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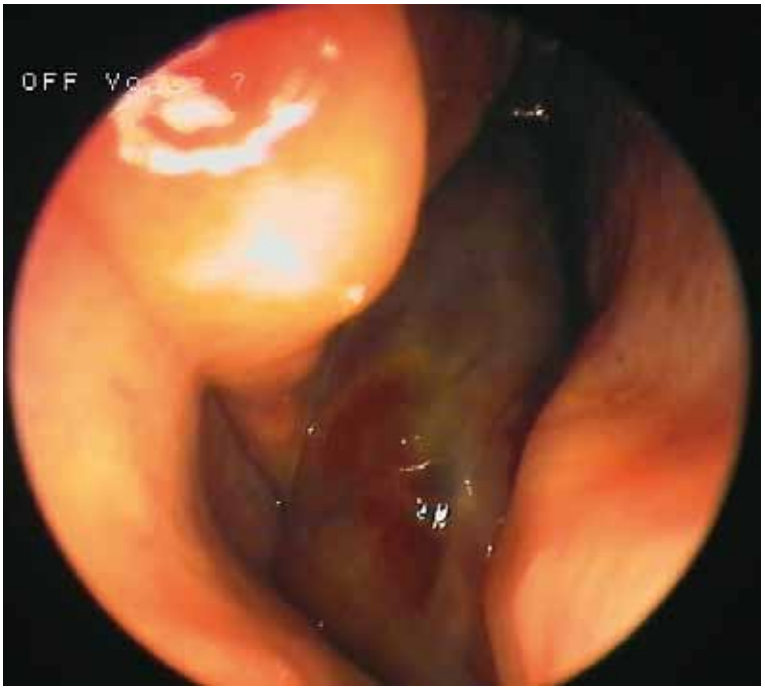

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Contents

EDITORIAL

Keep it cool on the ICU 188
R. Sie, A.R.J. Girbes

REVIEWS

Induction of a hypometabolic state during critical illness – a new concept in the ICU? 190
H. Aslami, N.P. Juffermans

Laparoscopic donor nephrectomy 199
R.C. Minnee, M.M. Idu

The effect of haemophilia and von Willebrand disease on arterial thrombosis: a systematic review 207
S. Biere-Rafi, M. Zwiers, M. Peters, J. van der Meer, F.R. Rosendaal, H.R. Büller, P.W. Kamphuisen

ORIGINAL ARTICLE

Reversible cardiac valvular disease in catastrophic antiphospholipid syndrome 215
C.C. Teunisse, A.J. Kalsbeek, S.T. de Vries, S.J. Huisman, J.E. Boers, A. Breeman, J.R. Beukhof

CASE REPORTS

Enteroviral encephalitis in a patient with a marginal zone lymphoma treated with rituximab 221
C. Schilthuizen, H.W.A. Berenschot, M-D. Levin

Primary squamous cell carcinoma of the thyroid years after radioactive iodine treatment 224
H. Yucel, N.C. Schaper, M. van Beek, B. Bravenboer

PHOTO QUIZZES

Just epistaxis? 227
R. Hofman, A.A.M. Franken, H.J. Rosingh

Red and wet 228
D.J. van Westerloo, N.P. Juffermans

A patient with renal cell carcinoma and thoracic pain 229
I.M.E. Desar, M.J. Sonderen, C.M.L van Herpen

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EDITORIAL

Keep it cool on the ICU

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Reduction of the proinflammatory response in cases of systemic and/or regional hypoperfusion and reperfusion, and sepsis has long been considered a promising method to preserve organ function. However, it only remains promising since so far studies intervening at a specific pathway have been disappointing in terms of patient outcome and survival.[1] Although our understanding of the proinflammatory and anti-inflammatory response in severe illness is growing, it is still very limited and the models on which the trials were designed too simplified. The systemic inflammatory response ultimately results in impaired tissue oxygenation and multiple organ failure. The current management of such patients with critical illness is primarily aimed at supportive care, providing adequate tissue perfusion and oxygenation in order to meet the high metabolic demands and correction of the cause.

As pointed out by Aslami and Juffermans, since mild therapeutic hypothermia (MTH) favourably interferes at many pathways for the pro-inflammatory response and apoptosis, it seems attractive to put forward MTH as a therapeutic intervention to reduce organ failure.[2] Additionally, MTH reduces tissue oxygen demands. Indeed, MTH has proven beneficial effects in patients after cardiopulmonary resuscitation in terms of neurological outcome. Lower levels of evidence exist for the beneficial effects of MTH in other forms of organ failure related to hypoperfusion and reperfusion, such as myocardial infarction (smaller infarct size), traumatic brain injury (decrease of intracranial pressure and improved neurological outcome), and major thoracic cardiovascular surgery (prevention of brain and spinal injury). Case series have demonstrated favourable effects of MTH in patients with severe pulmonary inflammation, ARDS.[3]

Recently, more attention has been given to the importance of mitochondrial dysfunction and bioenergetic failure during sepsis and shock. There is evidence that sepsis-induced mitochondrial dysfunction is associated with a loss or failed synthesis of mitochondrial DNA and mitochondrial recovery, as indicated by blood mitochondrial DNA levels. The latter has been shown to be associated with survival.[4] Preservation of mitochondrial dysfunction might, therefore, present a target for therapy. Baumgart *et al.* showed that, in a mice model, inhaled hydrogen sulphide (H_2S) adds to the preservation of mitochondrial function during hypothermia.[5] All this makes MTH a potential therapeutic intervention to reduce organ failure.

The practice of MTH is currently well feasible and established. However, 'dose-finding studies' on how low the temperature should be, and for how long, and at which rate the temperature should decrease and at a later stage increase to normotemperature, are all lacking. Randomised studies in intensive care medicine are very difficult to perform and to interpret, related to the diversity of the population and the obligatory multiple interventions.[6] MTH has not been studied in patients with sepsis. Known and unknown side effects of MTH may be anticipated when applied in sepsis. Since, especially in survivors, fever is present in patients with severe infection and sepsis, MTH is teleologically unattractive. Aslami and Juffermans adequately discuss the pros and cons of the use of MTH during critical illness. Inducing a hypometabolic state in order to inhibit the inflammatory response and preserve mitochondrial function is an appealing idea in view of the existing evidence. In this respect, hypothermia is the most attractive option. But, as in all therapies, the final outcome depends on the balance between favourable effects and negative side effects. The challenge is not only to investigate the effect of MTH, with or without H_2S, but also to investigate 'the dose' of MTH, as outlined above. That means that if MTH is ineffective if applied according to a specific protocol, e.g. starting MTH within two hours after diagnosing sepsis to 35°C for 48 hours with rewarming at a rate of 0.2°C/h, it will also be ineffective using another protocol, with other endpoints of temperature. Understanding of the mechanism will probably help to – finally – design the best possible studies. Until then, there is still a long way to Tipperary.

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REVIEW

Induction of a hypometabolic state during critical illness – a new concept in the ICU?

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ABSTRACT

Induced hypothermia after cardiopulmonary resuscitation provides organ protection and is currently considered standard of care in clinical practice. An increasing number of reports indicate that induced hypothermia is also beneficial in other conditions of hypoxia-induced organ injury, including brain injury, intestinal ischaemia-reperfusion injury and acute lung injury. The mechanism of the protective effect is thought to be caused by a reduction in metabolism. A hibernation-like state, characterised by hypothermia, bradypnoea and a reduction in metabolic rate, was induced in animals that normally do not hibernate, after inhalation of hydrogen sulphide. This state was termed a 'suspended animation-like state'.

In critically ill patients, an exaggerated systemic inflammatory response is common, which often results in multiple organ injury. Inducing a hypometabolic state during critical illness may limit organ injury by reducing oxygen consumption, constituting a fascinating new therapeutic perspective for the treatment of critically ill patients. In this manuscript, we describe mitochondrial dysfunction during critical illness and preclinical data that suggest a potential therapeutic possibility of lowering metabolism. In addition, we discuss issues that warrant further research before clinical applicability.

KEYWORDS

Critical illness, hydrogen sulphide, hypothermia, metabolism, suspended animation-like state

INTRODUCTION

Sepsis is the exaggerated systemic inflammatory response to infection, characterised by endothelial damage, microvascular dysfunction and vasodilatation, ultimately resulting in impaired tissue oxygenation and organ injury.[1] Systemic inflammatory response syndrome (SIRS) can also result in vasodilatory shock, with the same features as sepsis. SIRS can occur as a reaction to a variety of noninfectious insults, including severe trauma, cardiothoracic surgery and ischaemia-reperfusion injury. When untreated, the dysregulated host inflammatory response in sepsis and SIRS results in multiple organ failure (MOF). The development of MOF, including acute lung injury and acute kidney injury, contributes strongly to morbidity and mortality in the critically ill.[2]

The inflammatory response seen in MOF requires an acceleration of glycolytic adenosine triphosphate (ATP) supply by the mitochondria to maintain the heightened level of activity and to prevent ATP levels from falling below threshold level, the latter of which would compromise normal cell metabolism and trigger apoptotic cell death. Treatment of MOF traditionally consists of supportive care, ensuring adequate tissue perfusion and oxygenation to meet the high metabolic demands of severe inflammation, an approach that ignores oxygen consumption.

In this manuscript, the potential beneficial effects of reducing metabolism in critically ill patients are discussed. Inducing a hypometabolic state may limit organ injury by restoring the dysbalance between oxygen demand and consumption, which holds the promise of a novel therapeutic approach in critically ill patients.

CHANGES IN MITOCHONDRIAL FUNCTION IN SHOCK STATES

Mitochondrial function

Under aerobic conditions, cellular energy production depends on glycolysis in the cytoplasm, the Krebs cycle and the electron transport chain embedded in the inner mitochondrial membrane.[3] By glycolysis, highly energetic

molecules such as NADH and FADH2 are produced, which can enter the mitochondria together with fatty acids and amino acids. After oxidisation in the Krebs cycle, these products transfer electrons through the mitochondrial complexes, generating an electron current for the production of ATP by oxidative phosphorylation.[4] During ATP production, oxygen is reduced to water by cytochrome c oxidase, the terminal enzyme of the respiratory chain complex. During shock states, the aetiology of multiple organ failure in critical illness is thought to include a deficiency in tissue oxygen delivery, due to shunting in the microcirculation and to a failing cardiac output in relation to oxygen demand, causing inadequate oxygen supply to cells resulting in hypoxia, which affects cellular energy metabolism.[5] However, this view does not support several observations. Despite apparently sufficient oxygen delivery, signs of hypoxia and/or metabolic dysfunction have been found to persist. Rather then caused by microcirculatory hypoxia, the tissue distress seen in MOF may be caused by disturbances in cellular metabolic pathways. The finding of an increased tissue oxygen tension in the presence of metabolic acidosis in sepsis patients suggests that oxygen is available at the cellular level and that the predominant defect may be a decreased use of oxygen in the mitochondria.[6,7] This condition is termed 'cytopathic hypoxia' and is thought to play a role in cellular dysfunction and organ failure.

Mitochondrial dysfunction in MOF

Mitochondrial abnormalities have been found in models of sepsis, reporting loss of structural integrity of mitochondrial membranes and swelling.[8,9] Studies on mitochondrial function in models of sepsis have yielded variable results. Both an increase and a decrease in mitochondrial respiration have been reported (reviewed by Singer[10]). These conflicting results have been contributed to the use of different species or organs between models, as well differences in the degree of resuscitation. However, in long-term sepsis models, a decrease in mitochondrial function is a consistent finding. Preclinical findings of mitochondrial dysfunction include the cytotoxicity of proinflammatory mediators. Tumour necrosis factor alpha (TNF) and nitric oxide (NO), both produced in excess during sepsis, affect oxidative phosphorylation by inhibiting several respiratory enzymes in the electron transport chain, thereby inducing direct functional damage to the mitochondria.[11] In accordance, most laboratory models of sepsis have shown a decrease in mitochondrial activity and ATP generation,[12-14] and respiratory activity.[15-18] The clinical relevance of mitochondrial dysfunction was shown in patients with septic shock. Skeletal muscle ATP concentration, a marker of mitochondrial oxidative phosphorylation, is depleted in septic shock, together with structural changes in the mitochondria, which was associated with worse outcome.[17] Thus, bioenergetic failure (i.e. an inability to utilise oxygen) may be a mechanism underlying MOF in the critically ill.

Hypothesis of mitochondrial 'shutdown' during critical illness.

It has been proposed that mitochondrial energy alterations are part of the strategic defence.[19] The perceived failure of organs might instead be a potentially protective mechanism. Reduced cellular metabolism could increase the chances of survival of cells, and thus organs, in the face of an overwhelming insult. In this view, the modifications induced by sepsis should not be regarded solely as a failure of energy cell status, but as an integrated response. The decline in organ function may be triggered by a decrease in mitochondrial activity and oxidative phosphorylation, leading to reduced cellular metabolism, the process of which may be triggered by acute changes in levels of hormones and inflammatory mediators. The fact that organ dysfunction is reversible in survivors of MOF[19] suggests a window of opportunity in which strategies to improve mitochondrial function may be possible.

REGULATING CELLULAR SUBSTRATE DURING CRITICAL ILLNESS INFLUENCES OUTCOME

In recent years, some evidence has emerged that efforts to improve bioenergetic failure by regulating cellular substrate supply are beneficial in the critically ill. Hyperglycaemia is a common finding in critically ill patients, as a result of stress-induced insulin resistance and accelerated glucose production. Intensive insulin treatment aimed at maintaining normoglycaemia was shown to reduce mortality in patients on a surgical intensive care unit, as well as reduce inflammation and the occurrence of MOF.[20,21] The protective effect of normoglycaemia may occur via maintenance of mitochondrial integrity. In a post-mortem study, liver mitochondria from patients who were assigned intensive insulin therapy showed less morphological abnormalities when compared with patients assigned conventional therapy, which correlated with a higher activity of respiratory chain complexes I and IV.[22] In an experimental model of critical illness, it was found that mitochondrial dysfunction and organ damage was due to hyperglycaemic-induced cellular glucose overload and not to the actions of insulin.[23]

Another cellular substrate which has been studied to improve mitochondrial function in sepsis is succinate. Unlike complex I, complex II is relatively preserved during sepsis. Succinate is a component of the citric acid cycle and specifically donates electrons to complex II of the electron transfer chain, bypassing complex I. In an *ex vivo* rat model of sepsis, the addition of succinate was found to increase mitochondrial oxygen consumption.[24] The clinical significance of this strategy remains to be explored.

The effects of supplementing several amino acids have been studied in the critically ill too. Arginin, an NO donor, increases protein synthesis and improves immunological host defence during sepsis. Arginin-enriched enteral feeding formulae have been found to decrease the occurrence of multiple organ failure in critically ill trauma patients,[25] and to reduce mortality of septic patients.[26] Glutamine is a precursor of the antioxidant gluthathione. Improving the balance between overproduction of reactive oxygen species and depletion of antioxidants during sepsis may prevent the generation of peroxynitrite within the mitochondria, thereby restoring mitochondrial function. In accordance, in a model of sepsis, glutamine increased mitochondrial oxygen consumption, as exemplified by an increase in ATP synthesis.[27] In septic patients, a supplement containing glutamine dipeptides, antioxidative vitamins and trace elements resulted in faster recovery from organ dysfunction compared with control patients,[28] possibly by restoring low plasma levels of glutathione.[29]

HYPOTHERMIA AS A STRATEGY TO REDUCE ORGAN FAILURE

Induction of a hypometabolic state

Instead of enhancing oxygen delivery to meet enhanced demands, or regulating mitochondrial substrate, an alternative approach may be to reduce energy consumption. The regulated induction of a hypometabolic state, analogous to hibernation, may be beneficial in the imbalance between oxygen delivery and demand, thereby protecting the cells from severe bioenergetic failure and a critical fall in ATP.

Application of hypothermia in hypoxia-induced organ damage

Induced hypothermia by external cooling is a well-known beneficial preventive strategy in conditions causing tissue injury, such as cardiothoracic surgery and organ transplantation.[30] In addition, cooling the body to 32 to 34°C ameliorates neurological outcome when applied in patients who have suffered a cardiac arrest.[31] Other causes of hypoxic brain damage may also benefit from hypothermia, including stroke, traumatic brain injury and spinal cord injury.[32] Studies in experimental settings indicate that hypothermia may be protective in other organs suffering from of hypoxia-induced injury.[33-35] The beneficial effect is thought to occur via preservation of energy metabolism and reduction of the inflammatory response (*table 1*). Hypothermia reduces metabolism by 7% per grade, with reduction of ATP formation and reduction of cellular oxygen and cerebral glucose requirements. NO, which is produced in excess during sepsis, competes with oxygen in binding to cytochrome c oxidase in the mitochondrial membrane, thereby blocking the electron transport chain and resulting

Table 1. *Effects of H_2S-induced suspended animation and hypothermia on cardiovascular function, metabolism, inflammation and coagulation*

	H_2S-induced suspended animation	Induced mild hypothermia	References
Vascular system	Vasodilatation	Vasoconstriction	30, 55, 80, 88
Cardiac function	Decrease in heart rate Increase in stroke volume, No effect on contractility, Decrease or no effect on cardiac output	Decrease in heart rate Increase or no effect on contractility, Impaired or no effect on diastolic relaxation, Decrease or no effect on cardiac output	66, 74, 80, 89
Metabolism	Decrease in CO_2 production and O_2 consumption Inhibition mitochondrial respiration, no change in lactate production, increased rate of glucose oxidation Preservation of mitochondrial function	Decrease in brain CO_2 production and O_2 consumption Decrease in ATP production Increase in lactate production Increase or decrease in extraction ratio Breakdown of free fatty acids Decrease insulin secretion, insulin resistance	43, 56, 66, 90-92
Inflammation	Reduction MPO activity Decrease in chemokine levels Reduction lipid peroxidase	Decrease in production of free radicals Decrease in white cell count and neutrophil influx Decrease in chemokine levels Differential effect on levels of cytokines; decrease or increase in TNF and IL-6, no effect on IL-2, increase in IL-10 and IL-1 Decrease apoptosis	30, 67, 68, 86, 93-95
Coagulation	Not known	Increased generation of prostaglandins Decrease in platelet count Decrease in platelet adhesion Decrease in levels of thrombin-antithrombin complexes	96-98

in overproduction of free oxygen radicals. Mild to moderate hypothermia prevents the production of superoxide and subsequent formation of reactive oxygen and nitrogen species during ischaemia (*table 1*).[36] Another protective mechanism may include the prevention of apoptosis, which may also be linked to mitochondrial function.[32] The recovery of multiple organ failure has been found to be associated with improvement in mitochondrial respiration in survivors of septic shock.[17] As discussed above, both a lack of oxygen as well as an inability to utilise oxygen is likely to contribute to organ failure during critical illness. We hypothesise that hypoxic-induced organ damage in critically ill patients may benefit from induced hypothermia, by preservation of residual mitochondrial function or faster mitochondrial recovery after inflammation has resolved.

