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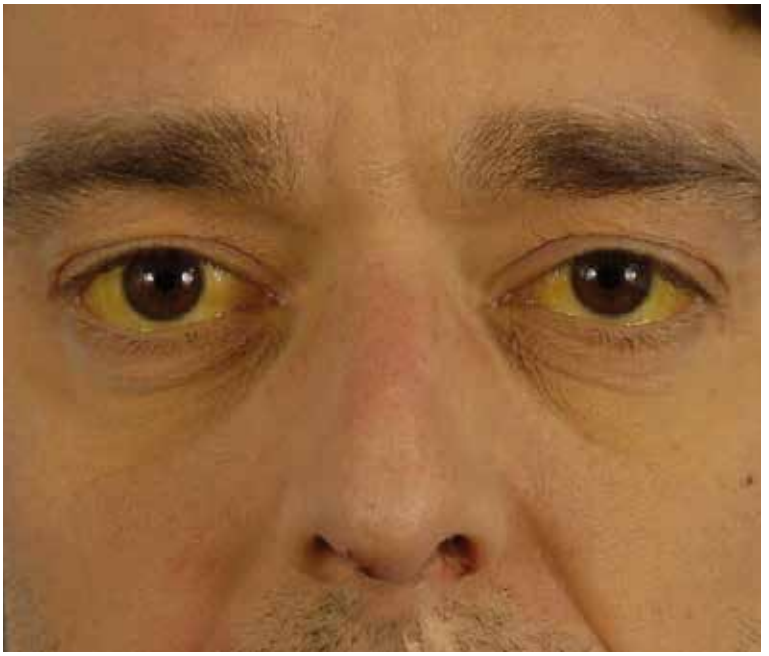

"Intense muscle aches after cleaning the boat on the Amsterdam canals: what is your diagnosis?"

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EDITORIAL

You only notice them if you understand them: Geriatric syndromes

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Ageing is acknowledged to result from a lifelong accumulation of molecular and cellular damage, caused by many mechanisms that are regulated by a complex maintenance and repair network.[1]
Multimorbidity and polypharmacy are the results of our good standard of care for ageing persons as present in many Western societies. Many healthcare expenses and efforts are currently devoted to providing healthcare to older persons. As the diversity among ageing persons is enormous and as the exact age of a person is easy to retrieve, higher age often lets clinicians make (wrong) judgments solely based on chronological age. Clinicians and researchers as well, therefore, need simple, easy to administer, valid, accurate, and reliable methods to detect the biological age of an older person, often referred to as his state of vulnerability or more preferred, frailty. As a problematic expression of ageing, age-related decline occurs in many physiological systems, which collectively results in vulnerability to sudden health status changes triggered by minor stressor events. This situation is often referred to as frailty.

FRAILTY

Frailty is a long-established clinical expression that implies concern about an elderly person's vulnerability and outlook. Frailty is often considered a state of increased vulnerability to poor resolution of homoeostasis after a stressor event, which increases the risk of adverse outcomes, including falls, functional decline, and delirium.[2]

For many years now, a debate is ongoing about whether frailty is best defined as a syndrome or a state. This has resulted in many frailty indices, models and definitions and in comparisons between them.[3,4] The bottom line is that the use of these indices and models could help to provide a more accurate and earlier identification of frail elderly persons eligible for interventions to improve outcomes in both primary care and in hospitals.

Ageing increases the presence of frailty, multimorbidity and disability, and large cohort studies show an overlap between the presence of these concepts. The CHS study population was used to investigate the overlap between frailty, disability, and multimorbidity.[5]
Frailty and multimorbidity as combination (defined as two or more chronic diseases) was present in almost 45% of the population. Disability, frailty and multimorbidity showed an overlap in almost 25% of the older participants.

COMPREHENSIVE GERIATRIC ASSESSMENT

Currently, most healthcare systems such as, for instance, outpatient clinics, emergency departments and hospital wards, are organised around single-system illnesses. However, many elderly people have multiorgan problems and more than one geriatric condition.[6]
As many older persons in need of acute care have multimorbid and geriatric conditions, interfering with clinical decision-making concerning diagnosis and treatment, all health domains of an ageing frail person should be taken into account. This should take place not only at the outpatient clinic, but at presentation to the emergency department or hospital ward as well.

