with age and APACHE II score. When we used CRP instead of sepsis in similar models (with 1682 patients in whom both APACHE II score and CRP were available) we found similar results: RDW remained an independent risk factor for mortality, while CRP was not (data not shown).

DISCUSSION

The aim of our study was to evaluate the relevance of RDW as an independent risk factor for mortality of critically ill patients. In this retrospective study of patients in a mixed general ICU we found that an increased variation in the size of a patient's erythrocytes (RDW) at admission to the ICU was an independent prognostic factor for in-hospital mortality after correction for APACHE II score, age, mechanical ventilation, sepsis and admission type. However, adding RDW to APACHE II only marginally increased the area under the ROC curve for the prediction of mortality. Although it has been suggested that RDW reflects inflammation, we did not find a correlation between RDW and CRP or between RDW and leucocyte count.

This study confirms the findings of the three previous studies on RDW and outcome in critically ill patients.[10-12] Bazick *et al.* studied over 50,000 patients from two tertiary academic hospitals and found RDW to be a strong and

Table 1. *Patient characteristics*

	All	Discharged alive	Died in hospital	P[2]
All patients	2915	2528 (86.7%)	387 (13.3%)	
Male	1665	1447 (86.9%)	218 (13.1%)	ns
Medical	1158	915 (79.0%)	243 (21.0%)	<0.001
Planned surgery	1418	1330 (93.8%)	88 (6.2%)	<0.001
Urgent surgery	339	283 (83.5%)	56 (16.5%)	<0.001
Age (years)	65.1 (16.0)	63.9 (16.2)	73.0 (12.3)	<0.001
<50	484	467 (96.5%)	17 (3.5%)	<0.001
50-59	443	404 (91.2%)	39 (8.8%)	<0.001
60-69	653	585 (89.6%)	68 (10.4%)	<0.001
70-79	841	711 (84.5%)	130 (15.5%)	<0.001
80-89	450	332 (73.8%)	118 (26.2%)	<0.001
>=90	44	29 (65.9%)	15 (34.1%)	<0.001
APACHE II exp mort[1]	10% (5-24)	9% (4-18)	44% (21-72)	<0.001
APACHE II score	12.0 (7.4)	12.1 (6.2)	22.3 (8.8)	<0.001
APACHE II 0-10	1154	1126 (97.6%)	28 (2.4%)	<0.001
APACHE II 11-20	1247	1104 (88.5%)	143 (11.5%)	<0.001
APACHE II 21-30	322	211 (65.5%)	111 (34.5%)	<0.001
APACHE II >30	107	29 (27.1%)	78 (72.9%)	<0.001
No sepsis on admission	2459	2188 (89.0%)	271 (11.0%)	<0.001
Sepsis on admission	456	340 (74.6%)	116 (25.4%)	<0.001
No mechanical ventilation	1846	1739 (94.2%)	107 (5.8%)	<0.001
Mechanical ventilation	1069	2528 (86.7%)	387 (13.3%)	<0.001
LOS ICU (days)	1.2 (0.8-3.3)	1.0 (0.8-2.9)	2.4 (0.9-6.0	<0.001
LOS hospital (days)	10 (6-18)	11 (7-19)	7 (3-15)	<0.001
Haemoglobin (mmol/l)	6.9 (1.3)	6.9 (1.3)	6.7 (1.5)	0.002
Haematocrit (l/l)	0.33 (0.06)	0.34 (0.06)	0.33 (0.07)	0.046
MCV (fl)	89.8 (6.0)	89.7 (5.9)	90.5 (6.7)	0.023
MCH (fmol/cell)	1.86 (0.14)	1.86 (0.14)	1.85 (0.15)	ns
MCHC (mmol/l)	20.7 (0.85)	20.7 (0.83)	20.5 (0.95)	<0.001
RDW (fl)	47.3 (6.4)	46.9 (6.2)	49.7 (6.9)	<0.001
Leucocytes (x 10^9/l)	11.9 (6.1)	11.8 (5.8)	12.6 (8.0)	ns
CRP (mg/l)[3]	48 (11-159)	41 (9-152)	88 (28-176)	<0.001

Data are presented as mean (SD), median (IQR) or number (percentage) as appropriate. [1]for 2830 patients fulfilling APACHE II inclusion criteria; [2]Chi square test, t test or Mann Whitney U test as appropriate; [3]CRP was available in 1743 patients; CRP = C-reactive protein; Exp mort = expected mortality; LOS = length of stay; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume; ns = not significant; RDW = red cell distribution width.

independent predictor of mortality. Wang *et al.* found RDW not only to be an independent predictor for mortality but also for hospital length-of-stay in a single centre study involving 602 patients. In their multicentre study of 17,922 ICU patients, Hunziker *et al.* found that RDW improved prognostication of SAPS I score. Newer and more frequently used models such as SAPS II, APACHE II or APACHE IV, however, were not included in these studies. The present study is the first to evaluate the predictive properties of RDW in addition to the commonly used APACHE II model.