Induced hypothermia in acute lung injury

In 30 to 60% of critically ill patients, acute lung injury occurs in the course of an exaggerated inflammatory host response during MOF.[37] The mechanisms that contribute to acute lung injury involve inflammatory processes as well as mechanical processes due to overstretching of alveoli. Reducing mechanical stress is a very beneficial strategy in these patients: the use of lower tidal volumes during mechanical ventilation has been found to reduce pulmonary damage in critically ill patients.[38] Besides too large tidal volumes, too frequent repetitive strain of respiratory cycles also may cause lung injury, as lowering of respiratory frequency attenuated lung damage in experimental models.[39] However, the use of low tidal volumes and lower respiratory rates is limited by the fact that the resulting low minute ventilation results in high levels of arterial pCO_2 and concomitant severe respiratory acidosis.

In animal models, induced hypothermia has been found to attenuate lung injury via reduction of neutrophil-mediated inflammation (*table 1*).[40,41] Hypothermia may also exert protective effects by its effect on metabolism. Reduced CO_2 production and O_2 demand may allow lower minute ventilation. Indeed, it was found that hypothermia enabled mechanical ventilation using a low respiratory rate, thereby attenuating lung injury in a rat model, a strategy which was termed 'lung rest'.[42]

The clinical significance of hypothermia during acute lung injury has been shown in an earlier study. In moribund patients with severe acute lung injury, hypothermia applied as a last resort was found to reduce mortality.[43] However, progress has been made since this trial, and treatment of the critically ill has changed considerably. Whether the beneficial effects of hypothermia can be reproduced in less severely ill patients with acute lung injury awaits exploration.

Hypothermia in acute kidney injury

Acute kidney injury shows a striking similarity to the inflammatory reaction observed in acute lung injury. An exaggerated inflammatory response, including the induction of cytokines and the initiation of coagulation, contributes to acute kidney injury.[44] Another defining feature is the damage to the microvascular endothelium and epithelium leading to altered blood flow and oxygen extraction, as well as an increased permeability to proteins and solutes. Notably, acute lung injury may induce acute kidney injury. Deterioration of kidney function in the course of acute lung injury carries a poor prognosis.[45] Data on the effect of hypothermia on hypoxia-induced kidney injury are limited to ischaemia-induced kidney injury associated with the use of cardiopulmonary bypass. In series of patients, hypothermia applied during cardiopulmonary bypass for aortic surgery has been reported to protect against renal failure.[46,47] It can be hypothesised that the protective effect of hypothermia found in models of acute lung injury also applies to acute kidney injury.

Hypothermia in gut ischaemia

In sepsis-induced multiple organ failure microcirculatory abnormalities may depress gut barrier function and contribute to bacterial translocation.[48] In critically ill patients, increased intestinal permeability was found to be predictive of the development of MOF.[49] In a model of intestinal ischaemia-reperfusion injury, hypothermia reduced the amount of injury, which was related to both a reduction in neutrophil infiltration as well as to a complete recovery of hepatic ATP synthesis.[35] The mechanism of this protective effect may have been inhibition of NO-mediated oxidative stress, as hypothermia attenuated NO production and prevented depletion of gut glutathione.[50] Interestingly, hypothermia applied during gut ischaemia shifted cardiac substrate utilisation from fatty acid oxidation to carbohydrate, as shown by an inhibition of carnitine palmitoyl transferase I activity.[51] The importance of mitochondrial dysfunction in this model was exemplified by the correlation between preservation of hepatic ATP levels and mortality.[52]

SUSPENDED ANIMATION AS A NOVEL STRATEGY TO REDUCE MULTIPLE ORGAN FAILURE

The concept of suspended animation

As induced hypothermia is not without complications, a more physiological approach to limit organ injury during critical illness could be the reduction of cellular energy expenditure, like hibernating animals when confronted with an environmental hypoxic insult. Hibernating mammals are thought to be tolerant to hypoxic conditions by a regulated suppression of ATP demand and ATP supply to a new hypometabolic steady state.[53]

A hibernation-like state has been induced in animals that normally do not hibernate, with the use of hydrogen sulphide (H_2S).[54] H_2S is commonly referred to as an environmental hazard. However, H_2S is endogenously produced from L-cysteine within the vasculature.[55] By competing with oxygen in binding to cytochrome c oxidase, H_2S can inhibit mitochondrial respiration, thereby reducing cellular oxygen consumption. Mice exposed to H_2S had a drop in core body temperature and a concomitant drop in metabolic rate, as measured by decreased O_2 consumption and CO_2 production.[56] After cessation of H_2S exposure, the mice awoke, without displaying neurological or behavioural deficits. Besides H_2S, nitric oxide and carbon monoxide are important gaseous signalling molecules, which act as an oxygen reducer and inhibit cytochrome c oxidase, similar to H_2S.[57] Carbon monoxide has also been used to induce suspended animation in nematodes.[58]

Suspended animation during severe hypoxia

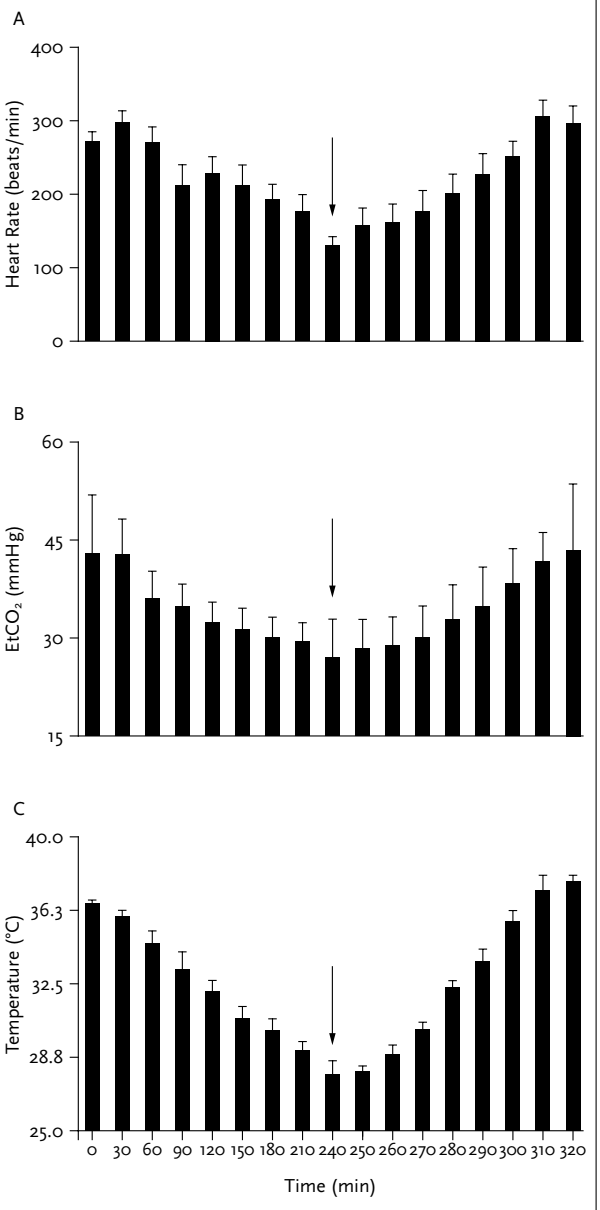
H_2S has been found to protect against myocardial ischaemia-reperfusion injury in nonhibernating doses,[59-61] as well as in a dose that preserved mitochondrial structure and function compared with controls,[62] via a vasorelaxant effect,[59] attenuation of inflammation[62] and reduction of apoptosis (*table 1*).[60,62] In a model of trauma-induced acute lung injury, H_2S at high doses attenuated lung injury, by decreasing proinflammatory cytokines and upregulating anti-inflammatory cytokines.[63] In addition, an antioxidant effect of H_2S was observed.

A possible protective effect of H_2S on oxygen deprivation is of particular interest in critically ill patients suffering from severe lung injury, in which potentially damaging high pressure mechanical ventilation is used to maintain oxygenation. Of interest in this respect are experiments with carbon monoxide. Inhibition of cytochrome c oxidase by carbon monoxide can protect nematodes against severe hypoxia by inducing suspended animation.[58] This has led to the suggestion that suppressing oxygen demand before oxygen supply falls short may protect the generation of reactive oxygen species and subsequent cell damage. In support of this hypothesis, it was shown that prior exposure of mice to H_2S increased survival during lethal hypoxia by a reduced oxygen demand.[64]

Suspended animation during anaesthesia and mechanical ventilation.

If the approach of lowering energy expenditure is to be applied in critically ill patients, these patients will be sedated and mechanically ventilated. Anaesthetic agents also suppress metabolic rate and can result in hypothermia. Studies exploring the effects of H_2S on metabolism in anaesthetised and mechanically ventilated animals found that both hypothermia and H_2S reduced oxygen consumption and CO_2 production (*table 1*),[65,66] but that mitochondrial integrity was preserved during H_2S exposure only and not in hypothermic controls, as measured by a reduction of cytochrome c-stimulated mitochondrial oxygen influx.[67] Also, H_2S resulted in a shift of substrate utilisation towards increased carbohydrate oxidation, an effect that was not observed in hypothermic subjects. This suggests that H_2S has distinct effects on metabolism, which are not induced by a mere fall in body temperature.

Figure 1. *Physiological changes during a suspended animation-like state in rats induced by infusion of 2 mg/kg/hr of an H_2S donor*



Arrow: cessation of the H_2S infusion. $EtCO_2$: end tidal CO_2. Data represented as mean ± SD of 8 animals.

Studies on the effect of suspended animation during disease states are still limited. Of interest, high doses of H_2S gas that reduced metabolism, exemplified by lower oxygen consumption, was found to be protective against stress-induced ulceration.[68] Also, preliminary data suggest that 'hibernating' doses of the H_2S donor NaHS reduced lung injury inflicted by mechanical ventilation, thereby providing evidence for protection of a hypometabolic state in a model that is relevant in the intensive care.[69] Importantly, all physiological changes induced by H_2S disappear after cessation (*figure 1*). The finding that the effects of H_2S are transient is an important finding with regard to suitability of clinical application. However, major issues remain that warrant exploration before clinical application.

HURDLES TO THE INDUCTION OF A HYPOMETABOLIC STATE

Risks of induced hypothermia during critical illness

Adverse events of induced hypothermia, in particular infections and bleeding, are not increased during hypothermia when compared with normothermia in patients after a cardiac arrest.[31] Therefore, in experienced hands, hypothermia is a safe treatment in these patients. However, hypothermia has not been applied before in multiple organ failure. It can be hypothesised that hypothermia hampers adequate immune response during bacterial infections, possibly leading to diminished clearance of bacteria. Indeed, a role for mediators that are produced during fever has been suggested for adequate host defence against bacteria.[70] In line with this, septic patients who develop hypothermia have a worse outcome compared with those who maintain a normal body temperature. Also, perioperative hypothermia is associated with increased surgical wound infections.[71] Several experimental studies, however, have reported favourable effects of mild hypothermia, increasing survival during sepsis[72] and after haemorrhagic shock.[73] The effect of controlled hypothermia in patients with sepsis has not been studied.

Hypothermia results in a prolonged bleeding time and diminished platelet aggregation. Although pulmonary bleeding is not a major feature of acute lung injury, hypothermia may increase the risk of bleeding from inflamed lung tissue with an altered morphology or may result in other types of bleeding. In addition, hypothermia reduces cardiac output, probably by reducing heart rate.[74] During sepsis or SIRS, relative myocardial dysfunction, in which oxygen delivering capacity is insufficient to meet oxygen demand, is a common finding. It remains to be determined whether the reduction of energy expenditure during hypothermia is sufficient to counteract an H_2S-induced decrease in cardiac output.

Feasibility of suspended animation in humans

Humans do not hibernate naturally and have a limited tolerance to inadequate oxygenation. In former times, when oxygen supply on earth was limited, life forms using sulphur as energetic substrate were abundant. The fact that humans produce H_2S within the vessel wall may suggest that the ability to switch to an alternative substrate, or go into a hypometabolic state with lowered oxygen consumption, may latently be present.[75] Several anecdotal reports on survival of deep and prolonged circulatory arrest, with good neurological recovery, may support this thought.[76-78]

An important issue is the clinical relevance of rodent models. Studies on H_2S-induced suspended animation in several larger animal models, including sheep and pigs[65,79] have yielded conflicting results, which may have resulted from differences in experimental set up, including the use of different H_2S donor compounds as well as the use of anaesthetic agents that may have influenced oxygen consumption. Conceivably, a difference in body mass may also contribute to these differences. Due to a large surface-to-mass ratio, rodents can rapidly reduce core body temperature, which may be difficult to induce in larger mammals and humans. However, in several experiments, the metabolic effect of H_2S occurred before core body temperature had dropped, suggesting that the suppressive effects of hibernation on metabolism are independent of the effects on body temperature.[79,80] Also, thermal inertia of large mammals did not prevent the induction of profound hypothermia in former experiments.[81] Therefore, although the induction of a suspended animation-like state is still far from clinical application, it may be feasible in large mammals and humans.

The dual role of H_2S in mediating inflammation.

The role of H_2S during systemic inflammation is a matter of debate, as H_2S in nonhibernating doses exerts both proinflammatory and anti-inflammatory effects. Inhibition of endogenous H_2S synthesis by DL-propargylglycine (PAG) demonstrated marked proinflammatory effects in various murine models; PAG inhibited production of cytokines and chemokines as well as leucocyte trafficking in sepsis models, thereby mediating or aggravating organ inflammation.[82-84] *In vitro*, H_2S has been found to promote apoptotic cell death.[85] In contrast, also anti-inflammatory effects have been found. H_2S donors reduced leucocyte-mediated oedema formation in a hindpaw oedema model.[63,86] More relevant to the intensive care is the finding that an H_2S donor improved survival in a murine model of smoke and burn-induced lung injury.[63] These differential effects of H_2S may be the result of variable doses and timing. Of note, anti-inflammatory effects of the H_2S donor NaHS in experimental pancreatitis were found to be dose-dependent.[87]

Practical hurdles

Although H_2S gas is highly flammable, this objection has been overcome with other flammable gases such as oxygen, as well as other 'toxic' gases, such as NO. In addition, H_2S has the smell of rotten eggs. In a closed system of mechanical ventilation, exposure of patients and personnel to the odour may be limited. Lastly, corrosion of tubes and metal parts may shorten durability of the mechanical ventilator.

CONCLUSION

Mitochondrial dysfunction plays a role in critically ill patients with MOF. Preclinical evidence suggests that inducing a hypometabolic state limits organ injury by inhibition of the inflammatory response and by preservation of mitochondrial function. Restoring the imbalance between oxygen demand and consumption may provide a fascinating novel therapeutic approach towards critically ill patients.

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REVIEW

Laparoscopic donor nephrectomy

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ABSTRACT

Living donor nephrectomy has been developed and promoted as a method to address the shortfall in kidneys available for transplantation. The classical method to procure a kidney from a living donor is the open donor nephrectomy performed through a flank lumbotomy incision. However, this classical method has negative short- and long-term side effects for the donor. These disincentives are a drawback for possible donors to donate a kidney. Therefore, transplant surgeons were stimulated to develop new and less invasive techniques. In this review several new open and laparoscopic techniques are described. Compared with open donor nephrectomy, laparoscopic donor nephrectomy has shown superior results in terms of postoperative pain, cosmetics, convalescence, and return to normal daily activities. No significant differences exist between the two approaches in terms of complication rates, cost-effectiveness and graft function. Nowadays, laparoscopic donor nephrectomy has become the preferred method for procuring kidney grafts of living donors in many centres.

KEYWORDS

Kidney transplantation, laparoscopy, minimally invasive surgical procedures, donor nephrectomy

INTRODUCTION

Living donor kidney transplantation is superior to deceased donor kidney transplantation because of better patient and graft survival rates, better cost-effectiveness and improved quality of life of the recipient.[1-3] However, the donor needs to undergo a major surgical operation for the benefit of another individual. In living kidney donation there are several surgical techniques for taking a renal allograft from a living donor. The classical method to procure a kidney from a living donor is the open donor nephrectomy performed through a flank lumbotomy incision. In 1995, Ratner *et al.* described the laparoscopic technique to perform a living donor nephrectomy.[4] The minimally invasive aspect of this technique was an important factor leading to the fast spread of this technique in the surgical community and this became the preferred method for procuring kidney grafts from living donors in many centres. Laparoscopic donor nephrectomy seems to be at least as safe and efficacious as open donor nephrectomy.[5,6] In the past years, several modifications to these two techniques of living donor nephrectomy have been described (*table 1*). Nowadays, the surgical technique of living donor nephrectomy varies greatly between transplant centres in European countries. An audit held in 2005 revealed that 40% of the living donor nephrectomies in Western Europe are performed laparoscopically.[7] In 2003, the percentage of laparoscopies in the United States was approximately 67%.[8]

To date, evidence of level I studies comparing the different available techniques are scarce. In the beginning of laparoscopic donor nephrectomy, patient selection bias may have existed, especially in reports from centres in which both open and laparoscopic donor nephrectomy were performed. The more complex donors at that time could have undergone open procedures. Therefore meta-analyses are also polluted with this bias and conclusions should be drawn with caution. In this review we describe different

Table 1. *Surgical techniques for living kidney donation*

Open donor nephrectomy technique
- Classical lumbotomy
- Muscle-sparing mini-incision donor nephrectomy
Laparoscopic transperitoneal technique (*)
- Laparoscopic donor nephrectomy
- Hand-assisted laparoscopic donor nephrectomy
Endoscopic retroperitoneal technique (*)
- Endoscopic retroperitoneal donor nephrectomy
- Hand-assisted Endoscopic retroperitoneal donor nephrectomy
*** These techniques can also be performed with robotic assistance.**

surgical techniques and intraoperative and postoperative factors related to laparoscopic donor nephrectomy.

SURGICAL TECHNIQUES

Open donor nephrectomy

The open donor nephrectomy through the lumbotomy approach has been the classical method of procuring kidney grafts from living donors for many decades. This technique is safe, both for the donor and for the kidney, and it is the gold standard all new techniques are compared to.