The most evidence-based manner to detect geriatric syndromes is comprehensive geriatric assessment (CGA).[7] Although this systematic assessment is a resource-intensive process and therefore reliable, more efficient and responsive screening methods for routine care are available that can be applied together with CGA in a two-step manner to improve ED outcomes. A number of these clinically sensible and easy to apply instruments have been developed to select older patients for CGA and different care pathways after presentation to an emergency department.[8,9]
The study by Schrijver *et al.*, presented in this issue of the Netherlands Journal of Medicine, shows that there

is still room for improvement in providing care after ED presentation, as many older patients present not only with an illness but also with one or more geriatric conditions.[10] The implementation of screening instruments may create more awareness of providing sufficient care after presentation of frail persons with geriatric syndromes to the ED to improve desirable outcomes, such as physical functioning.

OUTCOMES

A more patient-based approach instead of a disease-based approach would also have considerable clinical merit, as has recently been advocated by Reuben and Tinetti.[11] This approach would be the basis for a shift in the care of frail elderly patients towards a more appropriate goal-directed care, in which individually framed clinical outcomes that span organ systems are negotiated with patients and their relatives.

MEDICAL EDUCATION

The fuel for a system change or the key towards more insight is education. This is, however, an even more difficult goal to achieve.

A study by Brooks in 1993 showed that in order to put more effort into introducing training in geriatrics and gerontology into the medical curriculum, a number of barriers have to be overcome first: 1) some teachers, role models, paramedical personnel, etc. have positive attitudes but others may have negative attitudes regarding the elderly because of previous exposure or training (or lack of training); 2) students and residents may be unhappy that more materials will be added to their already crowded curriculum; 3) students and residents may be influenced by the experiences they have with relatives; 4) students and residents may strongly believe in existing myths about the aged, and some suffer from 'ageism'; 5) medical curriculum planners and department heads will probably not want changes in existing time schedules allocated to them; 6) all medical educators will agree, however, that gerontology and geriatric medicine needs to be studied and included in medical education, but few will want to sacrifice time or energy in that direction.[12]

The study by Tersmette *et al.*, also in this issue of the Netherlands Journal of Medicine, came even 20 years later to the same conclusions.[13] They assessed on the student level the topics addressed in the questions of the cross-institutional progress test (CIPT). In the CIPT, on average 1.5% of questions cover G&G. They also demonstrated that the Dutch National Guidelines Blueprint contains few specific Geriatrics and Gerontology (G&G) objectives. Currently, obligatory G&G courses in medical schools on average amount to 2.2% of the total curriculum measured as European Credit Transfer System units (ECTS). Only two out of eight medical schools have practical training during the Master phase in the form of an obligatory clerkship in G&G. So geriatric education in the Netherlands does not seem to be in line with current demographic trends. The National Guidelines Blueprint falls short of providing sufficiently detailed objectives for education on the care of older people. The geriatric content offered by medical schools is varied and incomplete, and students are only marginally tested for their knowledge of G&G in the CIPT. For nurses and other health professionals the same conclusions can be made. So haven't we learnt from the past?

NEAR FUTURE

Back to the past and redesigning the future fails because of the lack of a time machine. But knowledge gained in the past may help us to redesign the future and improve healthcare thinking and healthcare systems.[14,15] Fear of change should not keep us from doing the right thing and we all know what we have to do as age expectancy is still increasing and the number of very old citizens will be threefold higher in the next three decades.

Change is happening in the Western communities, in particular in the thinking about G&G education and caring for the aged. It will not take us another 20 years to change.

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REVIEW

Immunosuppressive drugs after solid organ transplantation

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ABSTRACT

In recent years solid organ transplantation has been rapidly developed as a therapeutic intervention that is life-saving and greatly contributes to a better quality of life in organ recipients. The rapid development has been made possible because of a drastic expansion in the immunosuppressive repertoire. Unfortunately, the side effects of these drugs can be severe, which is one of the reasons that life expectancy of transplant patients still significantly falls short of that of the general population. In this review manuscript we will discuss current and future immunosuppressive strategies that are employed in solid organ transplantation. Expanding our understanding of the human immune system will hopefully provide us with newer, smarter drugs that promote immunotolerance without the side effects observed today.