It has been suggested that the association between RDW and mortality can be explained by RDW being a marker of inflammation. However, we found no correlation between RDW and available markers of inflammation. RDW may also be increased by blood transfusions, by the presence of anaemia and by dietary status (iron, folate and vitamin B12). In our study, patients with one or more blood transfusions in the three months preceding admission to the ICU were excluded, but information on iron deficiency, folate or vitamin B12 was not available. An increase in RDW without a change in MCV implies an increase in

Table 2. *Univariate analysis of haematological parameters and mortality*

	Number	Hospital mortality	OR (95% CI)	P
RDW <43.20 fl	714	53 (7.4%)	Ref= 1	<0.001
RDW 43.20-46.09 fl	734	80 (10.9%)	1.53 (1.06-2.19)	<0.001
RDW 46.10-49.69 fl	729	84 (11.5%)	1.62 (1.13-2.34)	<0.001
RDW >49.70 fl	738	170 (23.0%)	3.73 (2.79-5.18)	<0.001
Haematocrit ≥0.37	807	100 (12.4%)	Ref=1	0.001
Haematocrit 0.33-0.36	719	85 (11.8%)	0.95 (0.70-1.29)	0.001
Haematocrit 0.29-0.32	848	101 (11.9%)	0.96 (0.71-1.28)	0.001
Haematocrit <0.29	541	101 (18.7%)	1.62 (1.20-2.19)	0.001
Haemoglobin ≥7.8 mmol/l	740	90 (12.2%)	Ref=1	<0.001
Haemoglobin 6.9-7.7 mmol/l	737	83 (11.3%)	0.92 (0.67-1.26)	<0.001
Haemoglobin 6.0-6.8 mmol/l	774	93 (12.0%)	0.99 (0.72-1.34)	<0.001
Haemoglobin <6.0 mmol/l	664	121 (18.2%)	1.61 (1.20-2.16)	<0.001
Leucocytes <7.94	728	109 (15.0%)	1.73 (1.25-2.39)	<0.001
Leucocytes 7.94-10.88	725	67 (9.2%)	Ref=1	<0.001
Leucocytes 10.89-14.51	731	88 (12.0%)	1.34 (0.96-1.88)	<0.001
Leucocytes ≥14.52	730	123 (16.8%)	1.99 (1.45-2.73)	<0.001
CRP <11 mg/l[1]	430	33 (7.7%)	Ref=1	<0.001
CRP 11-47 mg/l	440	79 (18.0%)	2.63 (1.71-4.05)	<0.001
CRP 48-158 mg/l	434	97 (22.4%)	3.46 (2.27-5.27)	<0.001
CRP >=159 mg/l	439	98 (22.3%)	3.46 (2.27-5.26)	<0.001
MCV <86.20 fl	713	96 (13.5%)	Ref=1	ns
MCV 86.20-89.59 fl	744	86 (11.6%)	0.84 (0.62-1.15)	ns
MCV 89.60-93,09 fl	721	88 (12.2%)	0.89 (0.66-1.22)	ns
MCV ≥93.10 fl	737	117 (15.9%)	1.21 (0.91-1.63)	ns
MCH ≥1.94	740	95 (12.8%)	Ref=1	ns
MCH 1.860-1.939	729	95 (13.0%)	1.02 (0.75-1.38)	ns
MCH 1.781-1.859	715	85 (11.9%)	0.92 (0.67-1.25)	ns
MCH <1.781	728	112 (15.4%)	1.23 (0.92-1.66)	ns
MCHC ≥21.30	729	75 (10.3%)	Ref=1	<0.001
MCHC 20.70-21.29	826	90 (10.9%)	1.07 (0.77-1.47)	<0.001
MCHC 20.20-20.69	675	91 (13.5%)	1.36 (0.98-1.88)	<0.001
MCHC <20.20	681	130 (19.1%)	2.06 (1.52-2.79)	<0.001

[1]C-reactive protein (CRP) was available in 1743 patients. All parameters except mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MVC) are associated with mortality. The association is linear (mortality either increases or decreases with increasing value of the parameter) except for leucocytes where the association is curvilinear (mortality is lowest in the range of 7.94-10.88 and is higher below and above that range). MCHC = mean corpuscular haemoglobin concentration; ns = not significant; RDW = red cell distribution width.

the number of smaller and an increase in the number of larger erythrocytes in the blood. The increased number of smaller erythrocytes may be explained by an increase in vesicle shedding by erythrocytes potentially due to membrane damage inflicted by alterations in the injured tissue capillary bed. Thus, accelerated vesicle shedding enhances erythrocyte clearance. To compensate this clearance may result in enhanced, erythrocyte production and the release of relatively large reticulocytes from the bone marrow. Future measurement of reticulocyte counts may indicate whether an increased RDW indicates enhanced ageing and reticulocyte release. Hence, the functional implications of RDW as an independent risk factor for mortality and morbidity needs further study.