The donor is positioned in a lateral decubitus position on the operating table and is flexed at the level of the umbilicus to expose the flank fully. The open donor nephrectomy is carried out retroperitoneally through a 15 to 25 cm flank incision below the 12th rib. Resection of the distal part of the lowest rib is frequently applied to allow sufficient access to the kidney. After transection of the three layers of abdominal muscles, Gerota's fascia is exposed and the kidney is freed from the surrounding tissues. The renal vessels are isolated and the ureter with sufficient periureteral tissue is divided as distally as possible. After the renal vessels are ligated, it is possible to immediately extract the kidney from the operative field and start cold perfusion on the back-table. In this way the warm ischaemia time is very short. With this procedure there is limited risk of postoperative intraperitoneal complications, such as adhesions, intestinal perforations, splenic injuries or bowel obstructions. However, open donor nephrectomy significantly injures the abdominal wall resulting in significant postoperative pain, a long hospital stay, cosmetic problems and slow convalescence.[9] In the long term, side effects include denervation of the abdominal wall, incisional hernias and less frequently intractable pain. These adverse events are a drawback for potential donors to donate a kidney. Therefore, in most centres, over the past decade the open donor nephrectomy has been replaced by less invasive techniques.

Minimally invasive open donor nephrectomy

After the introduction of the laparoscopic donor nephrectomy there was a stimulus for developing a minimally invasive modification of the classical open donor nephrectomy, and subsequently the muscle-sparing mini-incision donor nephrectomy was developed. This operation can be done via an anterior, flank or posterior approach with an incision of approximately 7 centimetres. With the donor placed in a lateral decubitus position and the operation table maximally flexed, a horizontal skin incision is made anterior to the 11th rib toward the umbilicus. The fascia and muscles of the abdominal wall are carefully split between the muscle fibres avoiding harm to the intercostal nerves between the internal oblique and transverse abdominal muscles. The peritoneum is displaced medially and Gerota's fascia is opened on the lateral side of the kidney. The working space is limited, therefore long instruments are used. The kidney is meticulously dissected and arterial and venous structures are identified. After dissection, the ureter is divided and sutured distally. The renal artery and vein are clamped and ligated.

This approach provides the safety of the conventional open technique. This minimally invasive open donor nephrectomy results in reduced blood loss, hospital stay and incision-related complications compared with the classical open donor nephrectomy. There is only a marginal increase in operation time without compromising graft and recipient survival.[10-12] Lewis *et al*. performed a prospective study comparing traditional open, minimal-incision, and laparoscopic donor nephrectomy. Blood loss was significantly higher for open donor nephrectomy (842±1439, 260±195, $p<0.0001$). Postoperative intravenous morphine requirements were twice as high after open donor nephrectomy than after the minimal-incision technique. Donors were able to do domestic tasks quicker after minimal-incision than after open donor nephrectomy (2±1 *vs* 4±3, $p<0.05$). No differences were found in recipient outcome.[11] However, on comparison with laparoscopic donor nephrectomy, minimal-incision donor nephrectomy resulted in slower recovery, more fatigue, a worse quality of life for the donor but with equal safety and function for donor and graft.[13-15]

LAPAROSCOPIC DONOR NEPHRECTOMY

The first laparoscopic donor nephrectomy was performed by Ratner and colleagues in 1995.[4]

With the donor placed in a lateral decubitus position and the operation table maximally flexed, 4 or 5 trocars are introduced. The abdomen is insufflated to 12 mmHg. The colon is mobilised and displaced medially. Gerota's fascia is opened and the renal vein and ureter, with sufficient periureteral tissue, are identified and dissected. The renal artery is identified. Branches of the adrenal, gonadal and lumbar veins are clipped and divided. The ureter is clipped distally and divided. Then, a low transverse suprapubic (Pfannenstiel) incision or midline incision is made creating a gate for extraction of the kidney later on. The renal artery and vein are divided using an endoscopic stapler or clips. The kidney is extracted through the extraction incision, and flushed with preservation fluid and stored on ice. Extraction of the kidney can be performed directly through the incision or by using a special endoscopic specimen retrieval bag.

Disadvantages of this technique include the steep and long learning curve, the risk of bowel injury from trocar insertion or during instrumentation, internal hernias or hernia through trocar sites and intestinal adhesions.[16] Injuries to the lumbar vein, renal artery

and aorta, pneumomediastinum, splenic injury, and adrenal/retroperitoneal haematomas have been reported.[17] Conversion rate from laparoscopic to open surgery is 1.8% (range 0 to 13.3%). Approximately half of the conversions to open are for bleeding or vascular injury.[18]

The laparoscopic technique results in a shorter vascular pedicle when compared with the open donor nephrectomy. The warm ischaemia time and operating time for laparoscopic donor nephrectomy is substantially longer than compared with open donor nephrectomy.

Simforoosh *et al*. reported the first randomised controlled trial between open and laparoscopic donor nephrectomy. They included 100 donors and reported no differences in complications and graft survival. Donors of the laparoscopic group were more satisfied and resumed their normal activities earlier.[2]

Recently, Nicholson *et al*. randomised 84 donors between open and laparoscopic donor nephrectomy (LDN). LDN results in less postoperative complications, less pain, shorter hospital stay, earlier return to employment without differences in renal function or allograft survival.[19]

Several meta-analysis compare open and laparoscopic donor nephrectomy.[15,18] The overall results demonstrate that the laparoscopic technique is associated with a significantly shorter hospital stay, fewer postoperative analgesic requirements, improved cosmetics and a quicker return to work as compared with open donor nephrectomy. In addition, compared with the open technique, laparoscopic donor nephrectomy is associated with less donor morbidity and similar allograft function and overall safety, but with increased costs.[18] Laparoscopic donor nephrectomy was compared with the mini-incision open donor nephrectomy in a study by Kok *et al*. In this randomised controlled trial comparing laparoscopic donor nephrectomy to mini-incision muscle splitting open donor nephrectomy, they reported longer warm ischaemia time (6 *vs* 3 min, p<0.001), less blood loss (100 *vs* 240 ml, p<0.001), less morphine (16 *vs* 25 mg, p=0.005) and shorter hospital stay (3 *vs* 4 days, p=0.003) in the laparoscopic group without a statistically significant difference in complication rate (intraoperatively 12 *vs* 6%, p=0.49, postoperatively both 6%) and graft survival.[13]

Hand-assisted laparoscopic donor nephrectomy

Hand-assisted laparoscopic donor nephrectomy was first utilised to minimise the learning curve of the total laparoscopic donor nephrectomy. In addition, the hand port provides addition safety to laparoscopic donor nephrectomy, because rapid control of eventual massive blood loss from major blood vessels is possible due to the hand assistance. Different incisions for hand introduction have been described, such as a Pfannenstiel incision, a midline supraumbilical, periumbilical or infraumbilical incision. The hand port can be used partly or totally during the operation.

The hand-assisted laparoscopic donor nephrectomy is done transperitoneally.[20] After open dissection of the distal ureter and gonadal vein through a 7 to 8 centimetre Pfannenstiel incision the nondominant operator's hand is introduced through a hand port and two trocars are placed. The insufflation pressure is maximally 12 mmHg. The right or left colon is then mobilised. The renal vein and artery are identified and the kidney is mobilised from the surrounding tissue. After transecting the ureter distally, the renal artery is transected with metal clips or an endoscopic stapler which is used to transect the renal vein. The kidney is extracted through the Pfannenstiel incision and cold flushed and preserved with preservation fluid.

Potential disadvantages are higher costs because of the hand port, a worse ergonomic position for the surgeon during operation, a higher rate of wound infections and increased traumatic injury to the transplant as a consequence of manipulation. Conversion to open surgery is 2.97% in the hand-assisted group.[21] The most common causes for conversion to open surgery include intraoperative haemorrhage or vascular injury, difficult kidney exposure or an obese donor, vascular staple malfunction, adhesions and loss of pneumoperitoneum. Potential advantages of hand-assisted laparoscopic donor nephrectomy over conventional laparoscopy include the ability to use tactile feedback, less kidney traction, rapid control of bleeding, fast kidney removal and shorter warm ischaemic periods.[21,22] Kokkinos *et al*. performed a meta-analysis which compared the total laparoscopic donor nephrectomy with the hand-assisted laparoscopic donor nephrectomy. They reported a significantly shorter warm ischaemic time, operation time and less blood loss for the hand-assistance technique. The hand-assisted technique also had a reduced intraoperative and postoperative complication rate when compared with the total laparoscopic technique, but these differences failed to reach statistical significance.[21]

In addition, the introduction of hand-assisted laparoscopic donor nephrectomy broadens the indications for laparoscopic living donor nephrectomy to include obese donors and donors who have had previous abdominal surgery.[23] Wolf *et al*. reported 47% less analgesic use (p=0.004), 35% shorter hospital stay (p=0.0001), 33% more rapid return to non-strenuous activity (p=0.006), 23% earlier return to work (p=0.037), and 73% less pain six weeks postoperatively (p=0.004) in the hand-assisted laparoscopy group compared with the open donor group.[24] Bargmann *et al*. showed no difference between the hand-assisted laparoscopy group and totally laparoscopy group in a randomised controlled trial regarding intra and postoperative complications.[22]

Retroperitoneoscopic donor nephrectomy

To limit and prevent possible intra-abdominal manipulation of the transperitoneal laparoscopic techniques, the retroperitoneal endoscopic donor

nephrectomy was developed. During this technique the peritoneal cavity is not opened. The technique has been described with and without hand assistance. The donor is placed in the full lateral position, and the retroperitoneal space is created using a balloon or the operators hand and maintained by carbon dioxide (CO_2) insufflation with a pressure of 12 mmHg. Dissection of Gerota's fascia, perirenal tissue and vascular structures are performed as described above. Potential disadvantages are emphysema such as pneumomediastinum, pneumothorax and pneumopericardium and gas embolism. Three comparative studies from Sweden comparing hand-assisted retroperitoneoscopic with laparoscopic donor nephrectomy revealed no differences in intraoperative and postoperative outcome for donor and recipient.[25-27] However, data on hand-assisted retroperitoneoscopic donor nephrectomy are scarce and more prospective data on this technique are needed.

Robotic-assisted donor nephrectomy

Horgan *et al.* described their first series of 12 patients undergoing robotic hand-assisted laparoscopic donor nephrectomy and compared it with the standard laparoscopic donor nephrectomy.[28] Robotic-assisted donor nephrectomy can be performed with or without hand assistance. The Da Vinci robotic system has three components: a console, a control tower and the surgical arm cart. The donor nephrectomy is performed with the patient placed in a decubitus position. The operating table is flexed to maximise the exposure of the kidney during the procedure. Four trocars are placed in the left or right side of the abdomen to allow placement of three articulated robotic arms, the robotic camera, and the standard laparoscopic instrument used for retraction and dissection during the procedure. The left or right colon is mobilised medially to expose the kidney. Dissection of Gerota's fascia, perirenal tissue and vascular structures are performed as described above.

There is only one small study comparing the robot-assisted donor nephrectomy to the open donor nephrectomy revealing no differences in intraoperative and postoperative outcome for donor and recipient.[29] This current lack of data has to be filled with prospective studies.

The advantage of this technique is the movement of the articulated arm of the robot reproduces the action of the human wrist, which provides more free mobility. A potential disadvantage is the costs.

INTRAOPERATIVE FACTORS

Left or right kidney

There is an ongoing discussion whether right or left donor nephrectomy is to be preferred. Most centres prefer to use the left kidney for living kidney donation because the renal vein is longer, which is advantageous during implantation.[30-32] However, some surgeons prefer the right kidney because it is easier to recover than the left kidney and the risk of splenic laceration is decreased.[33] A single-centre randomised controlled trial revealed no differences between left- and right-sided donor nephrectomy in donor hospital stay, donor quality of life, donor and acceptor complication rates, or graft survival. However, operation time for hand-assisted laparoscopic donor nephrectomy of the right kidney was significantly shorter (150 min, range 92 to 219) than that of hand-assisted laparoscopic donor nephrectomy of the left kidney (180 min, range 117 to 266, 95% confidence interval (CI) 3.93 to 46.38, p=0.021).[34] Right hand-assisted laparoscopic donor nephrectomy is justified if both kidneys have similar anatomy.

Multiple renal arteries and veins

Multiple renal arteries are present in 12 to 33%.[35,36] In earlier studies the implantation of kidneys with multiple arteries has been associated with an increased incidence of vascular and urological complications, such as thrombosis and ureteral ischaemia, and was considered a relative contraindication by some.[36,37] However, more recent reports state that renal transplantation can be performed safely in case of multiple arteries.[38-40] Special care has to be taken with the lower kidney pole accessory renal arteries as they often provide substantial blood supply to the renal pelvis and ureter in a transplanted kidney and otherwise giving urological complications.

Multiple renal veins are present in 5 to 10% of the donors.[35,36] Most of the small calibre accessory renal veins can safely be ligated, but occasionally reconstruction to gain length of a short right renal vein or repair of a damaged vein makes additional venous reconstruction necessary. It can be concluded that regardless of which technique (open or laparoscopic) used multiple vessels are not a contraindication.

WARM ISCHAEMIA TIME AND OPERATING TIME

Warm ischaemia time is the time the kidney remains at body temperature after its blood supply has been cut off but before cold perfusion is started. Compared with laparoscopic donor nephrectomy, open donor nephrectomy has a shorter warm ischaemia time by 102 seconds (95% CI 102.01 to 155.15, p<0.001).[18] Warm ischaemia time was shorter by 75 seconds in the hand-assisted group compared with the laparoscopic donor nephrectomy (95% CI 2.84 to 116.14, p<0.001).[21] In general, especially in the early years laparoscopic techniques had a longer warm ischaemia time than the open techniques but the hand assistance

and organ-retrieval bags have reduced these long warm ischaemia times. Nowadays, because of these adjuncts to laparoscopic techniques the warm ischaemia time is almost identical to open techniques. On the other hand, there is no clinically demonstrated negative effect on kidney function if the warm ischaemia time is less than 10 minutes, which is the case in almost all laparoscopic series.[41]

The open donor nephrectomy compared with the laparoscopic donor nephrectomy has a shorter operative time by 52 minutes (95% CI 39.73 to 64.12, p=0.001).[18] The hand-assisted group was on average quicker by 30 minutes compared with the laparoscopic donor nephrectomy (95% CI 3.84 to 56.22; p=0.02).[21] A recent systematic review stated that there appears to be sufficient evidence to conclude that both renal function and renal blood flow are decreased during pneumoperitoneum. The magnitude of the decrease is dependent on factors, such as preoperative renal function, level of hydration, level of pneumoperitoneum, patient positioning, and duration of pneumoperitoneum.[42]

Older donors

Due to the increasing organ shortage, more and more transplant centres are retrieving kidneys from older donors. Excellent results in younger donors encouraged them. With increased age more comorbidity such as hypertension and diabetes is manifested. Transplantation of kidneys from older donors has been associated with early hyperfiltration renal injury and shortened graft survival.[43,44] The use of older living donors remains controversial because of the physiological decline in glomerular filtration rate beginning in the third decade of life and an increased risk of surgical complications for the older kidney donor.[45,46] Garg *et al.* assessed a systematic review on proteinuria and reduced kidney function in living kidney donors.[47] They revealed that older age at the time of donation was associated with both lower pre- and post-donation glomerular filtration rate (GFR). However, the change in GFR after donation was not statistically associated with donor age at the time of donation. Boudville *et al.* performed a systematic review on hypertension after kidney donation and revealed a 5 mmHg increase in blood pressure within five to ten years after donation over that anticipated with normal ageing.[48] Age usually older than 60 years and older age at the time of donation were prognostic features associated with larger increases in blood pressure. The United Kingdom guidelines for living donor kidney transplantation stated that age alone is not an absolute contraindication to donation but the medical assessment of older donors (>60 years) must be particularly rigorous to ensure that they are suitable. Both donor and recipient should be made aware that the older donor may be at greater risk of perioperative complications and that the function and possibly the long-term survival of the graft may be compromised.[49]

However, studies have demonstrated similar graft survival rates of older and younger kidney donors.[50-53] Several studies revealed no differences in complication rates between older and younger donors.[54-56] In a prospective study surgical outcome and the quality of life were examined in older living donors, defined as 55 years and older. There were no significant differences in intraoperative and postoperative complication rates or in the one-year graft survival rate between younger and older donors. Elderly donors (n=34) had both significantly lower postoperative pain at rest at day 1 compared with the younger group (p=0.019) and a lower total pain score in the analysis for the whole follow-up period (p=0.002).

Obese donors

More and more transplant centres are faced with obese donors. However, obesity is recognised as an independent cardiovascular risk factor and has also been shown to be a significant risk factor for complications following major surgery, including living kidney donation.[57,58] Recently, obesity has been recognised as an independent risk factor for end-stage renal disease.[59] Compared with persons who had normal weight, obese persons had an increased adjusted relative risk for end-stage renal disease. In a retrospective study of 73 patients, Praga *et al.* reported that 13 out of 14 (92%) obese donors (BMI >30) developed proteinuria and renal impairment after a mean follow-up of ten years compared with 12% of nonobese donors.[60] A retrospective study involving 5304 donors revealed no differences in readmission and reoperation rates between normal and obese donors. Higher BMI was associated with higher blood pressure (p<0.01). At six months, decline in estimated glomerular filtration rate from baseline (p=0.63) and percent change in creatinine (p=0.11) did not differ significantly across groups. Delayed graft function was more common among recipients of kidneys from very obese donors (odds ratio 2.16, CI 1.20 to 3.89, p<0.01).[61] Nevertheless, obese donors are accepted in donor selection programmes. The United Kingdom guidelines for living donor kidney transplantation describe that obese patients should undergo careful preoperative evaluation to exclude cardiovascular, respiratory and renal disease. They should be counselled regarding the increased perioperative risk and potential long-term risk of renal disease and advised to lose weight prior to donation and encouraged to adopt a healthy lifestyle.[49]

A randomised controlled trial comparing two mini-incision techniques and judging the impact on the quality of life, pain, and safety of living kidney donors, revealed significantly longer incision length as well as higher blood loss in obese donors.[62] Open surgical nephrectomy in obese subjects is associated with higher rates of postoperative complications, primarily wound related.[58] A prospective study revealed a lower conversion rate in obese female

donors compared with obese male donors, due to different distribution of fatty tissue.[63] At this moment data are lacking on whether hand-assisted laparoscopic donor nephrectomy or total laparoscopic donor nephrectomy has additional advantages in kidney retrieval from obese donors.