KEYWORDS

Solid organ transplantation, renal transplantation, immunosuppressive drugs

INTRODUCTION

In 1954, the first successful renal transplantation was performed at the Peter Bent Brigham Hospital in Boston.[1] Because the donor and recipient were identical twins, there was no need for immunosuppression. This success underlined the surgical feasibility of organ transplantation and greatly stimulated research into immunosuppression, opening up the possibility to extend transplantation beyond identical twins.

In the 1950s, sublethal doses of total body irradiation (TBI) were combined with cortisone.[2] Although TBI did produce adequate immune suppression, it also resulted in profound bone marrow aplasia, which often led to patients dying from overwhelming infections.

The breakthrough came in 1959, when it was reported that 6-mercaptopurine (6-MP), which was already in use for acute lymphocytic leukaemia, suppressed the immune system.[3,4] Soon, the first clinical trial using a combination of corticosteroids and 6-MP was set up. It delivered one-year rates of allograft survival in the range of 40-50%.[5] A few years later, 6-MP was replaced by its prodrug azathioprine, which was equally effective but less toxic. Also, antithymocyte globulin (ATG) was introduced: first to treat corticosteroid-resistant rejection episodes and later as part of induction protocols. Several trials followed, producing rates of one-year graft survival of around 70%.[6-8]

In the early 1980s, the introduction of cyclosporine marked a new era in clinical transplantation, increasing one-year graft survival rates to well over 80%.[2]

In the last 20 years, the immunosuppressive repertoire has been extended significantly with the introduction of drugs, such as tacrolimus, mycophenolate mofetil and sirolimus, and monoclonal antibodies, such as basiliximab and alemtuzumab. These drugs have facilitated major improvements, especially in one-year graft survival, which now exceeds 90% in most centres. Unfortunately, long-term graft survival still lags behind, with only a very modest increase compared with the early days of transplantation medicine.

Here, we present an overview of the drugs currently used for immunosuppression after solid organ transplantation. Our focus is mainly on renal transplantation, but the general principles apply for all types of organ transplantation. We will discuss mechanisms of action, major toxicities and the place in the immunosuppressive regimen for these drugs. *Tables 1* and *2* provide a summary.

Table 1. *Overview of drugs currently used in solid organ transplantation*

Drug	Trade name	Place in treatment protocol	Comments
Glucocorticosteroids		Induction and maintenance; acute cellular rejection and AMR	Role in maintenance immunosuppression under investigation because of severe side effects during long-term use
Azathioprine	Imuran	Maintenance	Mainstay of immunosuppression together with glucocorticosteroids until 1980s, producing one-year graft survival of around 70%
MMF mycophenolic acid	CellCept Myfortic	Maintenance	MMF, introduced in early 1990s, was initially favoured over azathioprine, but newer trials show similar efficacy
Calcineurin inhibitors (Cyclosporine/ Tacrolimus)	Neoral Prograft/ Advagraf	Maintenance	CNIs were introduced in the 1980s-1990s and revolutionised maintenance immunosuppression. Tacrolimus has a lower risk of acute rejection and allograft loss than cyclosporin. The use of CNIs is limited by their side effects, especially nephrotoxicity
mTOR-inhibitors (Sirolimus/Everolimus)	Certican Rapamune	Maintenance	Place in maintenance immunosuppression still under investigation; often used to limit CNI nephrotoxicity
ATG		Induction; steroid-resistant rejection	Oldest available medication for induction and rejection treatment (apart from steroids), still highly effective but toxic
Alemtuzumab	MabCampath	Induction; steroid-resistant rejection	Place in induction and acute rejection treatment is still under investigation; appears to be similar to ATG while less toxic
Rituximab	MabThera	AMR, HLA-sensitised patients, ABO-incompatible transplantations	Has also been evaluated as induction agent, but unsuccessfully
Basiliximab	Simulect	Induction	Higher rejection rates one year post-transplantation than ATG, but less toxic
Belatacept	Nulojix	Maintenance	Promising new agent for maintenance immunosuppression, further studies are needed
Bortezomib	Velcade	AMR	Small, non-randomised trials suggest efficacy in AMR
Eculizumab	Soliris	AMR	May decrease AMR in highly sensitised individuals

AMR = antibody-mediated rejection; ATG = antithymocyte globulin; CNI = calcineurin inhibitor; MMF = mycophenolate mofetil; mTOR = mammalian target of rapamycin.