From a prediction perspective it appears that RDW is helpful in refining prognostic estimates for mortality and length of stay, but only to a minor extent (small increase in ROC area). Since RDW is a cheap laboratory

Table 3. *Logistic regression analysis in 2830 patients fulfilling APACHE II criteria of all significant predictors of mortality*

	Wald	p	OR (95% CI)
APACHE II	176	<0.001	1.14 (1.12-1.16)
Age	64	<0.001	1.04 (1.03-1.06)
Mechanical ventilation	31	<0.001	2.32 (1.72-3.12)
RDW	14	<0.001	1.04 (1.02-1.06)
Admission type	12	0.003	
-Urgent vs planned surgery (=ref)	7	0.008	1.76 (1.16-2.66)
-Medical vs planned surgery (=ref)	10	0.002	1.68 (1.21-2.33)

Final model, showing that even after correction for APACHE II score, age, mechanical ventilation and admission type, red cell distribution width (RDW) is an independent risk factor for mortality. When sepsis, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration or leucocytes were entered in this model instead of RDW, none of these were significant risk factors (data not shown).

measurement, its addition to risk prediction algorithms may still be cost-effective.[15] However, to achieve a significant improvement in current prediction scores of APACHE II and other prognostic factors, a larger set of new biomarkers is required.

We acknowledge several weaknesses and limitations in our study. Firstly, we performed a retrospective study and some possibly interesting data are lacking: reticulocyte count, iron status, folate and vitamin B12. Secondly, regarding the association between RDW and CRP, the latter was only available for 60% of patients and it is most likely that these patients were more seriously ill. Also the absence of other markers of inflammation, such as procalcitonin or interleukin-6, hampered further evaluation of the relationship between RDW and inflammation. The strengths of our study include the prospective collection of clinical and laboratory data, and the observation that different statistical analyses produced similar results.[16]

CONCLUSION

In conclusion, an increased variation in the size of erythrocytes on admission to the ICU, as indicated by RDW, is an independent prognostic factor for hospital mortality in critically ill patients. However, adding RDW to the APACHE II score only marginally improves mortality prediction. The biological mechanism behind this association is not one arising from inflammation as expressed by leucocyte count or CRP. This calls for further studies with more patients. Since RDW only marginally improves the prediction of mortality, a possible role of RDW in outcome prediction also warrants further analysis.

REFERENCES

1. Dabbah S, Hammerman H, Markiewicz W, et al. Relation between red cell distribution width and clinical outcomes after acute myocardial infarction. Am J Cardiol. 2010;105:312-7.
2. Pascual-Figal DA, Bonaque JC, Redondo B, et al. Red blood cell distribution width predicts long-term outcome regardless of anaemia status in acute heart failure patients. Eur J Heart Fail. 2009;11:840-6.
3. van Kimmenade RR, Mohammed AA, Uthamalingam S, et al. Red blood cell distribution width and 1-year mortality in acute heart failure. Eur J Heart Fail. 2010;12:129-36.
4. Tonelli M, Sacks F, Arnold M, et al. Relation Between Red Blood Cell Distribution Width and Cardiovascular Event Rate in People With Coronary Disease. Circulation. 2008;117:163-8.
5. Braun E, Domany E, Kenig Y, et al. Elevated red cell distribution width predicts poor outcome in young patients with community acquired pneumonia. Crit Care. 2011;15:R194.
6. Hampole CV, Mehrotra AK, Thenappan T, et al. Usefulness of red cell distribution width as a prognostic marker in pulmonary hypertension. Am J Cardiol. 2009;104:868-72.
7. Patel KV, Ferrucci L, Ershler WB, et al. Red blood cell distribution width and the risk of death in middle-aged and older adults. Arch Intern Med. 2009;169:515-23.
8. Patel KV, Semba RD, Ferrucci L, et al. Red cell distribution width and mortality in older adults: a meta-analysis. J Gerontol A Biol Sci Med Sci. 2010; 65:258-65.
9. Perlstein TS, Weuve J, Pfeffer MA, et al. Red blood cell distribution width and mortality risk in a community-based prospective cohort. Arch Intern Med. 2009;169:588-94.
10. Bazick HS, Chang D, Mahadevappa K, et al. Red cell distribution width and all-cause mortality in critically ill patients. Crit Care Med. 2011;39:1913-21.
11. Hunziker S, Celi LA, Lee J, et al. Red cell distribution width improves the simplified acute physiology score for risk prediction in unselected critically ill patients. Crit Care. 2012;16:R89.
12. Wang F, Pan W, Pan S, et al. Red cell distribution width as a novel predictor of mortality in ICU patients. Ann Med. 2011;43:40-6.
13. Felker GM, Allen LA, Pocock SJ, et al. Red cell distribution width as a novel prognostic marker in heart failure: data from the CHARM Program and the Duke Databank. J Am Coll Cardiol. 2007;50:40-7.
14. Lippi G, Targher G, Montagnana M, et al. Relation between red blood cell distribution width and inflammatory biomarkers in a large cohort of unselected outpatients. Arch Pathol Lab Med. 2009;133:628-32.
15. Steyerberg EW, Moons KG, van der Windt DA, et al. Prognosis Research Strategy (PROGRESS) 3: Prognostic Model Research. PLoS Med. 2013;10:e1001381.
16. Riley RD, Hayden JA, Steyerberg EW, et al. Prognosis Research Strategy (PROGRESS) 2: Prognostic Factor Research. PLoS Med. 2013;10:e1001380.