POSTOPERATIVE FACTORS

Complications

The described mortality risk for open and laparoscopic nephrectomy is 0.03%.[8,64] The complication rate of donor nephrectomy is approximately 10%.[6,18] Major complications, defined as Clavien[65] grade ≥3, are rare, ranging from 2.9 to 5.8%.[66-68] By comparison, pulmonary complications, including atelectasis, pneumothorax, pulmonary congestion, hypoxia, thrombophlebitis, intramural thrombus, and deep vein thrombosis, were reported more often after open donor nephrectomy than after laparoscopic donor nephrectomy. Wound complications including wound infection or abscess, wound haematoma, or seroma and incisional hernia were reported both for laparoscopic donor nephrectomy and open donor nephrectomy patients. Vascular complications, in particular injury to renal arteries and veins, were reported more often for laparoscopic donor nephrectomy patients, whereas fever, pain, and nausea were reported more often for open donor nephrectomy patients. In 2006, Kocak *et al.* described a graded classification scheme for reporting complications of laparoscopic donor nephrectomy, which may be useful for maintaining registry information on donor outcomes and when informing potential donors about the risks and benefits of this procedure.[69] In their analysis of 600 laparoscopic donor nephrectomies a complication rate of 7.2% was reported. These complications were scored in four grades. Grade 1 was defined as all events that, if left untreated, would have a spontaneous resolution or needed a simple bedside procedure (39.5%). Grade 2 complications differ from grade 1 in that they are potentially life-threatening and usually require some form of intervention, but do not result in ongoing disability (55.8%). Grade 3 complications are events with residual or lasting disability (4.7%). Grade 4 events are those resulting in renal failure or death because of any complication (0%).

Long-term follow-up

Long-term follow-up data are crucial for potential donors. In the open (donor) nephrectomy group numerous studies have revealed no increased risk in morbidity or mortality.[70,71] Forty-five year follow-up of World War II veterans who had undergone unilateral nephrectomy for trauma revealed no increased risk of hypertension or end-stage renal disease.[72] A recent cohort study from a single centre published long-term follow-up after kidney donation.[73] In total 3698 kidney donors were followed from 1963 to 2007. End-stage renal disease developed in 11 donors, a rate of 180 cases per million persons per year, as compared with a rate of 268 per million per year in the general population. Older age and higher body-mass index were associated with both a GFR <60 ml/min and hypertension. Survival appears to be similar to that in the general population.

In addition, the physical and mental quality of life of the donors was higher for the donors compared with a control group. Laparoscopic donor nephrectomy is a more recent technique and long-term follow-up data are not yet available.

Recipient graft function

One-year graft survival after laparoscopic donor nephrectomy ranges from 93 to 100% and after open donor nephrectomy from 91 to 100%.[6,18] Five-year graft survival after laparoscopic donor nephrectomy is 91% and after open donor nephrectomy 86%.[19]

To date, no long-term graft survival data between laparoscopic donor nephrectomy and open donor nephrectomy are available.

We might conclude that laparoscopic procurement of living donor kidneys does not have a clinically measurable negative effect on the kidney transplant.

Quality of life

The benefits of living kidney transplantations are well documented and a recently published systematic review revealed that most donors have a quality of life that is similar or even better when compared with the general population.[74] Most studies in which the donor's quality of life is evaluated report equivalent or better results if compared with healthy controls.[75-78] These results are linked to the intense medical evaluation of potential living kidney donors, resulting in the selection of only healthy and motivated individuals. Preoperatively, quality of life scores are higher than the age-matched healthy population. Postoperatively, the quality of life drops significantly; however, after three months it returns to the level at baseline.[34]

Several studies as described earlier in this review have demonstrated a better quality of life of donors after laparoscopic donor nephrectomy than after open donor nephrectomy.[14,79,80]

Costs

Laparoscopic donor nephrectomy has the potential to be more expensive due to the longer surgery and the use of disposable instruments. However, the shorter hospital stay and the donor's earlier return to work should negate the costs. Global hospital costs related to a living donor laparoscopic procedure depend on the balance between the length of the hospital stay and equipment costs.[6,81] Several studies have demonstrated the better cost-effectiveness of laparoscopic donor nephrectomy as compared with open donor nephrectomy. This result of the laparoscopic

technique can only be achieved if the length of hospital stay is short and there is a low complication rate.

CONCLUSION

Laparoscopic donor nephrectomy is a relatively new technique and has become a safe procedure. Various earlier contraindications to laparoscopic donor nephrectomy, such as right donor kidney, multiple vessels and anomalous vasculature, have been overcome with increasing experience. Compared with open donor nephrectomy, laparoscopic donor nephrectomy has shown superior results in terms of postoperative pain, cosmetics, convalescence, and return to normal daily activities. No significant differences exist between the two approaches in terms of complication rates, cost-effectiveness and graft function. Finally, the longer operating time and warm ischaemia time during laparoscopic donor nephrectomy showed no significant deleterious effect on graft survival. Laparoscopic donor nephrectomy has become the standard method for procuring kidney grafts of living donors in many centres.

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REVIEW

The effect of haemophilia and von Willebrand disease on arterial thrombosis: a systematic review

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ABSTRACT

Background: Patients with haemophilia and von Willebrand disease (VWD) may have a reduced cardiovascular mortality, due to a hypocoagulable state or decreased atherogenesis. We performed a systematic review to assess the association between haemophilia and VWD, and fatal and nonfatal arterial thrombosis and asymptomatic atherosclerosis.

Methods: Medline and PubMed were searched to identify studies that assessed the incidence of cardiovascular mortality and morbidity in haemophilia and VWD, and that measured asymptomatic atherosclerosis with intima media thickness (IMT) of the carotid and femoral arteries, or flow-mediated dilatation (FMD) of the brachial artery. Weighted standardised mortality ratios (SMR) and mean differences (WMD) were calculated and pooled using a random effects model.

Results: 15 longitudinal and cross-sectional studies consisting of 19,242 patients were included. Mortality due to arterial thrombosis was nonsignificantly reduced in patients with haemophilia compared with healthy controls (SMR 0.51, 95% CI 0.24 to 1.09). Haemophilia reduced nonfatal coronary events, and severe haemophilia offered better protection, but these results were based on a single study. No results were available for VWD. Although IMT of the carotid and femoral arteries was similar between VWD and haemophilia patients and healthy controls, atherosclerotic plaques of the large arteries were less prevalent in haemophilia patients. Only two studies assessed FMD and the results were inconsistent.

Conclusion: Haemophilia may reduce arterial thrombosis, but this association should be further studied in haemophilia patients with a higher prevalence of cardiovascular risk factors.

KEYWORDS

Atherosclerosis, arterial thrombosis, flow mediated dilatation, haemophilia, intima media thickness, von Willebrand disease

INTRODUCTION

The concept of risk factors in cardiovascular disease (CVD) has been well established. Smoking, hypertension, obesity, hypercholesterolaemia, diabetes mellitus, and a positive family history for CVD are all associated with an increased risk of morbidity and mortality due to CVD. A prothrombotic state contributes to the development of CVD.[1] Increased levels of fibrinogen, von Willebrand factor (VWF), and factor VIII have all been linked to arterial disease.[2,3] VWF is essential for platelet adhesion and aggregation. Furthermore, VWF acts as the carrier protein for coagulation factor VIII. Factor VIII contributes to the formation of a fibrin-rich clot, and also has a role in the formation of occluding thrombi in stenotic vessels. Patients with haemophilia A, who have a congenital deficiency of clotting factor VIII, are thought to be protected against mortality due to arterial thrombosis.[4,5] This protection may be due to hypocoagulability, which is associated with decreased thrombin generation and results in inhibition of thrombus formation.

On the other hand, haemophilia or VWD may also decrease the formation of atherosclerotic plaques. Studies evaluating preclinical atherosclerosis by measurement of intima media thickness (IMT) and flow-mediated dilatation (FMD) are, however, conflicting.[6,7] Moreover, autopsy findings have found extensive atherosclerotic plaques in subjects with VWD and haemophilia, and case reports have been published about patients with occlusive arterial thrombi.[8-15]

Although this subject was recently reviewed,[16] a systematic review assessing these studies has not been performed. We therefore systematically evaluated all literature to determine whether patients with haemophilia or VWD are protected against arterial thrombosis, and whether the prevalence of asymptomatic atherosclerosis is reduced. In order to thoroughly investigate this association, we first analysed fatal arterial thrombosis, followed by nonfatal events, and finally we addressed preclinical atherosclerosis.

METHODS

Data sources and study selection

We identified all published studies that evaluated the prevalence of atherosclerosis and arterial thrombosis in patients or carriers with haemophilia A or B, and VWD. A comprehensive literature search was conducted by a clinical librarian of Medline from 1950 to December 2009, and Embase from 1980 to December 2009. The following search terms were used: haemophilia, von Willebrand disease or coagulation disorder. The results of this search were combined with a subsequent search, in which the terms were arterial thrombosis, cardiovascular disease, arterial occlusive disease, atherosclerosis, cerebral vascular accident, stroke, myocardial infarction, acute coronary event, peripheral vascular event, peripheral artery disease, intima media thickness and flow-mediated dilatation. No language restrictions were initially applied to the search. A manual review of references from primary or review articles was performed to identify any additional relevant studies. The 'related articles' feature of PubMed was also used.

Study selection

Those studies that entailed observations of the same subjects over well-defined time periods were defined as longitudinal studies (e.g. prospective and retrospective cohort studies and registry studies). Longitudinal studies were included if they reported on mortality due to arterial thrombosis (i.e. ischaemic heart disease or ischaemic stroke) in carriers of haemophilia or patients with haemophilia or VWD. Carriers of haemophilia were also included in the analyses since they may have a bleeding tendency that can be similar to that of mild haemophiliacs. But, since pooling male haemophilia patients and female carriers may lead to heterogeneity, we analysed these groups separately. We further specified that every longitudinal study should either report a standardised mortality ratio (SMR) with 95% confidence interval (95% CI) or report sufficient data to estimate this. To adjust for differences in age with the reference population, SMRs were calculated, in which the incidence rates in the patients and the general population are standardised with the age distribution (in person-years) of the patients as weights. This leads to the calculation of an expected number of events, which is the number of events that would have happened in the patient group, if the population rates had applied to it. The SMR is the ratio of the observed over the expected number of events. Furthermore, all longitudinal studies reporting nonfatal arterial thrombotic events were also included. Only those cross-sectional studies that measured asymptomatic atherosclerotic disease in patients with haemophilia or VWD by means of ultrasonography, IMT or FMD were included in this review.

Study selection and quality assessment

After identifying relevant titles, the abstracts of these studies were read to decide if the study was eligible. The full article was retrieved when the information in the title or abstract appeared to meet the inclusion criteria of this systematic review. The list of articles was reviewed independently by two investigators (SB and MZ). Disagreement between the two reviewers was intended to be resolved by consensus or by the opinion of a third author if necessary. However, there was no disagreement during the review process.

Using a standardised data extraction sheet, the following data were collected from the articles: lead author, publication year, study design, sample size, years of follow-up, description of the study population, type of coagulation disorder and, if available, information on the classification of the coagulation disorders. In addition, the following baseline characteristics were extracted (if reported): mean age of the population and the number and proportion of male patients. From cross-sectional studies crude and adjusted (for cardiovascular risk factors) mean IMT thickness of carotid and femoral artery, and mean percent of flow-mediated dilatation were extracted. From longitudinal studies the SMR for fatal and nonfatal arterial thrombotic events was either calculated or extracted. The quality of cohort studies was assessed using a specific checklist consistent with the consensus recommendations by the Meta-analysis Of Observational Studies in Epidemiology group.[17] Cohort studies were assessed for quality according to four design features for each study: prospective data collection, consecutive patient enrolment, a clearly stated duration of follow-up and a description of losses during follow-up.

Statistical analysis

Review Manager (RevMan version 5.0, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2008) and the Stata statistical software package 10.0 (Stata Corp., College Station, TX) were used to pool data for each outcome variable. Summary estimates, including 95% CIs were calculated. The cross-sectional studies consisted of continuous outcome data; therefore, means and standard deviations of IMT were used to calculate a weighted mean difference in the meta-analysis. Data were pooled using the random effect model, where appropriate. X^2- and I^2 statistics were used to assess between-study heterogeneity. The SMRs in the longitudinal studies were pooled using a random effects model and this was added in a Poisson regression model by calculating the ratio of the number of observed over expected events, in which the number of observed events is the sum of the observed events in all studies, and the number of expected events is the sum of the expected number of events in all studies. As for the SMRs of the individual studies, confidence intervals for the pooled SMR were based on a Poisson distribution of the observed number of events, whereas the expected number was considered invariant.

Results

Our initial search yielded 4450 potential literature citations (*figure 1*). Of these, 4349 were excluded after scanning titles. A total of 86 studies were excluded after reading the articles: 60 studies were case reports, ten citations were letters, three were autopsy studies, two studies only reported venous thrombosis, another two were reviews, and in one study the SMR was not reported and could not be calculated. The interobserver agreement for study selection and quality assessment was 100%. So, 15 studies were included in the present review, with a total number of 19,242 patients, with 14,754 haemophilia A patients, 3408 haemophilia B patients, 965 carriers of haemophilia A and B, and 115 VWD patients (*table 1*).[4-7,18-28] The number of patients in the various studies ranged from 24 to 6018. The mean age of the patients in the various studies varied between 35 and 54 years. Eight longitudinal studies[22-26,28] and three cross-sectional studies[6,18,19] investigated males. In the remaining three cross-sectional studies the percentage of male subjects ranged between 43 and 60%.[7,20,21] In the remaining longitudinal study all patients were female carriers.[27]

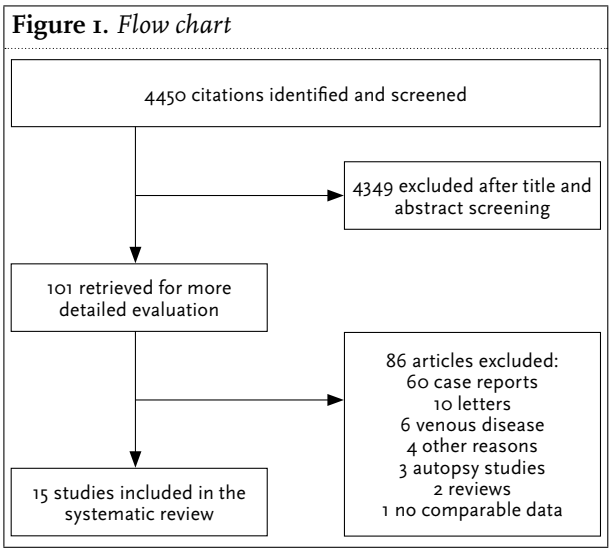


Figure 1. *Flow chart*

CARDIOVASCULAR MORTALITY IN PATIENTS WITH HAEMOPHILIA

All longitudinal studies[4,5,22-25,27,28] described cause-specific mortality. All studies reported on both haemophilia A and B patients, but the influence of the type of haemophilia was not analysed separately. One of these studies, by Srámek and colleagues, studied carriers of haemophilia A and B.[27] None of the longitudinal studies analysed patients with VWD. The duration of follow-up varied between two and 21 years. Follow-up was complete in two studies,[4,24] and nearly complete (lost to follow-up <5%) in six other studies.[5,22,23,25,27,28] In one study, no information on completeness of follow-up was provided.[26] Information on various parameters in the longitudinal studies was usually obtained through surveys supplemented with data from the haemophilia treatment centres, treating or attending physicians, municipal registries and death certificates. All studies compared cause-specific mortality with that of the general male population obtained through either the Central Bureau of Statistics or the World Health Organisation.

Most longitudinal studies[4,5,22-25,27] assessed cardiovascular mortality due to ischaemic heart disease, whereas one study[5] also mentioned mortality due to ischaemic stroke. One longitudinal study[28] reported on mortality due to circulatory disease without further specifying the underlying causes, and was therefore left out from the pooled analysis. Three longitudinal studies[4,22,23] assessed Dutch haemophilia patients from 1976 to 2001.

The mean age of mortality in the various studies ranged between 44 and 54 years. Overall, the number of fatal arterial thrombotic events was low. Compared with the general population, patients with haemophilia had a nonsignificant reduced mortality due to arterial thrombosis. For the seven studies, the overall SMR using a random effect model was 0.51 (95% CI 0.24 to 1.09). There was considerable, but not significant, heterogeneity between the studies (p=0.12) (*figure 2*). All longitudinal studies,[4,5,22-24,27] with the exception of the study by Soucie and colleagues,[25] found a reduced mortality due to arterial

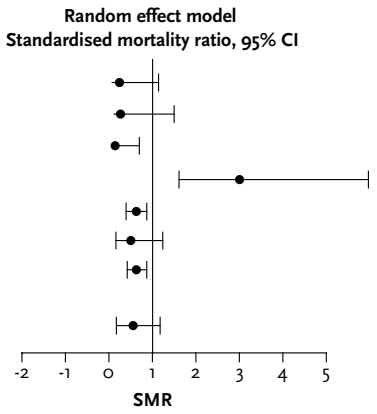
Table 1. *Characteristics of studies included in the systematic review*

Source	Population							Design	Outcome	Follow-up	
	Total	Haemophilia A	Haemophilia B	Severe	Mild	Moderate	VWD			Years	(n) loss to follow-up
Rosendaal et al. (1989)[4]	717	616	101	321	227	169	na	Longitudinal	Mortality	10.9	0
Koumbarelis et al. (1994)[24]	531	460	71	212	227	92	na	Longitudinal	Mortality	21	0
Triemstra et al. (1995)[22]	919	796	123	381	366	172	na	Longitudinal	Mortality	6.4	38
Walker et al. (1998)[28]	2450	1985	465	857	1127	465	na	Longitudinal	Mortality	15	53
Bilora et al. (1999)[20]	76	64	na	nr	nr	nr	12	Cross-sectional	IMT	na	na
Soucie et al. (2000)[25]	2950	2334	616	1252	902	717	na	Longitudinal	Mortality	2.6	71
Sramek et al. (2001)[18]	76	52	7	20	34	5	17	Cross-sectional	IMT	na	na
Bilora et al. (2001)[6]	40	25	na	nr	nr	nr	15	Cross-sectional	IMT	na	na
Sramek et al. (2003)[27]	965#	nr	nr	nr	nr	nr	na	Longitudinal	Mortality	15	33
Sramek et al. (2004)[7]	47	na	na	na	na	na	47	Cross-sectional	IMT	na	na
Kulkarni et al. (2005)[26]	3422	2.714	708	1419	1083	827	na	Longitudinal	Arterial events	6	nr
Plug et al. (2006)[23]	967	796	171	386	414	167	na	Longitudinal	Mortality	9	2
Bilora et al. (2007)[21]	24	na	na	na	na	na	24	Cross-sectional	IMT	na	na
Darby et al. (2007)[5]	6018	4874	1144	3222	1320	1476	na	Longitudinal	Mortality	21	194
Sartori et al. (2008)[19]	40	38	2	nr	nr	nr	na	Cross-sectional	IMT, FMD	-	-
Total	19,242	14,754	3408				115				

nr = not reported; na = not applicable; #: carriers of haemophilia.