Table 2. *Major toxicities of drugs currently used in solid organ transplantation*

Drug	Cardiovascular toxicity			Malignancies	Selected infections			Bone marrow suppression	Other
	Hypertension	Dyslipidaemia	DM		CMV[§]	EBV[#]	BKV[†]		
Glucocorticosteroids	++	+	++	-*				N	Cushingoid appearance, sleep disturbances, mood changes, impaired wound healing, osteoporosis
Azathioprine	N	N	N	+[$]				+	Hepatotoxicity
MMF	N	+	+	N		-	+	+	Gastrointestinal symptoms
Cyclosporine	++	+++	+	+				+	Nephrotoxic, neurotoxic, gum hyperplasia
Tacrolimus	+	++	++	+			+	+	Nephrotoxic, neurotoxic, gum hyperplasia
mTOR-inhibitors	N	+++	+	-	-			+	Impaired wound healing, flulike syndrome, acne
ATG				+	+	+		++	Cytokine-release syndrome
Alemtuzumab				-		+		+	Mild cytokine-release syndrome, induction of autoimmune disease
Rituximab				-		-		+	Infusion reactions
Basiliximab				-		+		+	Hypersensitivity reactions
Belatacept	++	++	+	+				+	
Bortezomib				-				+	Neurotoxicity
Eculizumab								+	Very expensive

***Used in many treatment protocols for malignancies; however, an increased risk of malignancy has also been described; [$]historically associated with an increased risk of malignancy, when azathioprine was given in high dosages. The risk association for currently used lower dosages is less clear; [§]CMV infections are increased with all immunosuppressive medications; however mTOR-inhibitors are thought to decrease the risk, whereas ATG increases the risk; #EBV increases the risk of PTLD (post-transplant lymphoproliferative disorder). Induction or rejection therapy with polyclonal and monoclonal antibodies increases the risk of PTLD. PTLD can be treated with rituximab; [†]The risk of a BKV infection is mostly dependent on the total load of immunosuppression, but MMF and tacrolimus appear to increase the risk.**
ATG = antithymocyte globulin; BKV = BK virus; CMV = cytomegalovirus; DM = diabetes mellitus; EBV = Epstein-Barr virus; MMF = mycophenolate mofetil; mTOR = mammalian target of rapamycin.