Netherlands
The Journal of Medicine

Cervical dysplasia associated with the use of natalizumab

L. Rolfes[1,2]*, B. Lokhorst[1], J. Samijn[3], E. van Puijenbroek[1]

[1]Netherlands Pharmacovigilance Centre Lareb, 's-Hertogenbosch, the Netherlands, [2]Department of Pharmacy: Pharmacotherapy and Pharmaceutical Care, University of Groningen, Groningen, the Netherlands, [3]Department of Neurology, Maasstad Hospital, Rotterdam, the Netherlands, *corresponding author: tel.: +31 (0)73-6469700, fax: +31 (0)73-6426136, e-mail: l.rolfes@lareb.nl

Dear editor,

Natalizumab is a monoclonal antibody against anti-α4-integrin, which was registered in the Netherlands in June 2006. It is indicated for severe and highly active relapsing-remitting multiple sclerosis (MS) in adult patients. In the product information of natalizumab it is stated that opportunistic infections such as progressive multifocal encephalopathy due to reactivation of the John Cunningham (JC) virus, herpes infections and a fatal case of herpes encephalitis have occurred during therapy. Human papilloma virus (HPV) expression is not mentioned and until now no cases of HPV expression or cervical dysplasia in association with natalizumab have been reported.

HPV is the major cause of cervical intraepithelial neoplasia (CIN), the premalignant lesion of cervical carcinoma. Usually, acquired HPV infections are cleared by the immune system by 90% of women within two years. However, in immunocompromised patients persistent HPV infections, namely high-risk HPV types 16 and 18, may lead to progression of cervical squamous epithelial lesions. The risk of invasive carcinoma is increased in CIN-I (mild dysplasia), CIN-II (moderate dysplasia) and CIN-III (includes both severe dysplasia and carcinoma in situ).

A case series is presented where the use of natalizumab may have led to persistent HPV infections which resulted in cervical dysplasia in four patients with MS.

METHOD AND MATERIALS

The Netherlands Pharmacovigilance Centre Lareb maintains the voluntary adverse drug reaction (ADR) reporting system in the Netherlands. Lareb receives reports of ADRs spontaneously from healthcare professionals, pharmaceutical companies, and patients. Qualified assessors review each ADR report case-by-case. Reports are sent to the European Medicines Agency (EMA) and are submitted to the worldwide database of the World Health Organisation (WHO).

RESULTS

Until 1 June 2013, Lareb received four reports of cervical dysplasia associated with the use of natalizumab. Cervical HPV testing for all four patients was positive but we have no information on HPV typing. Histological examination revealed CIN-II (cases 1-3) and CIN-III (case 4) lesions.

Case 1

The first report concerns a 30-year-old woman with cervical smear (PAP-IIIB), 45 months after starting natalizumab, 300 mg once a month for MS. Concomitant medications were modafinil, drospirenone/oestrogen, sumatriptan, and temazepam. Natalizumab therapy was interrupted for an unknown period, as the MS was stabilised at that time, one year before observation of the cervical dysplasia. Her past drug therapy was interferon beta-1a. The patient has a medical history of migraine and urinary tract infection. The patient has a family history of breast and colon carcinoma.

Case 2

The second report concerns a 33-year-old woman with dysmenorrhoea 20 months after starting natalizumab, dosage unknown. She had a cervical smear using thin layer cytology and was graded as PAP-IIIA. The patient also had a positive JC-virus test. Natalizumab was discontinued. Past drug therapy was glatiramer, which was administrated for two years.

Case 3

A 30-year-old woman had a pap smear which was examined with thin layer cytology showing the presence of PAP-IIIB 35 months after starting natalizumab, dosage unknown. A control cytology was staged PAP-II. Natalizumab was continued. She had been using interferon beta-1a in the past.

Case 4

The final case concerns a 28-year-old woman who was treated with natalizumab with an unknown dosage for a period of nine months when a cervical pap smear was performed as part of the Dutch population screening programme. Thin layer cytology was graded PAP-IIIB. Natalizumab was discontinued. She had been using interferon beta-1a and glatiramer in the past.

DISCUSSION

These reports suggest a possible relation between natalizumab and cervical dysplasia. Three out of four reports were reported by one reporter (J.S.), who noted that over the last year three out of 16 patients treated with natalizumab were diagnosed with cervical dysplasia. Cervical dysplasia or HPV infections in association with natalizumab have not been described before in literature. Regarding other biologicals acting on the immune system, two cases of genital HPV infections have been published: one regarding infliximab and one regarding etanercept.[1]

The mechanism by which natalizumab may cause cervical dysplasia is not fully elucidated. Natalizumab is known to cause opportunistic infections. From the literature it is shown that clearance of the HPV virus is far less likely to occur among immune-compromised patients, who are therefore at increased risk for developing HPV-related malignancies.[2] It is for these reasons conceivable that immune suppression caused by the use of natalizumab is responsible for the persistence of an HPV infection and subsequently cervical dysplasia. To what extent the past drug therapy contributed to the development of the cervical dysplasia is not clear. In the literature, no information was found on interferon beta-1a or glatiramer in association with cervical dysplasia or HPV infections. However, given the mechanism an effect of interferon cannot be excluded.