Figure 2. *The influence of haemophilia on cardiovascular mortality*

Study	Events Observed	Expected	SMR	95% CI
Rosendaal 1989	1	5	0.20	0.0-1.1
Koumbarelis 1994	1	4	0.25	0.0-1.4
Triemstra 1995	0	5.2	0	0.0-0.58
Soucie 2000	8	2.7	3	1.50-5.80
Sramek 2003	39	60.5	0.64	0.47-0.88
Plug 2006	6	12	0.50	0.20-1.10
Darby 2007	104	167	0.62	0.51-0.76
	Heterogeneity p=0.12			
	Overall SMR		95%	0.24-1.09



SMR = standardised mortality ratio.

thrombosis in carriers and patients with haemophilia, but due to the low number of events, confidence intervals were wide. Exclusion of the study with haemophilia carriers resulted in a SMR of 0.59 (0.33 to 1.05). When restricting analyses to studies with a follow-up period of ten years or longer and that had complete follow-up,[4,5,24] the association between a lower cardiovascular mortality and haemophilia became more consistent (overall SMR 0.59, 0.48 to 0.72). In the only study that investigated the influence of haemophilia on ischaemic stroke,[5] the SMR was 0.63, with a wide confidence interval (0.17 to 1.62). The influence of the severity of haemophilia on cardiovascular mortality was assessed in one study, by Darby and colleagues.[5] A similar reduction in cardiovascular mortality was found in patients with severe, moderate and mild haemophilia but, again, the number of fatal cardiovascular events was too

low to detect potential differences (1.2% in patients with severe haemophilia, and 1.9% for patients with moderate and mild haemophilia).

NONFATAL CARDIOVASCULAR DISEASE

Only one study investigated the influence of haemophilia on nonfatal arterial thrombotic events.[26] The occurrence of ischaemic heart disease (consisting of acute myocardial infarction, acute/subacute coronary syndrome, angina pectoris, and chronic heart disease) in 3422 patients with haemophilia was based on hospital discharge diagnosis, and was compared with general US males based on the National Hospital Discharge survey. From 1993 until 1998, ischaemic heart disease was reported 79 times in 48 patients, corresponding with a prevalence of approximately 2.3%. Among 45- to 64-year-old haemophilic men, the discharge rate (per 1000) for ischaemic heart disease was 24.1, 50% lower compared with that of US males (48.9/1000). This difference was 28% among patients of 64 years and older (127.3 *vs* 175.6 respectively). In addition, the incidence of IHD was higher in patients with mild haemophilia (3.4%) than in moderately severe (0.7%) or severe (0.4%) types of haemophilia (p<0.001). When haemophilia A and B were analysed separately, IHD was more prevalent in patients with haemophilia B (2.4%) than haemophilia A (1.1%) (p<0.05). No studies assessed the prevalence of peripheral arterial disease or stroke.

PREVALENCE OF ASYMPTOMATIC ATHEROSCLEROSIS IN HAEMOPHILIA AND VWD

Of the six cross-sectional studies[6,7,18-21] that assessed endothelial function or preclinical atherosclerotic disease, one study[19] involved patients with haemophilia A and B, two studies[6,20] involved patients with haemophilia A and VWD, one study haemophilia A and B and VWD[18], whereas one study[21] described patients with type IIb VWD, and one study[7] investigated type III VWD patients. None of the studies were performed on either the same or an overlapping patient group. The ultrasonographers were not blinded for the severity of the bleeding disorder in any of the studies.

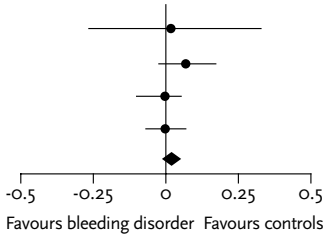
Intima media thickness

Four studies assessed the presence of asymptomatic atherosclerosis by measuring IMT of the carotid artery,[7,18,19,21] with a total of 187 patients (99 haemophilia patients and 88 patients with VWD) and 290 control subjects, whereas three studies also assessed the femoral artery.[7,19,21] Overall, mean IMT of the carotid artery was 0.75 mm in patients with haemophilia and VWD and 0.74 mm in healthy controls, matched for age and sex (WMD 0.01 mm, 95% CI -0.02 to 0.04). The mean IMT of the femoral artery was 0.75 mm in patients with a coagulation disorder and 0.79 mm in controls (WMD -0.04 mm, 95% CI -0.10 to 0.02). Statistical heterogeneity was not present in either of the two analyses (*figure 3*).

The presence of atherosclerotic lesions and extent of the occlusion of the arteries was assessed by ultrasonography in two studies,[6,20] without quantification of the IMT. The presence of atherosclerotic plaques was analysed in various arteries (common carotid, bifurcation, brachial, femoral and abdominal aorta) of patients with haemophilia A or VWD. In the first study, atherosclerotic plaques of the carotid artery were present in 13.1% of 76 patients with haemophilia A and VWD (mean age 58 years), and in 27.2% of the 77 controls (p<0.05).[20] This difference was more pronounced in patients older than 60 years. In the second study,[6] atherosclerotic plaques in the abdominal aorta were present in 7.5% of the 40 patients with haemophilia A and VWD (mean age 48 years) and in 27.5% of the 40 controls (p<0.001). 12.5% of the 40 patients and 42.5% of the 40 control subjects had plaques in the leg arteries (p<0.001).

Figure 3A. *Carotid IMT in patients with haemophilia and VWD compared with healthy controls*

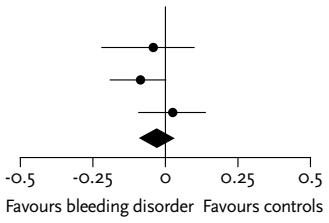
	Bleeding disorder			Controls				Mean difference, random, 95% CI
Study or subgroup	Mean	SD	Total	Mean	SD	Total	Weight	
Bilora, et al. 2007	0.87	0.38	24	0.86	0.21	24	3.0%	0.01 [-0.16, 0.18]
Sartori, et al. 2008	0.74	0.09	24	0.7	0.11	24	28.0%	0.04 [-0.02, 0.10]
Sramek, et al. 2001	0.76	0.18	76	0.77	0.18	142	36.0%	-0.01 [-0.06, 0.04]
Sramek, et al. 2004	0.63	1.17	47	0.63	0.09	84	33.1%	0.00 [-0.05, 0.05]
Total (95% CI)			171			274	100.0%	0.01 [-0.02, 0.04]



Heterogeneity: Tau²=0.00, Chi²=1.80, df=3 (P=0.61), I²=0%; Test for overall effect: Z=0.51 (P=0.61).

Figure 3B. *Femoral IMT in patients with haemophilia and VWD compared with healthy controls*

Study or subgroup	Bleeding disorder Mean	SD	Total	Controls Mean	SD	Total	Weight	Mean difference, random, 95% CI
Bilora, et al. 2007	0.92	0.23	24	0.96	0.23	24	21.0%	-0.04 [-0.17, 0.09]
Sramek, et al. 2001	0.75	0.31	76	0.83	0.3	142	45.5%	-0.08 [-0.017, 0.01]
Sramek, et al. 2004	0.59	0.31	47	0.57	0.23	84	33.5%	0.02 [-0.08, 0.12]
Total (95% CI)			171			274	100.0%	0.01 [-0.02, 0.04]



Heterogeneity: Tau²=0.00, Chi²=2.19, df=2 (P=0.33), I²=9%; test for overall effect: Z=1.22 (P=0.22).

Four cross-sectional studies[6,18-20] analysed the relation between the severity of the coagulation defect and atherosclerosis. In the study by Srámek and colleagues[18] mean IMT of the femoral arteries was similar in patients with moderate and severe haemophilia (0.74 mm, 95% CI 0.64 to 0.87) and in individuals with a mild form (0.76 mm, 95% CI 0.69 to 0.84). Also in the study by Sartori and colleagues, IMT of the carotid artery was not different between patients with moderate to severe compared with mild haemophilia.[19] Two studies by Bilora *et al.*[6,20] showed less atherosclerotic plaques in patients with a more severe coagulation defect.

Flow-mediated dilatation

Endothelial dysfunction, assessed with flow-mediated dilatation (FMD), was analysed in two studies.[19,21] FMD was lower in 40 haemophilia patients compared with 40 controls (3.8±5.2% *vs* 20.3±13.0%, p<0.0001) in the first study, and this difference remained after adjustment for viral infections.[19] In this study, severity of haemophilia did not affect FMD. In the second study, the percentage of vasodilatation was 15.2±3.1% in 24 type IIb VWD patients and 14.1±2.9% in 24 controls, which was not different.[21]

DISCUSSION

Our findings suggest that mortality due to ischaemic heart disease is 50% lower in patients with haemophilia when compared with the general population. This association is more consistent in studies with longer and complete follow-up. Asymptomatic atherosclerosis as measured by IMT of the carotid and femoral arteries in patients with VWD and haemophilia was not different from healthy matched controls, but atherosclerotic plaques of the large arteries were less prevalent in these patients. Interestingly, in the only (large) study assessing nonfatal cardiovascular events, haemophilia not only reduced cardiovascular events, but patients with severe haemophilia had fewer events than those with mild or moderate haemophilia. Haemophilia B patients had more cardiovascular events than those with type A. The influence of VWD on cardiovascular events has not been studied.

The potential beneficial effect of haemophilia on arterial thrombosis could be the result of reduced thrombin formation. Thrombin is the key player in both fibrin formation and platelet activation. Thrombin cleaves fibrinogen to form fibrin, but can also trigger platelet activation through protease-activated receptors (PAR) 1 and 4.[29] This may lead to the formation of thrombi and ultimately to vascular occlusion. Importantly, thrombin may also influence the process of atherosclerosis. Tissue factor and PARs are highly expressed in human atheroma and are induced in response to injury in animal models.[30] *In vitro,* PAR activation induces leucocyte chemotaxis, smooth muscle cell proliferation and migration, which may lead to arterial remodelling and stenosis of the injured artery.[31] In addition, coagulation factors and PARs are also involved in inflammatory responses and repair after injury.[32] These data suggest that local arterial damage may trigger both platelet activation and thrombin formation, which may further lead to arterial remodelling. It may well be that patients with haemophilia, who have a decreased thrombin formation, are relatively protected from these atherosclerotic processes.

Our systematic review cannot answer the question whether the association between haemophilia and arterial thrombosis is causal. The observed reduction in mortality, for instance, could be explained by a difference in cardiovascular risk factors between individuals with and without haemophilia. As previously shown by Rosendaal and colleagues, this does not seem to be an explanation.[33] As a prerequisite for causality, the association between haemophilia and arterial thrombosis seems biologically plausible. In addition, the association was consistent in different studies: only the study by Soucie and colleagues found no reduction in cardiovascular mortality.[25] Furthermore, there seemed to be a dose-response gradient, in the sense that a more severe deficiency of factor VIII offered better protection. Although this was not

apparent in two longitudinal studies,[5,27] a more severe type of haemophilia also seemed to reduce nonfatal events and atherosclerotic plaques compared with mild haemophilia.[6,18-20,26] However, these data were based on a small number of patients and events, affecting the strength of the association. Finally, also carriers of haemophilia had a 36% reduction in mortality due to ischaemic heart disease.[27]

Life-long hypocoagulability may be an interesting model to investigate the role of haemostasis in the occurrence of arterial thrombosis and the formation of atherosclerotic plaques. Further studies on this subject could serve two goals. First, the role of coagulation in the formation of atherosclerotic plaque formation could be further explored. If low factor VIII levels reduce atherogenesis and this is mediated through decreased thrombin generation, specific anticoagulants, such as thrombin inhibitors, may be beneficial. Next, if patients with haemophilia and cardiovascular risk factors are not protected against atherosclerosis, cardiovascular prevention will also be applicable to haemophilia patients, since the life expectancy of these patients has considerably increased.[34]

However, we noticed some methodological drawbacks of the studies included in this systematic review. In general, the number of fatal cardiovascular events was low, mostly due to the relatively young age of the patients, and therefore confidence intervals were wide. Nonfatal cardiovascular events, which are more prevalent than fatal ones, were only reported in one longitudinal study.[26] Furthermore, the low life expectancy of haemophilia patients as a result of bleeding complications and poor treatment regimens in the 1970s and 1980s may have affected cause-specific mortality ratios. Also HIV, a well-known cardiovascular risk factor, may have influenced cardiovascular mortality, but was seldom mentioned in the studies. However, Darby and colleagues showed a 40% reduction in cardiovascular mortality in haemophilia patients without HIV.[5] Also in the IMT studies, the number of participants was small, whereas the detection of significant differences in IMT requires large populations. Together with the relative young age of the participants and a low prevalence of cardiovascular risk factors, an inverse association between hypocoagulability and atherosclerosis is difficult to detect. Since IMT is clearly affected by age[35] and atherosclerotic risk factors,[36] the question whether a hypocoagulable state protects against atherosclerosis should be studied in older patients with a higher prevalence of cardiovascular risk factors.

In conclusion, this systematic review suggests that patients with haemophilia have a reduced cardiovascular mortality. Whether this reduction is mediated by a lesser formation of atherosclerosis should be investigated in patients with a higher prevalence of cardiovascular risk factors.

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ORIGINAL ARTICLE

Reversible cardiac valvular disease in catastrophic antiphospholipid syndrome

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ABSTRACT

Catastrophic antiphospholipid syndrome (CAPS) is a severe form of antiphospholipid syndrome (APS). It frequently leads to multiorgan failure with an approximate mortality rate of 50%. The heart is involved in about 50% of the patients with CAPS. We report two cases with CAPS and severe heart manifestations, documented by echocardiography. Both women show regression of the valvular regurgitation under treatment. Valve replacement therapy was no longer necessary.

In earlier studies and case reports, cardiac valve involvement had been characterised by valve thickening and vegetations. We suppose that (sometimes reversible) microvascular disturbances lead to valvular regurgitation via papillary muscle dysfunction and myocardial stunning.

KEYWORDS

Antiphospholipid syndrome, catastrophic antiphospholipid syndrome, echocardiography, heart valve diseases, therapeutics, valve regurgitation

INTRODUCTION

Antiphospholipid syndrome (APS) is an autoimmune disease, in which the presence of antiphospholipid antibodies (aPL) is associated with recurrent arterial and venous thrombosis and/or recurrent obstetric morbidity.[1] The clinical manifestations are heterogeneous and mortality is extremely high (~50%) when the disease accelerates to a 'catastrophic' course. Less than 1% of all patients with APS present with this catastrophic form.[2,3]

Catastrophic APS (CAPS) develops by definition within a week and is characterised by: a) clinical evidence of multiple organ involvement (three or more), b) histopathological evidence of multiple small vessel occlusion (a minority also have large vessel thrombosis), and c) persistent presence of aPL, usually in a high titre; only one of the three types of antibodies (lupus anticoagulant (LAC), anticardiolipin antibodies (aCL) and anti-β2 glycoprotein-I antibodies (anti-β2 GPI)), is enough for diagnosis.[2,3]

In approximately 50% of the patients with CAPS the heart is involved.[4] The major cardiac manifestations in APS are heart failure due to cardiac myopathy, macrovascular and microvascular disease, and valvular involvement. Valvular abnormalities are deemed to be due to thickening or vegetations (Libman-Sacks or noninfective endocarditis).[5] The latter is the most common cardiac manifestation in CAPS. The mitral valve is most frequently involved, followed by the aortic valve.[6]

CASE REPORTS

We present two patients with heart valve regurgitation during the acute phase of CAPS, who show regression of the valve lesions under therapy.

Patient A

A 38-year-old woman of 60.4 kilograms presented to the cardiology unit with a two-week history of dyspnoea, orthopnoea and general malaise with 6 kg weight loss, preceded by arthralgias. There was no significant medical history, apart from two early spontaneous abortions, both before the tenth week of gestation. She did not use any medication, smoked approximately 30 cigarettes per day and denied alcohol and drug abuse.

On physical examination, the patient appeared ill and dyspnoeic. Her temperature was 37°C, blood pressure 190/140 mmHg, heart rate 115 beats/min and peripheral oxygen saturation 98% while breathing ambient air. Auscultation of the heart revealed a loud (grade III/VI)

holosystolic murmur on the apex without thrill or S3. Examination of the skin showed purplish discoloration in a patchy distribution over the lower extremities, characteristic of livedo reticularis. Findings on other organ systems were non-contributory; endocarditis stigmata were absent. Laboratory test results are shown in *table 1* and *2*.

A presumptive diagnosis of bacterial endocarditis was made, and a work-up for valve replacement was started.

According to the American Society of Echocardiography (ASE) the severity of a mitral valve regurgitation is quantified by specific and supportive signs. The specific signs include the regurgitation index or RJA/LAA index, i.e. ratio of the regurgitant jet area (RJA) to the left atrial area (LAA), both obtained in the same plane as the maximum regurgitant flow (<20% is mild, 20 to 40% is moderate, >40% is severe). The vena contracta is also an important parameter. This is defined as the narrowest central flow region of a jet that occurs at, or just downstream to, the orifice of a regurgitant valve (<0.3 cm is mild, 0.3 to 0.7 cm is moderate, >0.7 cm is severe). Two other important specific signs of a severe mitral regurgitation are the systolic reversal of flow in the pulmonary veins or a prominent flail of one of the mitral valve leaflets or a ruptured papillary muscle.

Transoesophageal cardiac ultrasound revealed a severe mitral regurgitation (RJA/LAA index >50%, vena contracta 0.74 cm and systolic reversal in the pulmonary vein) and diffuse hypokinesis of the left ventricle. The mitral valve leaflets showed signs of thickening, without vegetations (*figure 1*). Since repetitive blood cultures remained negative, an autoimmune pathogenesis was considered and tested for (*table 3*). A renal biopsy showed severe acellular vasculitis with thrombotic microangiopathy (TMA) and extensive fibrous intimal hyperplasia (FIH) (*figures 3* and *4*). A coronary angiogram showed no signs of macroscopic coronary artery disease.