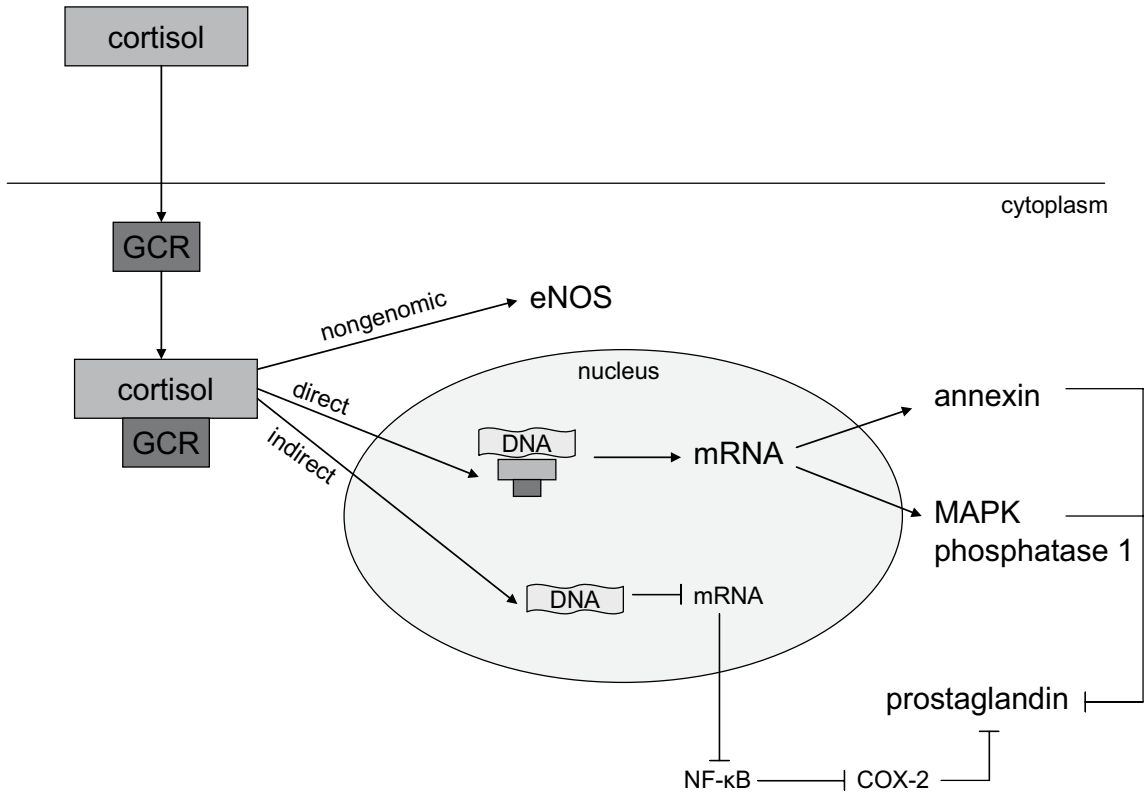
GLUCOCORTICOSTEROIDS

Since their discovery just after World War II, glucocorticosteroids have become one of the most widely used drugs in modern medicine. Glucocorticosteroids inhibit inflammation through three mechanisms: direct genomic effects, indirect genomic effects and nongenomic mechanisms,[9] illustrated in *figure 1*. Direct genomic effects occur when the cortisol-glucocorticosteroid receptor complex moves to the nucleus and affects transcription. Two important examples are the induction of annexin 1 and MAPK phosphatase 1. Each of them inhibits prostaglandin synthesis, which in turn inhibits inflammation. Indirect genomic effects take place when the glucocorticoid-receptor complex interacts with other transcription factors. NFκB is inhibited through this mechanism, leading to a decrease of COX-2, which also inhibits prostaglandin synthesis. Nongenomic effects, i.e. effects not mediated by changes in gene expression and transcription, may explain why glucocorticosteroids can also act very rapidly. The best-described non-genomic mechanism involves the activation of endothelial nitric oxide synthethase (eNOS), which appears to protect against ischaemia and reperfusion-induced injury in mice.[10]

The net result of these pathways is a neutrophilic leukocytosis, accompanied by dramatic transient reductions in circulating eosinophils, monocytes, and lymphocytes.[11] Circulating T cells rapidly decline, due to a combination of effects, including redistribution,[12] inhibition of pro-inflammatory cytokines[9] and induction of apoptosis.[13,14] B cells are less affected and antibody production is largely preserved.[15]

Because of their wide scope of immunosuppressive effects, glucocorticosteroids are used both for induction and maintenance immunosuppression and for treatment of acute rejection episodes. However, there are several well-known side effects. In addition to opportunistic infections, these include a Cushingoid appearance, sleep disturbances, mood changes, hyperglycaemia, hypertension, alterations in lipid metabolism, impaired wound healing and osteoporosis. Therefore, the role of corticosteroids, especially their long-term use in maintenance immunosuppression, is a subject of active research. In a recent systematic review of 29 randomised controlled trials, steroid avoidance and steroid withdrawal strategies in renal transplantation were not associated with increased mortality or graft loss despite an increase in acute rejection episodes. However, follow-up was

Figure 1. *Mechanism of action of glucocorticosteroids*



COX-2 = cyclo-oxygenase 2; eNOS = endothelial nitric oxide synthethase; GCR = glucocorticosteroid receptor; MAPK = mitogen-activated protein kinase; NF-κB = nuclear factor kappa-B.

limited, varying from six months to five years.[16] Another meta-analysis[17] also concluded that acute rejections were increased in steroid avoidance protocols, without affecting graft or patient survival.