In the literature, an increase in cancer risk, including cervical related cancers, was seen in immune suppressed patients.[3,4] Adami *et al.*[3] explored the cancer risk following organ transplantation in Sweden using a cohort design. The standardised incidence ratio (SIR) was used to estimate the relative risk of tumours for different categories. A small statistically non-significant difference was seen for cervical cancer *in situ* following organ transplantation (SIR 1.3 with 95% confidence interval 1.0-1.8) and for cervix cancer *uteri* (SIR 2.0 95% confidence interval 0.7-4.7). Exploring the cancer incidence before and after kidney transplantation, Vajdic *et al.*[4] found a greater than threefold increase in risk for cervix cancer. For both studies the SIR for cervix cancer was not increased before transplantation; immune suppression may therefore be responsible for the increased risk.

For now, the number of reports of cervical dysplasia associated with natalizumab is low. More reports would make a causal relationship between the use of natalizumab and cervical dysplasia more likely. In order to make an estimation of the incidence of the possible ADR, controlled studies are needed.

CONCLUSION

The reports presented point towards a possible relation between the use of natalizumab and an increased risk for the development of cervical dysplasia. On the basis of these reports it cannot be concluded that frequent monitoring of these women is required. However, attention for this possible association is warranted.

Competing interests/funding: Johnny Samijn is a member of advisory board of Biogen Idec. Other authors have none to declare.

REFERENCES

1. Antoniou C, Kosmadaki MG, Stratigos AJ, Katsambas AD. Genital HPV lesions and molluscum contagiosum occurring in patients receiving anti-TNF-alpha therapy. Dermatology. 2008;216:364-5.
2. Aubin F, Martin M, Puzenat E, Magy-Bertrand N, Segondy M, Riethmuller D, et al. Genital human Papillomavirus infection in patients with autoimmune inflammatory diseases. Joint Bone Spine. 2011;78:460-5.
3. Adami J, Gabel H, Lindelof B, et al. Cancer risk following organ transplantation: a nationwide cohort study in Sweden. Br J Cancer. 2003;89:1221-7.
4. Vajdic CM, McDonald SP, McCredie MR, et al. Cancer incidence before and after kidney transplantation. JAMA. 2006;296:2823-31.

Netherlands
The Journal of Medicine

Cyanide intoxication by apricot kernel ingestion as complimentary cancer therapy

L. Seghers[1]*, M. Walenbergh-van Veen[2], J. Salome[1], P. Hamberg[1]

Department of [1] Internal Medicine and [2]Hospital Pharmacy, Sint Franciscus Gasthuis, Rotterdam, the Netherlands, *corresponding author: tel.: +31 (0) 10 4616161, fax: +31 (0)10 4612692, e-mail: leonard_seghers@hotmail.com

Dear editor,

Complementary and alternative medicine (CAM) is frequently used in patients with cancer, up to 90% within one year for at least part of their therapy.[1,2] Both beneficial and adverse effects of such therapy remain controversial, and CAM has even been associated with shorter overall survival.[3]

The use of amygdalin, usually in the form of laetrile, has been one of the most popular alternative 'cancer cures' in the past 40 years.[4] Importantly, laetrile and amygdalin are pharmacologically distinct compounds and their names are often used interchangeably. Amygdalin is a cyanogenetic glycoside compound[5] found in the pits of many fruits and can be applied, e.g. via ingestion of apricot kernels. Laetrile is an acronym from laevorotatory and mandelonitrile, used to describe a purified form of amygdalin.

Briefly, the working mechanism of amygdalin has been proposed to rely on the specific vulnerability of malignant cells to cyanogenic glycosides because of 1) a higher level of beta-glucosidases and beta-glucuronidase as compared with normal cells, leading to a more rapid intracellular release of cyanide from amygdalin and 2) a deficiency in rhodanese, an enzyme that converts cyanide into the harmless compound thiocyanate.

There is circumstantial evidence that amygdalin is a potential anti-cancer drug, mostly based on *in vitro* experimental studies,[6,7] although no clinical evidence supporting these findings has emerged over the past decades.[8] Moreover, it was even associated with toxic blood cyanide levels and reduced overall survival when used in the form of laetrile as described.[9] Despite this, use of amygdalin is recently increasingly advocated as an anticancer therapy.[8] It can be applied by intravenous administration, tablet or apricot kernel ingestion.[9]

In the present case report we describe the occurrence of elevated liver chemistry tests in correlation with chronic cyanide intoxication after apricot kernel ingestion as CAM in a patient suffering from metastatic colon carcinoma.

Case

A 58-year-old man receiving palliative chemotherapy (capecitabine, oxaliplatin, and bevacizumab) because of metastatic colon carcinoma visited our outpatient clinic before the start of the sixth cycle of chemotherapy. He did not report any symptoms. However, peripheral blood analysis revealed abnormal liver chemistry tests (*figure 1*). Progression of liver metastases was ruled out as an ongoing tumour response was observed on computed tomography. He was not on any relevant concomitant medication.

The patient then mentioned the use of 70 apricot kernels per day, which he chopped thoroughly. He had taken them for 45 days in a row up to a week before the outpatient visit. He discontinued the use as it was too much work to chop them.