The patient only met two out of the necessary four ARA criteria for the diagnosis of systemic lupus erythematosus (SLE), namely proteinuria and thrombocytopenia. Her anti-double-stranded DNA antibody (anti-dsDNA) titres were only marginally elevated.

CAPS, however, was a reasonable diagnosis: presence of aPL, thrombocytopenia, renal TMA, cardiac involvement, livedo reticularis and her obstetric history all fitted very well with this diagnosis. Moreover, these manifestations developed within one week, apart from her abortions. Therefore this patient received the diagnosis of CAPS.

She was initially treated with therapeutic dosages of low-molecular-weight heparin (LMWH), intravenous pulses of methylprednisolone of 1000 mg for three days, intensive plasma exchange (consisting of four litres on five consecutive days) and oral cyclophosphamide (2 mg/kg/day).

Table 2. *Results of urinalysis*

Variable	Patient A	Patient B	Reference range
pH	6.0	6.0	5.5-6.5
Screening dipstick			
- Nitrites	Negative	Negative	Negative
- Albumin	Positive	Positive	Negative
- Glucose	(4+)	(2+)	Negative
- Ketones	Negative	Negative	Negative
- Leucocytes	Negative	Positive	Negative
- Erythrocytes	Negative	(3+)	Negative
	Positive	Positive	
	(1+)	(3+)	
		Positive	
		(1+)	
Creatinine clearance (ml/min)	41.5		70-130
Total protein (g/24 hour)	1.76		0-0.15
Sediment (no. per high-power field)	2-5		0-2
- Erythrocyte count	10-20	5-10	0-4
- Leucocyte count	0		0
- Casts			

Table 1. *Results of laboratory tests*

Variable	Patient A	Patient B	Reference range
Erythrocyte sedimentation rate (mm/hr)	117	>120	0-20
Haemoglobin (mmol/l)	7.2	6.3	7.5-10
Mean corpuscular volume (fl)	82	85	80-100
Leucocyte count (x10^9/l)	5.3	18.3	4-10
- Neutrophils	4.0	14.3	1.5-9
- Lymphocytes	1.0	0.7	1.0-4.0
- Monocytes	2.0	0.2	0.2-0.8
- Eosinophils	<0.1	<0.1	<0.4
- Basophils	<0.1	<0.1	<0.2
- Schistocytes		0	0
Thrombocyte count (x10^9/l)	137	70	150-400
C-reactive protein (mg/l)	36	525	<5
Creatinine (µmol/l)*	121	112	50-90
Lactate dehydrogenase (U/l)	262	289	<250
Alkaline phosphatase (U/l)	68	225	0-120
Gamma-glutamyltransferase (U/l)	15	25	<40
Aspartate aminotransferase (U/l)	18	23	0-40
Alanine aminotransferase (U/l)	9	48	0-45
Amylase (U/l)	58	21	0-100
Creatine kinase (U/l)	43	27	<170
Creatine kinase MB isoenzymes (U/l)	9	9	<24
Troponin T (ng/ml) §	<0.05	<0.05	<0.05
NT-pro-BNP (pg/ml)	11451		<125
Activated partial thromboplastin time (sec)	48	41	20-35
Prothrombin time (INR)	1.1	1.0	0.9-1.1
Antithrombin III activity (%)		75	80-120
D-dimer (µg/ml)		7.16	<0.5
Fibrinogen (g/l)		4.9	2.0-4.0

*After 1 week the creatinine concentration rose to 188 and 117 for patient A and B, respectively. After six months there was a gradual decline in creatinine concentration to 135 and 70, respectively. §There was no follow-up of the cardiac enzymes.

Figure 1. *Echocardiography, apical four chamber view. A: Thickening of the mitral leaflets. B: Severe mitral regurgitation.*

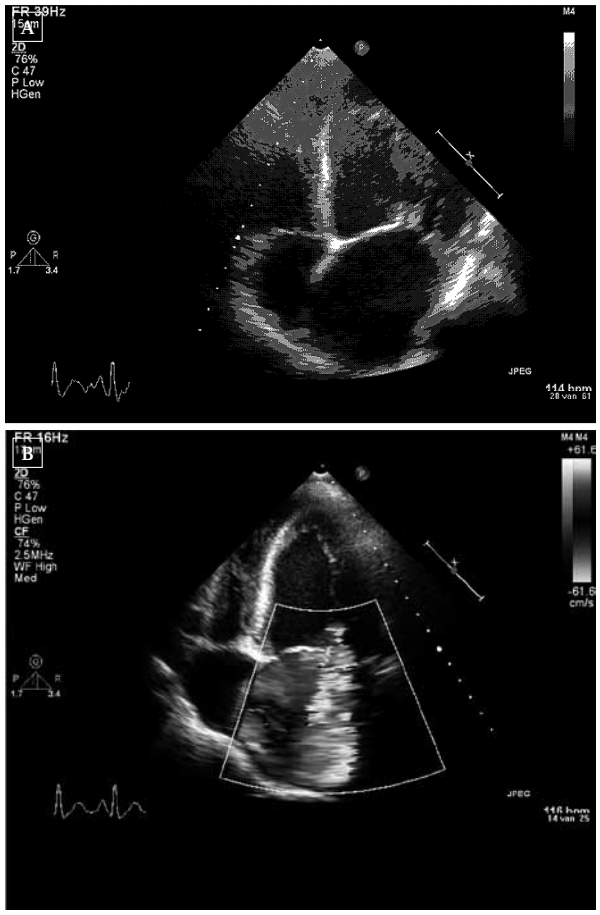
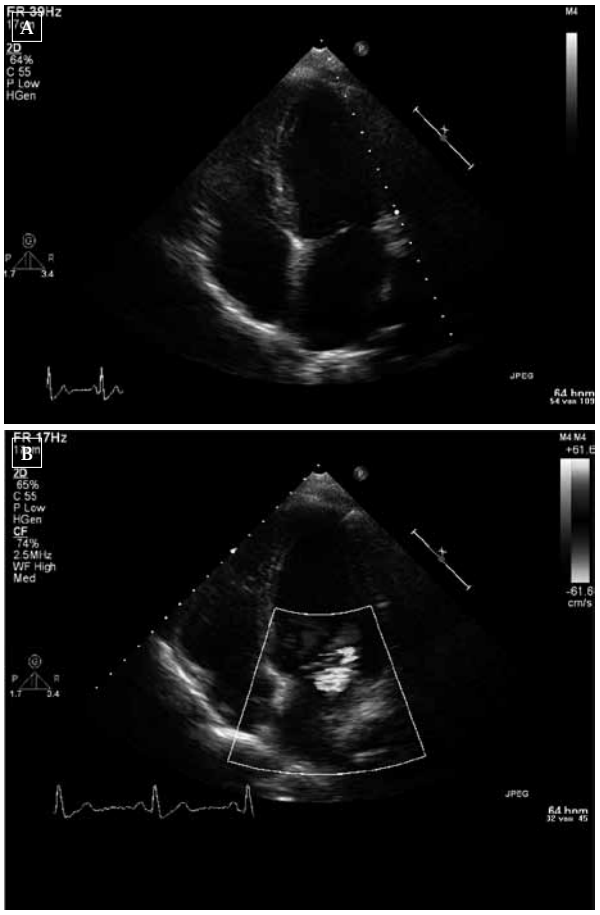


Figure 2. *Echocardiography, apical four chamber view. A: Minimal mitral valve thickening. B: Moderate mitral regurgitation.*



During follow-up, LAC could not be detected due to oral anticoagulation. IgG-aCL were still present, 11 months after the acute phase.

Nevertheless her clinical condition improved. We saw complete resolution of the haematological abnormalities and improvement of GFR and proteinuria. Moreover, the dyspnoea disappeared. The patient was dismissed with a maintenance dosage of cyclophosphamide (2 mg/kg/day) and prednisolone (20mg/day), acenocoumarol, cotrimoxazole (480 mg/day) and lisinopril. A second cardiac ultrasound – eight weeks later – showed significant regression of the mitral valve regurgitation, with an RJA/LAA index of less than 40%, a smaller vena contracta (0.59 cm) and no longer any signs of a systolic reversal of flow in the pulmonary veins (*figure 2*). Her serum creatinine peaked on day 3 of admission to 188 µmol/l [this was before start of cotrimoxazole], and stabilised at 94 µmol/l (14 months after admission with a MDRD[4] clearance of 58 ml/min/1.73 m^2, and a completely normal urinalysis). At follow-up three months after presentation the patient had a greatly improved renal function and a stable cardiac condition. Unfortunately her renal function did not recover to normal due to the chronic ischaemic nephropathy also seen in the renal biopsy. After three months therapy, cyclophosphamide was switched to azathioprine (1.5 mg/kg/day), and she was weaned from prednisolone. The scheduled valve replacement surgery could be cancelled.

Patient B

A 39-year-old woman, of Indonesian descent, presented at the emergency department with progressive abdominal pain in the right upper quadrant and nausea. Five days earlier her general practitioner had prescribed nitrofurantoin for an alleged cystitis. Further questioning revealed progressive exercise intolerance for a few months. Her medical history revealed four spontaneous births, and three missed abortions. Her regular medication consisted of alprazolam, diclophenac, omeprazole and tramadol for five days. She denied smoking, alcohol or drug abuse.

On physical examination, the patient appeared ill and apathic. The temperature was 35.9°C, blood pressure was 128/83 mmHg, and heart rate 105 beats/min. Neurological examination showed normal consciousness (Glasgow Coma Score E4-M6-V5), without signs of meningism or localising

Table 3. *Results of immunological tests*

Variable	Patient A	Patient B	Reference range
Direct antiglobulin (Coombs) test	Positive		Negative
Complement (g/l)			
- C3	1.0	0.6	0.9-1.8
- C4	0.2	0.3	0.1-0.4
Cryoglobulins	Negative	Negative	Negative
Autoantibodies			
- Antinuclear antibodies	Negative	Negative	Negative
- Anti-dou ble-stranded DNA antibody (U/ml)	Dubious	Negative	Negative
- Anti-smooth muscle antibody	Negative	Negative	Negative
- Anti-extractable nuclear antigens	Negative	Negative	Negative
- Anti-neutrophil cytoplasmic antibody	Atypical	Negative	No fluo-rescence
- Anti-PR3 and anti-MPO	Negative	Negative	Negative
- Lupus anticoagulant*	Positive*	Positive*	Negative
- IgG anticardiolipin antibody§	Positive	Negative	Negative
- IgM anticardiolipin antibody§	Dubious	Negative	Negative
- IgG anti-β2 glycoprotein-I antibody	Not tested	Negative	Negative
- IgM anti-β2 glycoprotein-I antibody	Not tested	Negative	Negative

***In patient A, tests for lupus anticoagulant could not be repeated while under anticoagulant therapy. * In patient B, lupus anticoagulant remained detectable two weeks after the acute phase; afterwards the test could not be repeated while under anticoagulant therapy. §In patient A, IgG anticardiolipin antibodies remained detectable for more than 11 months; IgM anticardiolipin antibodies became undetectable.**

Figure 4. *Renal biopsy, glomerulus with extensive fibrin deposition and stasis of red blood cells.*

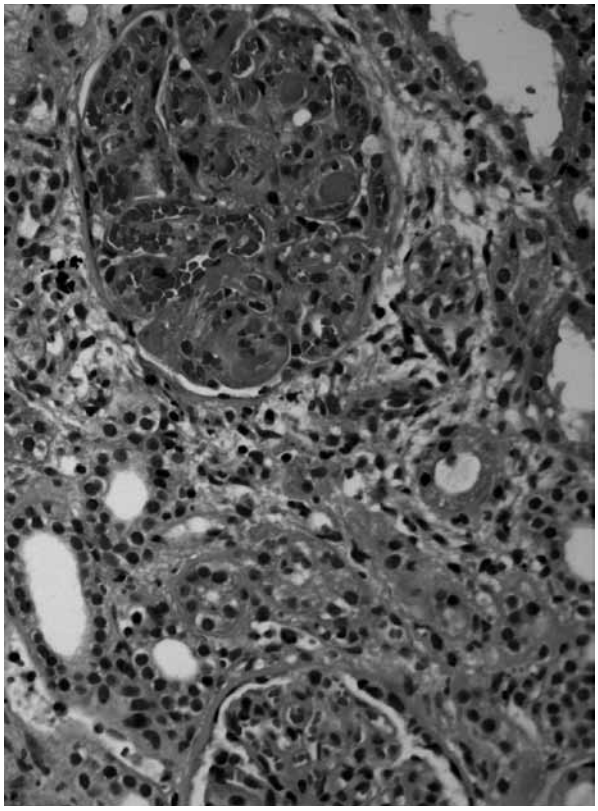
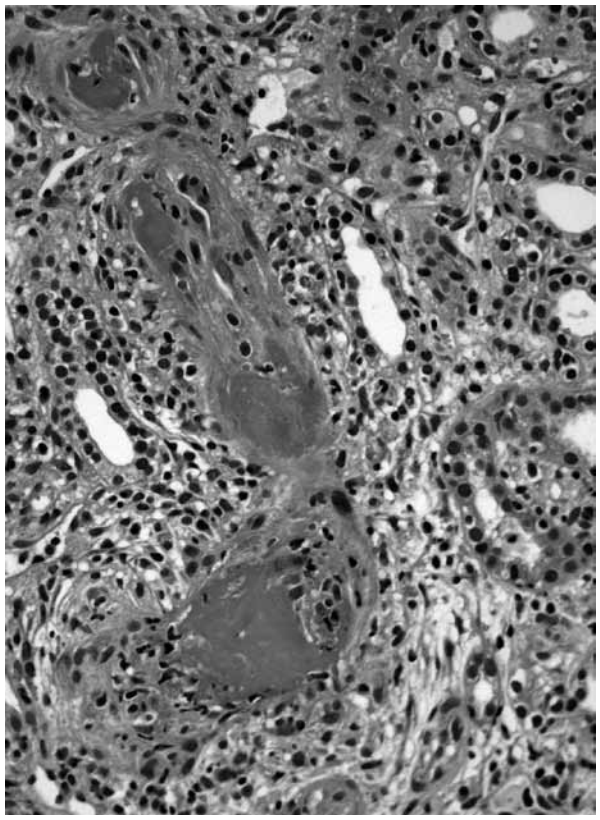


Figure 3. *Renal biopsy, arteriole with fibrinoid necrosis (original magnification: 40x).*



signs. Abdominal examination revealed normal peristalsis and diffuse tenderness with guarding on palpation of the right hypochondrion, and pain in the right costo-phrenic region. Vaginal and rectal examination were normal.

Laboratory test results are shown in *tables 1* and *2*. Ultrasonography of the abdomen did not show any abnormalities; chest radiography showed interstitial lung disease. A presumptive diagnosis of urosepsis with impending respiratory insufficiency due to ARDS was made and the patient was admitted to the intensive care unit. Antibiotic therapy with amoxicillin/clavulanic acid was started, which was later changed into ciprofloxacin when blood and urine cultures showed *Escherichia coli*. Major cerebrovascular disease was excluded with an MRI. Although quick resolution of fever followed after starting antibiotic therapy, stupor, abdominal pain, respiratory insufficiency, and thrombocytopenia persisted. CT angiography of the abdomen and thorax was performed, showing a triangular-shaped perfusion defect in the upper pole of the left kidney (renal infarction) and bilateral pleural effusions, without signs of pulmonary embolism or mesenterial thrombosis. Transthoracic cardiac ultrasound revealed a severe tricuspid regurgitation (vena contracta 0.74 cm, no signs of flow reversal in the liver veins, but a dense triangular jet density with an early peaking), a dilated right ventricle and atrium, a moderate mitral valve

regurgitation (RJA/LAA 20 to 40%, vena contracta 0.54 cm, no signs of systolic flow reversal in the pulmonary veins) with thickened valve leaflets and a diffuse left ventricular hypokinesis.

The increase of her APTT could not be corrected by addition of normal plasma, suggesting an inhibitor; this inhibitor turned out to be LAC.

This finding in combination with persistent thrombocytopenia, increased level of D-dimers, unremitting organic psychosyndrome, respiratory insufficiency and renal infarction, led to the diagnosis of CAPS (*table 3*). LAC was persistently present two weeks after the acute phase; later tests for LAC could not be repeated because of anticoagulant therapy. Anti-CL and anti-β2 GPI were negative and have remained negative for up to three months after the acute phase.

Treatment with LMWH was started and later replaced by acenocoumarol. Thrombocytopenia, which had been progressive in spite of resolution of sepsis, only improved after full anticoagulation with LMWH. The same was true for the organic psychosyndrome, which we attributed to cerebral microvascular disease. The interstitial lung disease and elevated pulmonary artery pressure could have been due either to adult respiratory distress syndrome (ARDS) due to sepsis or to CAPS. Cardiac ultrasound – eight weeks later – was normal besides a mild mitral valve regurgitation (RJA/LAA <20%, vena contracta 0.23 cm).

We omitted immunosuppression in this patient because of quick resolution of the symptoms under anticoagulation. While under treatment with full-dose acenocoumarol and without immunosuppression, patient remained in good condition during her follow-up of more than 24 months.

DISCUSSION

Both our patients had CAPS with hypokinesis of the left ventricle and valvular regurgitation as cardiac manifestations. An underlying mechanism of the formation of valvular vegetations is circulating aPL, which stimulates thrombin formation on endothelium.[6] Complement and aCL immunoglobulin deposits are also found in the subendothelial connective tissue of the deformed valves. This results in valve thickening, fusion, and rigidity causing dysfunction.[6]

We speculate that microvascular thrombosis in the myocardium and the papillary muscles could be an alternative explanation for the reversible regurgitation and hypokinesis in our patients. Unfortunately, there was no follow-up of the cardiac enzymes in our patients, as a marker for cardiac ischaemia.

Heart failure in patients with secondary APS (i.e. due to SLE) is thought to be the result of myocarditis due to immune complex formation and complement activation. Granular deposits of complement and immunoglobulin are found in myocardial blood vessels and muscle bundles.[7]

Septic myocardial depression in humans is characterised by reversible biventricular dilation and decreased systolic contractile function.[8] Cytokines, especially TNFα and IL-1β, are implicated as potent myocardium depressant factors in sepsis. Recently it has become clear that a multitude of cytokines may contribute to the multiorgan damage during the thrombotic storm in CAPS.[9] And thus could also have caused part of the cardiac manifestations in our patients.