He was evaluated for cyanide intoxication at the emergency department but fortunately no lactic acidosis was detected. The level of thiocyanate, the hepatic cyanide metabolite, became available a few days later and showed a high level within the toxic range. Serial measurements of thiocyanate levels are shown in the perspective of the liver chemistry demonstrating a time-dependent correlation (*figure 2*).

We measured a thiocyanate concentration of 71 mg/l a week after the last intake of kernels. Assuming first-order kinetics and a calculated elimination half-life of 9.5 days, thiocyanate concentration on the last day of intake would have been a toxic 118 mg/l.

DISCUSSION

In the present case report, we demonstrate, for the first time, elevated liver chemistry tests as a result of chronic cyanide intoxication after apricot kernel ingestion. Thus far, elevated liver chemistry tests have not been reported as an effect of cyanide intoxication. In our patient there is a clear time-dependent relationship between the levels of

the toxic cyanide metabolite and the liver chemistry tests after apricot kernel ingestion.

To our knowledge, therapeutic use of amygdalin has only been associated with liver failure in one case concerning cirrhotic liver disease.[10] Elevated liver chemistry tests are not a common adverse effect of cyanide intoxication in humans, although chronic cyanide ingestion was reported to cause degenerative changes in the liver of rabbits and pigs.[11,12]

Most common reported side effects of amygdalin use in humans are nausea, vomiting, hyperpnoea, headache, palpitation, cyanosis and obtundation.[9,13] None of these effects were reported by our patient.

In our patient liver chemistry tests were normal before he started using apricot kernels. In this perspective, the disturbed liver chemistry tests during follow-up strongly suggest a correlation with the apricot kernel ingestion. Liver chemistry abnormalities do not affect amygdalin pharmacology or metabolism.[13] Two drugs from the chemotherapy regimen that our patient received rarely cause disturbed liver chemistry tests, and in our patient it is unlikely that this would have been the cause as the prior four cycles were administered without this adverse effect. Additionally, he was re-exposed to oxaliplatin and capecitabin without detrimental effects to the liver chemistry.

Figure 2. *Level of thiocyanate (left Y-axis), the measurable metabolite of cyanide as a result of amygdalin metabolisation, compared with ASAT and ALAT values (right Y-axis). Values above a thiocyanate concentration of 60 mg/l are within the toxic range. Time in months. The first data point represents the values at initial presentation with abnormal liver chemistry tests*

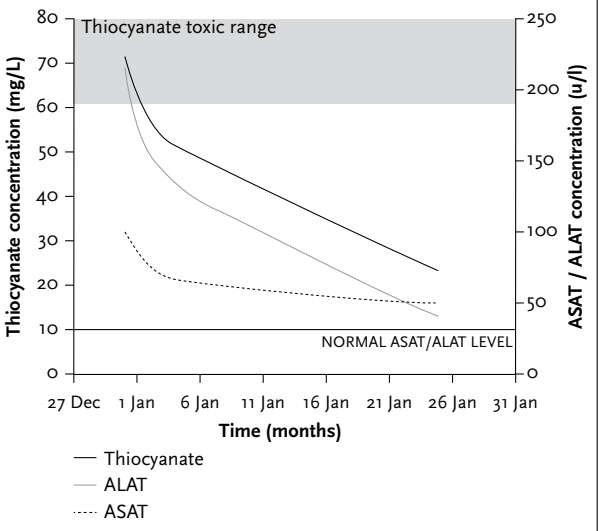
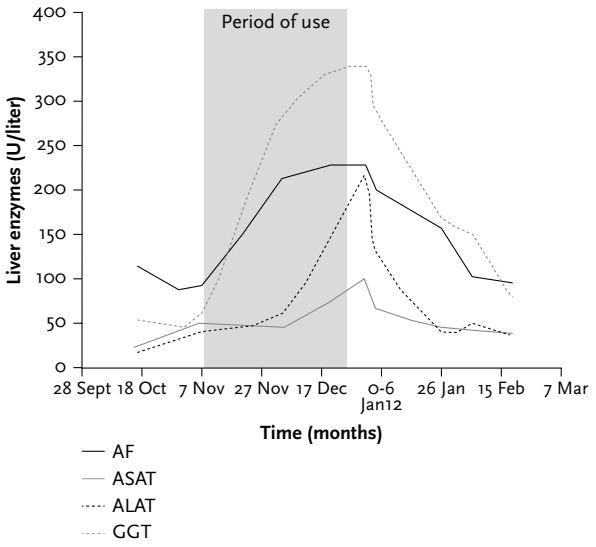


Figure 1. *Level of the four different liver enzymes (U/litre) over time (in months). The grey area indicates the period of use of apricot kernels. Throughout this period there is an increase of the four liver enzyme values, whereas these values rapidly decrease when the use of apricot kernels is discontinued*



Despite the fact that the apricot kernel ingestion had already been halted for one week, there were still toxic levels of thiocyanate, however not resulting in lactic acidosis.

It is striking that with the large number of apricot kernels ingested per day, our patient did not present with an acute cyanide intoxication but a chronic intoxication. In theory, cyanide binds to the ferric ion of mitochondrial cytochrome oxidase, which causes an almost total inhibition of cytochrome oxidase activity, leading to anaerobic metabolism and subsequent lactic acidosis, the hallmark of acute cyanide intoxication. The latter was absent in our patient and we do not have a proper explanation for this fact. Nevertheless, we demonstrate a clear time-dependent relationship in the levels of the cyanide metabolite and the liver chemistry tests.

With an estimated daily intake of 1.4-2 g of amygdalin, based on an amygdalin content of 4.5-6.5% on dry weight,[14] maximal daily cyanide exposure in our patient would be 0.12 g, assuming all the cyanide is released from amygdalin. This would lead to a near-lethal plasma cyanide concentration of 3 mg/l (distribution volume of 0.41 l/kg, lethal concentration >3 mg/l).[15] In practice the degree of cyanide release is much lower and depends on the intensity of grinding of the kernels.

In conclusion, this case report illustrates for the first time that a daily intake of 70 apricot kernels during more than six weeks induces abnormal liver chemistry tests without other toxicity signs. As a remark, this case report stresses the importance of physicians being aware of the use of complementary and alternative medicine when commencing anti-cancer therapy and indicates that unexplained abnormalities in the follow-up of cancer patients should prompt the question about the use of complementary medicine.

REFERENCES

1. Gansler T, Kaw C, Crammer C, Smith T. A population-based study of prevalence of complementary methods use by cancer survivors: a report from the American Cancer Society's studies of cancer survivors. Cancer. 2008;113:1048-57.
2. Hunt KJ, Coelho HF, Wider B, et al. Complementary and alternative medicine use in England: results from a national survey. Int J Clin Pract. 2010;64:1496-502.
3. Risberg T, Vickers A, Bremnes RM, Wist EA, Kaasa S, Cassileth BR. Does use of alternative medicine predict survival from cancer? Eur J Cancer. 2003;39:372-7.
4. Holland JC. Why patients seek unproven cancer remedies: a psychological perspective. CA: Cancer J Clin. 1982;32:10-4.
5. Bruneton J. Pharmacognosy, 2nd ed. Lavoisier, Paris. 1999:520-3.
6. Chang HK, Shin MS, Yang HY, Lee JW, Kim YS, Lee MH, et al. Amygdalin induces apoptosis through regulation of Bax and Bcl-2 expressions in human DU145 and LNCaP prostate cancer cells. Biol Pharmaceut Bull. 2006;29:1597-602.
7. Chen Y, Ma J, Wang F, et al. Amygdalin induces apoptosis in human cervical cancer cell line HeLa cells. Immunopharmacol Immunotoxicol. 2013;35:43-51.
8. Milazzo S, Ernst E, Lejeune S, Boehm K, Horneber M. Laetrile treatment for cancer. Cochrane Database Syst Rev. 2011:CD005476.
9. Moertel CG, Fleming TR, Rubin J, et al. A clinical trial of amygdalin (Laetrile) in the treatment of human cancer. New Engl J Med. 1982;306:201-6.
10. Leor R, Michaeli J, Brezis M, Stessman J. Laetrile intoxication and hepatic necrosis: a possible association. South Med J. 1986;79:259-60.
11. Manzano H, de Sousa AB, Soto-Blanco B, Guerra JL, Maiorka PC, Gorniak SL. Effects of long-term cyanide ingestion by pigs. Vet Res Commun. 2007;31:93-104.
12. Okolie NP, Osagie AU. Liver and kidney lesions and associated enzyme changes induced in rabbits by chronic cyanide exposure. Food Chem Toxicol. 1999;37:745-50.
13. Moertel CG, Ames MM, Kovach JS, Moyer TP, Rubin JR, Tinker JH. A pharmacologic and toxicological study of amygdalin. JAMA. 1981;245:591-4.
14. Femenia A, Rossello C, Mulet A, Canellas J. Chemical composition of bitter and sweet apricot kernels. J Agricult Food Chem. 1995;43:356-61.
15. RIVM. Available from: http://www.vergiftigingen.info.

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.

Manuscripts
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.

Submission
All submissions to the *Netherlands Journal of Medicine* should be submitted online through Manuscript Central at http://mc.manuscriptcentral.com/nethjmed. Authors should create an account and follow the instructions. If you are unable to submit through Manuscript Central contact the editorial office at m.m.levi@amc.uva.nl, tel.: +31 (0)20-566 21 71, fax: +31 (0)20-691 96 58.

Preparation of manuscripts
Type all pages with double spacing and wide margins on one side of the paper. To facilitate the reviewing process, number the lines in the margin and the pages.
Subheadings should not exceed 55 characters, including spaces.
Abbreviations: Measurements should be abbreviated according to SI units. All other abbreviations or acronyms should be defined on the first appearance in the text. Use a capital letter for generic names of substances and materials.
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).

Divide the manuscript into the following sections: Title page, Abstract, Keywords, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables and Figures with Legends.