Likewise, reversible pulmonary hypertension, which can be ascribed to either CAPS[10] or sepsis,[8] may have aggravated regurgitation of the tricuspid valve in patient B.

Echocardiography, especially transoesophageal, has shown to be an important technique that allows diagnosis of cardiac manifestations.[11] However, we expect that cardiac MRI will become a very important noninvasive modality, which can provide useful information regarding the presence of viable myocytes in patients with ischaemic or nonischaemic cardiac diseases.[12] In this setting, cardiac MRI may be an escape for myocardial biopsy.

Some recent guidelines for the management of patients with (C)APS are given in *table 4*.

Table 4. *Recommendations for the management of patients with (C)APS*

1. Treat the precipitating factor: infection, malignancy, autoimmune disorders
2. The mainstay of therapy for APS is anticoagulation; immunosuppression alone is not enough[13,14]
3. High-dose anticoumarin therapy does not seem to be necessary in view of recent information: INR 2.5 to 3.0 will do[15,16]
4. Do not forget to do enough blood cultures to exclude bacterial endocarditis
5. Because of its high mortality, catastrophic APS should be treated aggressively. Consider intensive immunosuppression with plasma exchange and/or intravenous immunoglobulins, pulses with methylprednisolone and cyclophosphamide in life-threatening CAPS (although there are no randomised controlled trials to support this policy)[15,17]
6. In case of operations, do not interrupt anticoagulation for longer than strictly necessary: operations are well-known precipitating factors for (a new bout of) CAPS[14,15,16]
7. Do not rush for cardiac surgery (see this paper!)

CONCLUSION

In our case reports we describe regression of valve regurgitation and left ventricular hypokinesis under intensive treatment for CAPS. Partial and complete regression of the cardiac manifestations have been described earlier, but reports are conflicting. We hypothesise that reversal of the valvular disease in our patients is (partially) due to the effect of anticoagulant (and

possibly immunosuppressive) therapy on microvascular disease.

We expect that an MRI is an important noninvasive way of providing more information about microvascular cardiac disease.

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CASE REPORT

Enteroviral encephalitis in a patient with a marginal zone lymphoma treated with rituximab

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ABSTRACT

A 64-year-old woman with a progressive marginal zone lymphoma for which she had received induction therapy with six courses of rituximab and fludarabine presented with fever while receiving maintenance therapy with rituximab. In addition to the fever she complained of nausea, vomiting, weight loss and fatigue. After an extensive diagnostic procedure no cause was found for the fever. Finally, additional testing showed a positive polymerase chain reaction (PCR) for enterovirus in the cerebrospinal fluid and faeces. Because the immunoglobulin G level of our patient was 4.06 g/l (normal values 5.2 to 16 g/l), she was treated with intravenous immunoglobulins (IVIg) weekly with the goal to maintain an IgG level above 10 g/l. This resulted in a significant rise in anti-enteroviral antibodies from 10 IE/ml to 106 IE/ml. One month after treatment with IVIg, while withholding the rituximab, the PCR for enterovirus on faeces was negative and antibodies to the enterovirus in the serum had returned to normal levels. Rituximab can cause a prolonged B-cell deficiency resulting in hypogammaglobulinaemia. We believe that treatment with ritxumab may have played a significant role in the development of this rare central nervous system infection.

What was known on this topic?
Rituximab can induce a long-lasting depletion of B cells which results in hypogammaglobulinaemia. Several case reports describe severe opportunistic infections in patients treated with rituximab in combination with immunosuppressive agents or chemotherapy. Enteroviral encephalitis after treatment with rituximab has been described in several case reports before.

What does this add?
Rituximab is part of the standard therapy for patients with B-cell lymphomas and is usually well tolerated. However, rituximab can cause a prolonged B-cell deficiency. In this case report we describe a patient with enteroviral encephalitis after therapy with rituximab. We propose treatment with rituximab may have predisposed our patient. To our knowledge our patient is the first patient with enteroviral encephalitis to be successfully treated with intravenous immunoglobulins. With the increasing use of rituximab we recommend clinical awareness for enterovirus infections in these patients, if no other nonspecific signs can be found.

KEYWORDS

B cells, enteroviral encephalitis, hypogammaglobulinaemia, marginal zone lymphoma, rituximab

INTRODUCTION

Severe enteroviral infections are mainly observed in patients with congenital immunodeficiencies. Most viral infections are controlled by the cellular immune system. However, enteroviruses are generally controlled by neutralising antibodies. Rituximab is a chimeric anti-CD20 molecule and induces a long-lasting depletion of peripheral B cells which may result in hypogammaglobulinaemia. We describe a patient with marginal zone lymphoma and enteroviral encephalitis after treatment with rituximab.

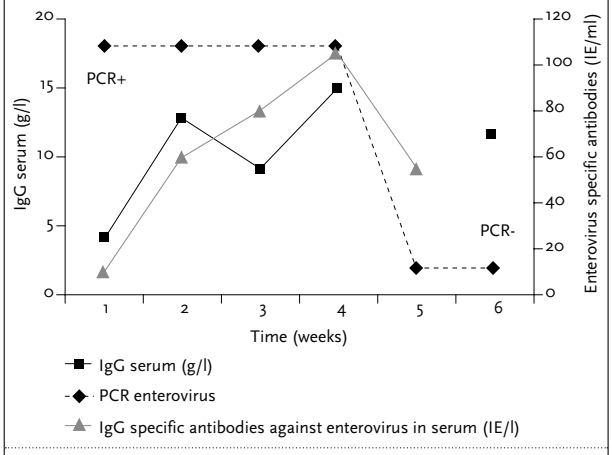
CASE REPORT

A 64-year-old female with a marginal zone lymphoma presented at our hospital with fever, nausea, vomiting, weight loss and fatigue. One month earlier she was admitted to the internal medicine department with fever caused by an upper respiratory infection. Her general medical history included extirpation of the uterus and a hemithyroidectomy. The haematological history noted a stage IV marginal zone lymphoma for which she had been treated three years before with eight cycles of R-CVP (rituximab 375 mg/m^2 day 1, cyclophosphamide 750 mg/m^2 day 1, vincristine 1.4 mg/m^2 day 1 and prednisone 40 mg/m^2 day 1-5) which resulted in a partial remission. Approximately 18 months before, the lymphoma became progressive and therefore our patient was treated with fludarabine (25 mg/m^2) and rituximab (375 mg/m^2) induction for six courses, followed by rituximab 375 mg/m^2 every three months as maintenance therapy for the next two years until one month before presentation. On admission, the patient received levothyroxin (0.125 mg/day), mirtazapin (30 mg/day), movicolon (2 sachets daily) and metoclopramide (10 mg in case of complaints of nausea). The patient was not in acute distress, her blood pressure was 98/59 mmHg, heart rate 109 beats/min and her temperature was 37.3 °C. At physical examination no abnormalities were found. During admission the patient's temperature varied between 37.3 and 40 °C. Laboratory findings revealed a haemoglobin of 8.4 mmol/l (7.5 to 10 mmol/l), a platelet count of 150 x 10^9/l (150 to 400 x 10^9/l) and a white blood cell count of 5.1 x 10^9/l (4.3 to 10 x 10^9/l) with lymphopenia of 0.5 x 10^9/l (1 to 4 x 10^9/l). Renal and liver functions were unremarkable. Our differential diagnosis included infection, progression of the lymphoma, autoimmune disease or drug fever. After admission a diagnostic procedure was performed. A chest-X-ray, an X-orthopantogram, MRI cerebrum and gastroscopy revealed no abnormalities. CT scanning of thorax and abdomen showed some pleural effusion but no other abnormalities pointing to residual marginal zone lymphoma. A PET scan was performed and showed an increased uptake of FDG in the thoracic region most probably due to local tension of the pectoral muscles. Besides depletion of B cells (2%), bone marrow investigation showed no signs of infection or recurrence of lymphoma. Cultures of urine and blood were repeatedly negative. Serological tests on Epstein-Barr virus (EBV), Cytomegalovirus (CMV), *Mycoplasma pneumoniae*, *Chlamydia trachomatis* and human immunodeficiency virus (HIV) revealed an EBV infection in the past. Because of persistent complaints of nausea and vomiting we also performed a cerebrospinal fluid puncture to exclude cerebral localisation of the lymphoma. The number of cells and protein was increased, further cytological investigation revealed pleiocytosis consisting of 90% of T cells, 0.3% of B cells and 9.7% of granulocytes. There was no evidence of marginal lymphoma cells. Extensive tests showed a positive polymerase chain reaction (PCR) for an enterovirus in the cerebrospinal fluid. The PCR for enterovirus was also positive on the patient's faeces. At that time, the immunoglobulin G of our patient was 4.06 g/l (5.2 to 16 g/l). She was treated with nonspecific intravenous immunoglobulins (40 g) once a week which resulted in an increase of specific antibodies against the enterovirus from 10 IE/ml to 106 IE/ml. The method used to measure enterovirus specific antibodies is a commercially available test from Serion Immundiagnostica & Institut Virion\ Serion GmbH, which is located in Würzburg, Germany (product number: Enterovirus IgG quantitativ (ESR133G)). Our goal was to keep the immunoglobulin G above 10 g/l (*figure 1*). Follow-up consisted of weekly PCRs on faeces and serological testing of the blood serum (*figure 1*). One month after starting treatment with IVIg the IgG level of our patient was normal and the enterovirus could no longer be found by PCR on the faeces. The level of antibodies against the virus in blood serum had returned to normal levels. She is still being treated with IVIg once monthly until one year after the last rituximab infusion. Clinically she is doing well without symptoms and no other signs of progression of the lymphoma.

DISCUSSION

To our knowledge we present the first patient with enteroviral encephalitis following treatment with rituximab

Figure 1.



The gray line represents IgG level in time. Treatment with intravenous immunoglobulins started on T=0 and was repeated weekly for the first month. The black line represents IgG specific enterovirus antibodies. After treatment with intravenous immunoglobulins enteroviral specific antibodies levels rise from 10 IE/ml to 106 IE/ml. The dotted line represents results of the weekly performed PCRs on the faeces. The patient is currently treated with IVIg monthly until one year after the last exposure to rituximab.

who has been successfully treated with IVIg. The human enteroviruses are classified into five sub-genera based on differences in host range and pathogenicity.[1] Enterovirus infection can occur in all age groups, although infections are mostly seen in infants and children. The virus is transmitted from person to person through ingestion of faecally contaminated material. Infection with an enterovirus can lead to a wide spectrum of clinical manifestations. More than 90% of the non-polio enteroviruses are asymptomatic or give undifferentiated fever.[2] Most viral infections are controlled by the cellular immune system. Enteroviruses, however, cause an infection that is controlled mainly by neutralising antibodies. Severe enterovirus infections have been described in patients with hereditary or acquired defects in B-lymphocyte function (X-linked agammaglobulinaemia, common variable immunodeficiency).[3] The enteroviruses can cause persistent central nervous infections in these patients. Clinical symptoms in immunocompromised patients can be mild or even absent and include headache, lethargy, papilloedema, seizure disorders, motor weakness, tremors and ataxia. Symptoms can fluctuate in severity, they can progress or disappear.

Since the beginning of the 1980s, patients with congenital agammaglobulinaemias are treated with intravenous immunoglobulins (IVIg). This treatment appears to prevent chronic enterovirus infections.[4] Successful treatment with IVIg in hypogammaglobulinaemic patients with enteroviral meningoencephalitis has been reported.[4,5] Several case reports describe the occurrence of meningoencephalitis following treatment with rituximab.[6-11] Rituximab is a chimeric anti-CD 20 molecule and induces a rapid and long-lasting depletion of the peripheral B-cell pool, which can last for up to 24 months.[8] Padate *et al.* describe a case of enteroviral meningo-encephalitis in a patient with non-Hodgkin's lymphoma after therapy with rituximab.[6] This patient was treated with IVIg weekly to maintain immunoglobulin G at 10 g/l. At first the patient responded symptomatically; unfortunately, his neurological situation deteriorated and the patient died.[6]

Rituximab is usually well tolerated and is part of the standard therapeutic regimen in most B-cell lymphomas.[12,13] However, several case reports have documented severe and opportunistic infections in patients treated with rituximab combined with chemotherapy or immunosuppressive agents.[6,8,14,15] One of these opportunistic infections may be an enterovirus infection. This infection may be accompanied by nonspecific signs such as fever, lethargy and fatigue. Because enterovirus infections are usually encountered in adults who lack B cells and because the pleiocytosis of the cerebrospinal fluid consisted mainly of T cells we do not think that the pretreatment with fludarabine was causative for the enterovirus infection. We propose that hypogammaglobulinaemia due to rituximab predisposed our patient for developing enteroviral encephalitis. With the increasing use of rituximab we therefore recommend screening for enterovirus infections in these patients, if no other nonspecific signs can be found.

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CASE REPORT

Primary squamous cell carcinoma of the thyroid years after radioactive iodine treatment

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ABSTRACT

Primary squamous cell carcinoma (SCC) of the thyroid gland is a rare diagnosis, since there is no squamous epithelium in the thyroid gland. SCC of the thyroid is highly aggressive with a poor prognosis. We present a case of primary SCC of the thyroid: this 88-year-old male patient had a history of hyperthyroidism which was treated with radioactive iodine 25 years earlier. Whether this treatment could be related to SCC of the thyroid is not clear. We treated our patient with thyroidectomy and subsequent intensified radiotherapy. Six months after treatment our patient is doing well and there is no sign of local reoccurrence. Our work-up is described, including the differentiation from metastatic disease. The origin of squamous cell carcinoma in the thyroid is uncertain; we discuss some theoretical considerations. We conclude that after excluding metastatic disease, thyroidectomy combined with radiotherapy is the treatment of choice.

KEYWORDS

Thyroid carcinoma, squamous cell carcinoma, treatment

INTRODUCTION

Primary squamous cell carcinoma (SCC) originating in the thyroid gland is very rare, since there is no squamous epithelium in the thyroid gland.[1] When SCC is found in the thyroid gland the first consideration is metastasis of another primary site. Still, there are some exceptional situations where squamous cells can be seen in thyroid tissue, for instance such as embryological remnants, in inflammatory processes and cancers. Until now there have been approximately 150 cases of primary SCC reported in the English literature. In most cases it behaves aggressively, clinically identical to undifferentiated thyroid cancer with poor outcome.[2] We report a patient with primary SCC of the thyroid, 25 years after treatment with radioactive iodine.

CASE

An 88-year-old man presented with a swelling in the neck at our outpatient clinic. He had noticed this swelling for a few days and it had been rapidly increasing in size. His previous medical history revealed hyperthyroidism 25 years ago, due to a toxic nodule of the right thyroid gland. The patient was treated with radioactive iodine with subsequent hypothyroidism. Furthermore, he had hypertension and chronic renal failure, with an MDRD of 33 ml/min. The swelling in his neck gave rise to dyspnoea and dysphagia. His weight was stable and he had no history of smoking. On physical examination his blood pressure was 140/70 mmHg with a pulse rate of 70 beats/min. A hard mass of 4 x 3 cm was palpable in the region of the left thyroid gland without detectable lymph nodes in the neck or elsewhere. Examination of heart, lungs and abdomen was normal. Ultrasound examination of the thyroid gland showed multiple small nodules on both sides, and in the left thyroid gland there was a large nodule of 4 x 2.5 cm in diameter, partially solid and partially cystic. Fine needle aspiration (FNA) of this nodule was performed. Cytological examination showed squamous cell carcinoma. At this time metastatic disease from another primary location was considered. CT scan of the lungs, MRI of the head and neck region, laryngoscopy, oesophagogastroduodenoscopy and positron emission tomography combined with computer tomography of the total body did not reveal another primary tumour. A thyroidectomy was

performed and pathological examination showed a poorly differentiated SCC with a diameter of 4.5 cm with dubious infiltration of the thyroid capsule and no lymph node metastasis (T3N0M0). There were no signs of follicular carcinoma, papillary carcinoma or muco-epidermoid carcinoma. It was concluded that this 88-year-old male patient had a primary squamous cell carcinoma of the left thyroid gland.

CYTOLOGICAL AND HISTOLOGICAL EXAMINATION

FNA showed atypical squamous cells with keratinisation in a background of necrosis with polymorphonuclear leucocytes (*figure 1A*), suggestive of squamous cell carcinoma. Histology showed fields of atypical squamous cells with mitosis (*figure 1B*). There was no evidence of associated papillary carcinoma, follicular carcinoma, anaplastic carcinoma, follicular adenoma, muco-epidermoid carcinoma or squamous metaplasia in colloidal nodules.

Figure 1A. *Cytology of thyroid gland, Giemsa staining, shows two atypical squamous cells (arrows)*

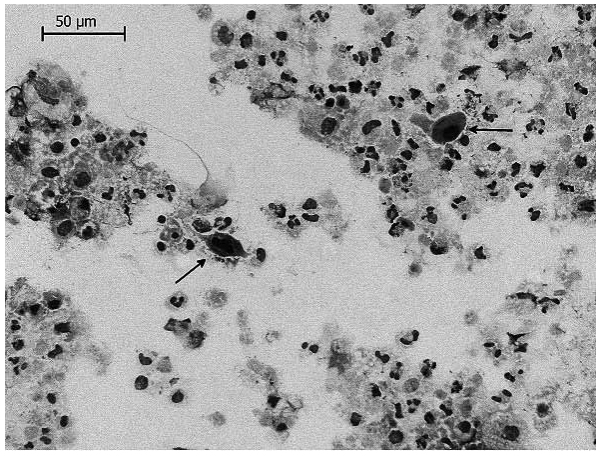
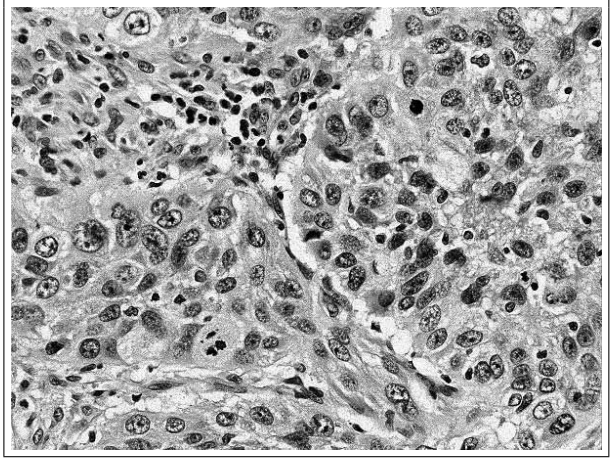


Figure 1B. *Histology of thyroid gland, fields of atypical squamous cells with mitosis*



DISCUSSION

This case represents a rare form of thyroid cancer, namely squamous cell carcinoma. In a histological review of 600 primary thyroid carcinomas, primary SCC accounted for 0.7%.[3]

In our region of the Netherlands, consisting of approximately two million citizens, there were 532 cases of thyroid carcinoma during the years 1998-2007. Only one case of SCC was reported in this period. We calculated that SCC accounts for 0.38% of all cases of thyroid carcinoma. The proportion of papillary, follicular, medullary and anaplastic cancer was 67.5, 22.0, 3.5 and 5.1%, respectively.[3] The origin of SCC in the thyroid is uncertain, but there are some theoretical considerations. Some reports suggest that squamous cells can be derived from embryonic remnants such as the thyroglossal duct or an ultimobranchial body. Another theory is that thyroiditis or inflammation may trigger metaplasia of follicular epithelial cells. Squamous metaplasia can also be seen in papillary, follicular, medullary and anaplastic thyroid carcinomas.[5,6] Our patient had no other type of thyroid cancer on pathological examination. Interesting to note is that our patient had a history of hyperthyroidism which was treated with radioactive iodine 25 years earlier. Whether these could be related to each other is not clear. There are some case reports suggesting that there might be a relationship between radioactive iodine and anaplastic carcinoma.[7,8] No reports have been published in the literature suggesting a relationship between radioactive iodine treatment and SCC of the thyroid. The behaviour of SCC of the thyroid is aggressive. It is a fast growing tumour with poor outcome, with a mean survival of 8.6 months.[9] The treatment is similar to the treatment of anaplastic carcinoma and consists of thyroidectomy and radiotherapy.[10,11] We treated our patient with thyroidectomy and subsequent intensified radiotherapy, 35 sessions of 2 gray. Six months after treatment our patient is doing well and there is no sign of local reoccurrence.

CONCLUSION

In case of SCC of the thyroid, it is important to rule out other primary sites of SCC with a fast and thorough work-up, since SCC of the thyroid is highly aggressive

and has an extremely poor prognosis. After excluding metastatic disease, thyroidectomy combined with radiotherapy is the treatment of choice.

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Just epistaxis?

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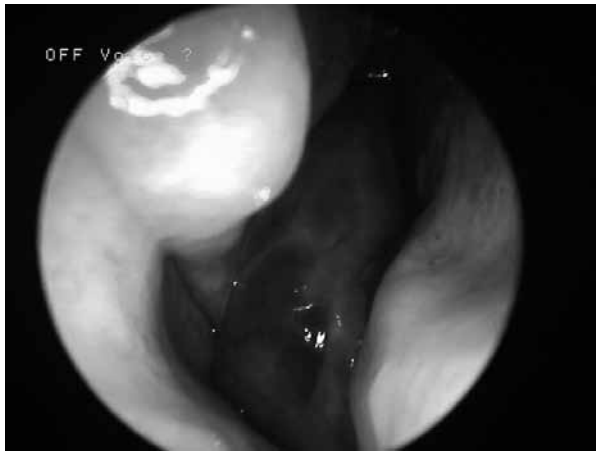
CASE REPORT

A 74-year-old woman with epistaxis at the right side of the nose was referred to the ENT department by the general practitioner. For six weeks she had experienced daily dropwise blood loss at the right side of the nose. She had no complaints of disturbed nasal breathing, rhinorrhoea, visual disturbances or headaches. She was taking anticoagulants for a cardiac condition. She had not suffered from epistaxis before.

Anterior rhinoscopy did not show any lacerations of the nasal mucosa in locus Kiesselbachi. Nasal endoscopy showed a smooth mass that almost completely obstructed the lumen in the right dorsal nasal cavity and nasopharynx. Small superficial veins were visible on the mass (*figure 1*). Palpation of the neck did not reveal any lymphoid nodules.

Figure 1. *Mass in dorsal part of nasal cavity and nasopharynx seen with nasal endoscopy. The mass is located in the choane. On the right side the nasal septum is visible. The left top shows the middle nasal turbinate*



After cessation of the anticoagulants for a week a biopsy of the mass was taken under general anaesthesia. The resulting epistaxis was managed by tamponade of the right nasal cavity. Also magnetic resonance imaging (MRI) of the head was performed.

The scan showed a giant mass (>6 cm) destroying the sella region, invading the sphenoid sinus and nasopharynx. The mass was growing half way to the anterior skull base and passed the cerebral pons caudally. No compression of the optic chiasm was seen (*figure 2*).

WHAT IS YOUR DIAGNOSIS?

See page 230 for the answer to this photo quiz.

Figure 2. *Sagital CT scanning slide of the right nasal cavity showing a mass destroying the sella region, invading the sphenoid sinus and nasopharynx. *sphenoid sinus; **bulging of the mass in the nasal cavity and nasopharynx*

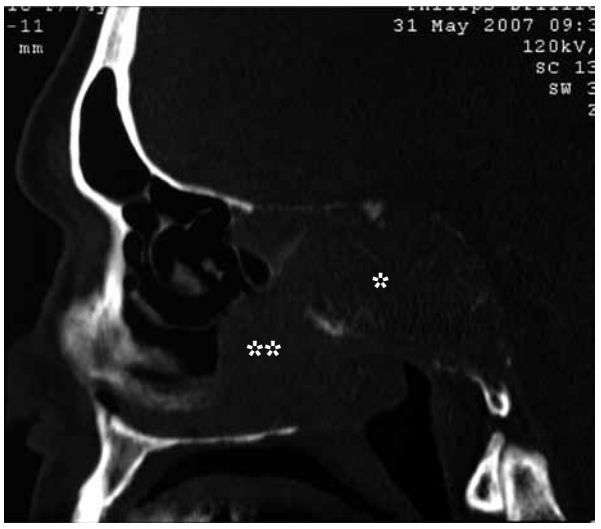


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PHOTO QUIZ

Red and wet

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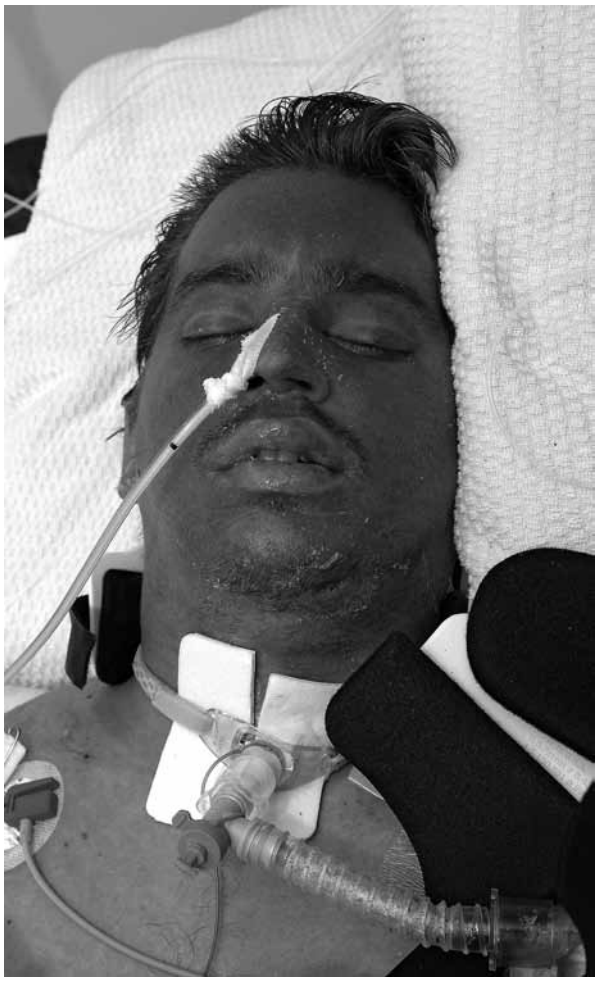
CASE REPORT

A 28-year-old man was admitted to our critical care department after a head-on collision with a car while riding his motorcycle. He suffered severe cerebral contusions as well as several major injuries including severing of this brachial vasculature, for which a Dacron interposition was inserted. Early in his admission, blood cultures grew coagulase-negative *Staphylococci* for which vancomycin was started with the intent to continue vancomycin for six weeks to prevent infection of this vascular prosthesis. Five weeks after admission, sputum cultures grew *Staphylococcus aureus* for which treatment with flucloxacillin was initiated. At the same time he was started on amitriptyline (for neuropathic pain) as well as metoprolol (for refractory sinus tachycardia). Six weeks after admission he developed a severe generalised erythema which was most notable in the face (*figure 1*). This was accompanied by generalised oedema, shock, fever, diarrhoea, renal failure, hepatitis and acute lung injury.

WHAT IS YOUR DIAGNOSIS?

See page 231 for the answer to this photo quiz.

Figure 1. *Generalised redness accompanied by severe oedema and multiorgan failure*



***The patient's guardians provided written permission for publication of this photo.**

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PHOTO QUIZ

A patient with renal cell carcinoma and thoracic pain

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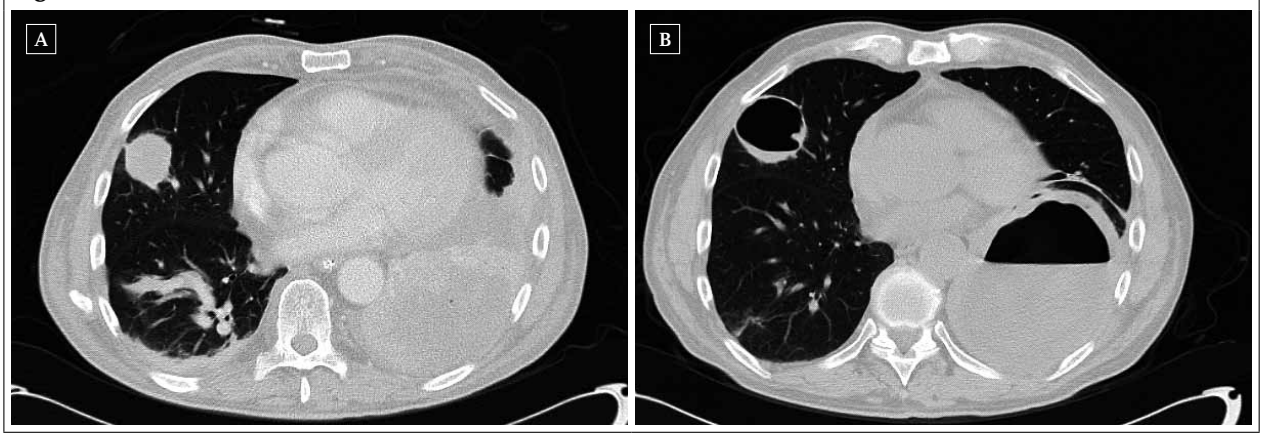
CASE REPORT

A 65-year-old man was diagnosed with metastatic clear cell renal cell carcinoma with sarcomatoid differentiation. Within weeks after a palliative nephrectomy, the patient's condition deteriorated. Computed tomography (CT) scan showed progressive disease of the pulmonary metastases (panel A) and we started treatment with the oral angiogenesis inhibitor sunitinib 50 mg once daily, 4 weeks on, 2 weeks off. Sunitinib is a multiple tyrosine kinase inhibitor targeting the vascular endothelial growth factor receptor, platelet derived growth factor receptor, c-kit, neurotrophic factor receptor RET and FMS-like tyrosine kinase 3. Three weeks after starting treatment, the patient presented at the emergency ward with right thoracic pain, dyspnoea and cough. Laboratory studies showed a haemoglobin of 6.2 mmol/l (was 6.1 mmol/l 5 days before), leucocytes of 7.3 x 10^9/l (was 9.7 x 10^9/l 5 days before), C-reactive protein 176 mg/l (was 144 mg/l 9 days before) and a D-dimer of 6234 ng/ml. A new CT was performed (panel B).

WHAT IS YOUR DIAGNOSIS?

See page 232 for the answer to this photo quiz.

Figure 1.



DIAGNOSIS

Histological examination of the biopsy showed pituitary adenoma. Accessory staining for prolactin was positive. Consultation with the endocrinologist revealed no symptoms related to hyperprolactinaemia or hypopituitarism. Hormonal function evaluation, however, showed extremely elevated serum prolactin levels: 697000 mU/l (normal: <500 mU/l) and a mild secondary hypothyroidism without any signs or symptoms (thyroid-stimulating hormone (TSH) 0.89 mU/l and free T4 9.8 pmol/l).

The patient was treated with a dopamine agonist; serum prolactin levels decreased to 4000 mU/l after six months. Control MRI was performed and showed shrinkage of the mass. She had no recurrence of epistaxis and her nasal airway was not obstructed.

Prolactinomas are pituitary adenomas that express and secrete prolactin (PRL) to variable degrees; they are almost invariably benign, but are nevertheless frequently clinically significant. Prolactinomas are generally classified according to size as microadenomas (less than 10 mm in diameter) or macroadenomas (more than 10 mm in diameter). Prolactinomas are called giant prolactinomas when they reach a size of >40 mm diameter and show invasive growth on neuroimaging.[1-3]

Epistaxis is a very rare primary presenting symptom of a giant prolactinoma. Ghannam *et al.*[4] described a case of a TSH-secreting pituitary adenoma first presenting with nasal bleeding. In 1985, Lessard *et al.*[5] described a unilateral intranasal extension of a pituitary adenoma. Epistaxis was not a presenting symptom.

The diagnosis of prolactin-producing pituitary adenoma is made on a combination of radiological (MRI) and histopathological findings and elevated prolactin levels. Symptoms of prolactinomas are closely related to hyperprolactinaemia (galactorrhoea and hypogonadism) and the location and size of the mass. In most cases mass effects are due to suprasellar extension with invasion of the optic chiasm which causes visual disturbance.

All patients with macroadenoma and most patients with microadenoma require treatment. Dopaminergic agonists such as bromocryptine and cabergoline are the first-line, preferred therapy. Transsphenoidal surgery is an option in individuals who cannot tolerate a dopamine agonist or in whom the drug is ineffective.[3,6] In macroadenomas medical treatment causes rapid shrinkage of the lesion and cessation of prolactin secretion.[5,6] Treatment of giant prolactinomas with dopamine agonists may result in cerebrospinal fluid liquorrhoea.[2]

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ANSWER TO PHOTO QUIZ (PAGE 228)

RED AND WET

DIAGNOSIS

Initially, the diagnoses of vancomycin-related red man syndrome and severe penicillin allergy were suspected. Red man syndrome has often been associated with rapid infusion of the first dose of the drug and was initially attributed to impurities found in vancomycin preparations.[1] Our department has strict protocols in place regarding the vancomycin infusion rate and since vancomycin-induced red man syndrome is almost exclusively related to rapid infusion of the drug, this diagnosis was unlikely. Furthermore, since the patient had been treated with penicillins before, an allergic reaction to penicillin was deemed unlikely, although it could not be excluded. The day after the initiation of the syndrome, peripheral blood leucocytes were markedly elevated at 46 x 10^9/l and the blood smear showed 36% eosinophils. The clinical picture of erythema, systemic symptoms and marked eosinophilia was most consistent with the drug rash with eosinophilia and systemic symptoms (DRESS) syndrome.[2] Recently started medications, as noted above, were immediately discontinued and the patient was treated with steroids, and H1 and H2 receptor blockers. Hereafter, the erythema and systemic symptoms regressed over the course of several days. A few weeks later the patient was re-challenged with vancomycin (because of infectiological necessity at the time) as well as penicillins, which did not result in recurrence of the rash. Therefore the occurrence of DRESS syndrome in this patient was most likely related to the administration of amitriptyline or metoprolol. Of these, the DRESS syndrome has only been described secondary to amitriptyline use.[3] Unfortunately, a definite diagnosis of drug allergy by positive patch tests to amitriptyline was not demonstrated due to the fact that the patient was transferred to another hospital.

Drug-induced rash with eosinophilia and systemic symptoms is a life-threatening adverse reaction characterised by skin rashes, eosinophilia and multiorgan failure. The syndrome develops two to six weeks after initiation of administration of a specific drug. So far, the only undisputed way to treat severe hypersensitivity reactions is prompt withdrawal of the offending drug.[4] Therefore, early recognition of the syndrome is paramount to prevent organ failure.

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ANSWER TO PHOTO QUIZ (PAGE 229)

A PATIENT WITH RENAL CELL CARCINOMA AND THORACIC PAIN

DIAGNOSIS

The differential diagnosis of thoracic pain in this patient included a pulmonary embolus, pneumonia, pneumothorax, pleural effusion, and pleural metastases or bleeding within a metastasis. An angiographic CT scan excluded a pulmonary embolus and showed no signs of pneumonia, pneumothorax or pleural effusions. However, the CT scan showed extensive central necrosis of the lung metastases with cavitation (panel B), of which the largest was located in the area of the patient's pain. The CT image is characteristic for a good response of metastases of renal cell carcinoma to antiangiogenic treatment, although the size of the metastases did not decrease.

For the evaluation of treatment response of cancer, the Response Evaluation Criteria in Solid Tumours (RECIST) are used.[1] RECIST is based on the sum of one-dimensional measurements of the greatest diameter of the tumour and/or metastases. In the presented patient, according to RECIST, stable disease (+9%) was established. However, the sunitinib-induced extensive necrosis and cavitation illustrate the limitations of the RECIST guidelines for the evaluation of response to targeted therapies. The effect of targeted therapies as angiogenesis inhibitors and antivascular drugs can be underestimated by using the tumour size based RECIST guidelines.[2] In case of first-line treatment with sunitinib in RCC patients, the observed objective response rate is 47%, with a progression-free survival of 11 months, compared with five months for interferon alpha.[3] However, first-line single-agent treatment with sorafenib[4] in metastatic renal cell cancer failed to achieve significant objective response rates according to the RECIST criteria, but did result in a significant increase in progression-free survival, demonstrating its clinical efficacy.

Attempts are made to achieve more sophisticated imaging techniques or evaluation criteria. Striking examples are the PET criteria, also called PERCIST.[5] and the Choi criteria for gastrointestinal stromal tumours (GIST).[6] The Choi criteria add tumour density to tumour size, which makes it possible to assess tumour necrosis, an early feature of antiangiogenic therapies.

In this patient, the sunitinib was continued and analgesics were added. After this episode the condition of the patient gradually improved, the cough and pain disappeared and his weight increased. Unfortunately, five months later he developed cerebral metastases and died shortly thereafter.

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