The *Title page* should include authors' names, degrees, academic addresses, correspondence address, including telephone number, fax number, e-mail address and grant support. Also the contribution of each author should be specified.
The title should be informative and not exceed 90 characters, including spaces. Avoid use of extraneous words such as 'study', 'investigation' as well as priority claims (new, novel, first). Give a running title of less than 50 characters. If data from the manuscript have been presented at a meeting, list the name, date and location of the meeting and reference and previously published abstracts in the bibliography. Give a word count (including references, excluding tables and legends) at the bottom of this page.

The *Abstract*, not exceeding 250 words, should be written in a structured manner and with particular care. In original articles, the Abstract should consist of the following paragraphs: Background, Methods, Results and Conclusion. They should briefly describe the problem being addressed in the study, how the study was performed and which measurements were carried out, the most relevant results, and what the authors conclude from the results.

Keywords: Include three to five keywords in alphabetical order.

The *Introduction* should be brief and set out the purposes for which the study has been performed.

The *Materials and methods* should be sufficiently detailed so that readers and reviewers can understand precisely what has been done without studying the references directly. The description may be abbreviated when well-accepted techniques are used.

The *Results* should be presented precisely, without discussion.

The *Discussion* should directly relate to the study being reported. Do not include a general review of the topic, but discuss the pertinent literature.

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

References should be numbered consecutively as they appear in the text (after the punctuation and in square brackets). Type the reference list with double spacing on a separate page. References should be in the language they are published in, conform the 'Vancouver' style for biomedical journals (N Engl J Med. 1991;324:424-8).

Journal abbreviations should conform to the style used in the Cumulated Index Medicus. Examples:

1. Smilde TJ, van Wissen S, Wollersheim H, Kastelein JJP, Stalenhoef AFH. Genetic and metabolic factors predicting risk of cardiovascular disease in familial hypercholesterolemia. Neth J Med. 2001;59:184-95.
2. Kaplan NM. Clinical Hypertension. 7th ed. Baltimore: Williams & Wilkins; 1998.
3. Powell LW, Isselbacher KJ. Hemochromatosis. In: Braunwald E, Fauci AS, Kasper DL, et al., editors. Harrison's Principles of Internal Medicine. 15th edition. New York: McGraw-Hill; 2001. p. 2257-61.

Please note that all authors should be listed when six or less; when seven or more, list only the first three and add et al. Do not include references to personal communications, unpublished data or manuscripts either 'in preparation' or 'submitted for publication'. If essential, such material may be incorporated into the appropriate place in the text. Recheck references in the text against the reference list after your manuscript has been revised.
The use of bibliographic software programmes that are designed to generate reference lists such as Reference Manager© or Endnote© is highly encouraged. Authors can use the predefined output 'Vancouver' style from these programmes.

Tables should be typed with double spacing each on a separate page, numbered consecutively with Arabic numerals, and should contain only horizontal lines. Provide a short descriptive heading above each table with footnotes and/or explanation underneath.

Figures must be suitable for high-quality reproduction (>300 DPI). Submit line drawings made in Word or other computer programmes but not in a PowerPoint file. Colour figures are occasionally possible and will be charged to the authors. *Legends for figures* should be typed, with double spacing, on a separate page.

Case reports
Case reports containing concise reports on original work will be considered for publication. Case reports which are relevant for understanding the pathophysiology or clinical presentation of disease may also be accepted under this heading. Selection of case reports will be based on criteria as outlined in a special report by the editors (Drenth et al. The case for case reports in *the Netherlands Journal of Medicine*. Neth J Med. 2006;64(7):262-4). We advise potential authors to take notice of the instructions in this report. Articles published in this section should be no longer than 1000 words, and supplied with a summary of about 60 words, preferably no more than two figures and/or tables, and no more than 15 references. In addition, we require that authors of case reports answer the following two questions (Neth J Med. 2008;66(7):289-90): 1) What was known on this topic? and 2) What does this add? The answers will appear in a separate box in the text.

Mini reviews
Mini reviews are concise notes that bring the reader up to date with the recent developments in the field under discussion. The review article should mention any previous important reviews in the field and contain a comprehensive discussion starting with the general background of the field. It should then go on to discuss the salient features of recent developments. The authors should avoid presenting material which has already been published in a previous review. The manuscript should be divided as follows: title page, abstract and main text. The text may be subdivided further according to the areas to be discussed. The text should not exceed 2500 words.

Letters to the editor (correspondence)
Letters to the editor will be considered by the editorial board. Letters should be no more than 400 words. Please use SI units for measurements and provide the references conform the Vancouver style (N Engl J Med. 1991;324:424-8). No more than one figure is allowed. For letters referring to articles previously published in the Journal, the referred article should be quoted in the list of references.

Photo quiz
A photo quiz should not exceed 500 words and include no more than two figures and four references conform the Vancouver style. Abbreviations of measurements should be quoted in SI units.

Book reviews
The editorial board will consider articles reviewing books.

Reviewing process
After external and editorial review of the manuscript the authors will be informed about acceptance, rejection or revision. We require revision as stated in our letter.

Proofs
Proofs will be sent to the authors to be carefully checked for printer's errors. Changes or additions to the edited manuscript cannot be allowed at this stage. Corrected proofs should be returned to the editorial office within two days of receipt.

Offprints
These are not available. The first author receives a sample copy of the Journal with the published article.