ANTIMETABOLITES

Antimetabolites are purine and/or pyrimidine inhibitors, blocking DNA synthesis. Two well-known examples are azathioprine and mycophenolate mofetil, which are both used for maintenance immunosuppressive treatment. Azathioprine (Imuran®) was among the first drugs to be used in solid organ transplantation. It is metabolised to 6-MP, which interferes with DNA synthesis. Its immunosuppressive action *in vivo* seems to be mediated mainly by its inflammatory properties.[18,19] However, it is also thought to stimulate T-cell apoptosis.[20] *Figure 2* summarises the mechanism of action of azathioprine and other immunosuppressive drugs.[21-25]

The main side effects of azathioprine are bone marrow suppression and hepatotoxicity. Historically, its use has also been associated with malignancies. However, whereas older data show a clear correlation, newer data analysing combined immunosuppressive medications in which azathioprine is generally used in lower dosages, are less clear-cut.[26]

Mycophenolate mofetil (MMF, CellCept®) was first used in the early 1990s.[27] MMF is a prodrug that is rapidly metabolised to its active metabolite mycophenolic acid. A few years ago, mycophenolic acid also became available directly as Myfortic®. MMF inhibits lymphocyte function by blocking purine biosynthesis via inhibition of the enzyme inosine monophosphate dehydrogenase.[28] In most eukaryotic cells, blocking inosine monophosphate dehydrogenase has little effect on cell division because purines can also be generated from nucleotide breakdown products, the so-called purine salvage pathway. Because B and T lymphocytes lack this pathway, MMF is a more selective antiproliferative agent than azathioprine.[29,30] Because of this, and because MMF is less hepatotoxic and is not associated with malignancies,[26] it was expected to replace azathioprine in the immunosuppressive repertoire, especially when several studies found that acute rejection rates for prednisolone/MMF/cyclosporine were lower than for prednisolone/azathioprine/cyclosporine.[31,32] However, all these studies used older preparations of cyclosporine. Studies using the newer micro-emulsification formulation (Neoral®) have shown similar efficacy and adverse effects for the two regimens.[33,34]

When deciding between azathioprine and MMF, several additional factors come into play. For example, azathioprine should be used with caution in patients treated with allopurinol, because this drug inhibits xanthine oxidase, resulting in an accumulation of active azathioprine metabolites.[35] MMF, on the other hand, causes more dyslipidaemia and diabetes mellitus and is associated with an increased risk for BK nephropathy.[36]

CALCINEURIN INHIBITORS

Cyclosporine (Neoral®) and tacrolimus (Prograf®, Advagraf®) are calcineurin inhibitors (CNIs). Cyclosporine binds to cyclophilin, whereas tacrolimus binds to FK-binding protein. Both result in calcineurin inhibition, which in turn inhibits translocation and activation of nuclear factor of activated T cells (NFAT), leading to downregulation of IL-2, 3 and 4, TNF-alpha, CD40L, G-CSF, IFN-γ and others.[37]

The development of CNIs – first cyclosporine and later tacrolimus – revolutionised the treatment of solid organ transplant patients, significantly improving graft survival rates. Both have become cornerstones of maintenance immunosuppression. However, nephrotoxicity has proven to be a major problem. Two mechanisms are at play.[38] The first mechanism, endothelial injury leading to heightened mesangial cell contractility, is potentially reversible, but the second mechanism, interstitial fibrosis, which may occur as early as three months after transplantation, is irreversible.

Over the last two decades, tacrolimus has gradually become the more widely used CNI, because it is associated with a lower risk of acute rejection and allograft loss, as shown by several studies and confirmed by meta-analysis.[39,40] Toxicity profiles are similar: both increase the risk of malignancy; cyclosporine is associated with slightly higher rates of hypertension, hyperlipidaemia and gum hyperplasia, whereas tacrolimus has more prominent neurological side effects and more cases of drug-induced diabetes and BK nephropathy.[36]

mTOR INHIBITORS

mTOR stands for mammalian target of rapamycin. Inhibition of this target prevents the transduction of the signal initiated by binding of IL-2 to its IL-2 receptor. This signal targets the mTOR complex, which has a key role in the regulation of various processes in the cell affecting cell growth and division.[41] In addition to immunosuppressive properties, mTOR inhibitors also have antiproliferative properties and are used for oncological indications. Sirolimus and everolimus (an active metabolite of sirolimus) are the main drugs in this class. Major side effects include thrombocytopenia, hyperlipidaemia and impaired wound healing. Sirolimus monotherapy is generally not nephrotoxic, but in combination with CNIs, significant nephrotoxicity has been described, due to increased blood levels of CNIs.[42,43] CMV infections, on

the other hand, seem to occur less frequently with mTOR inhibitors.[44,45]

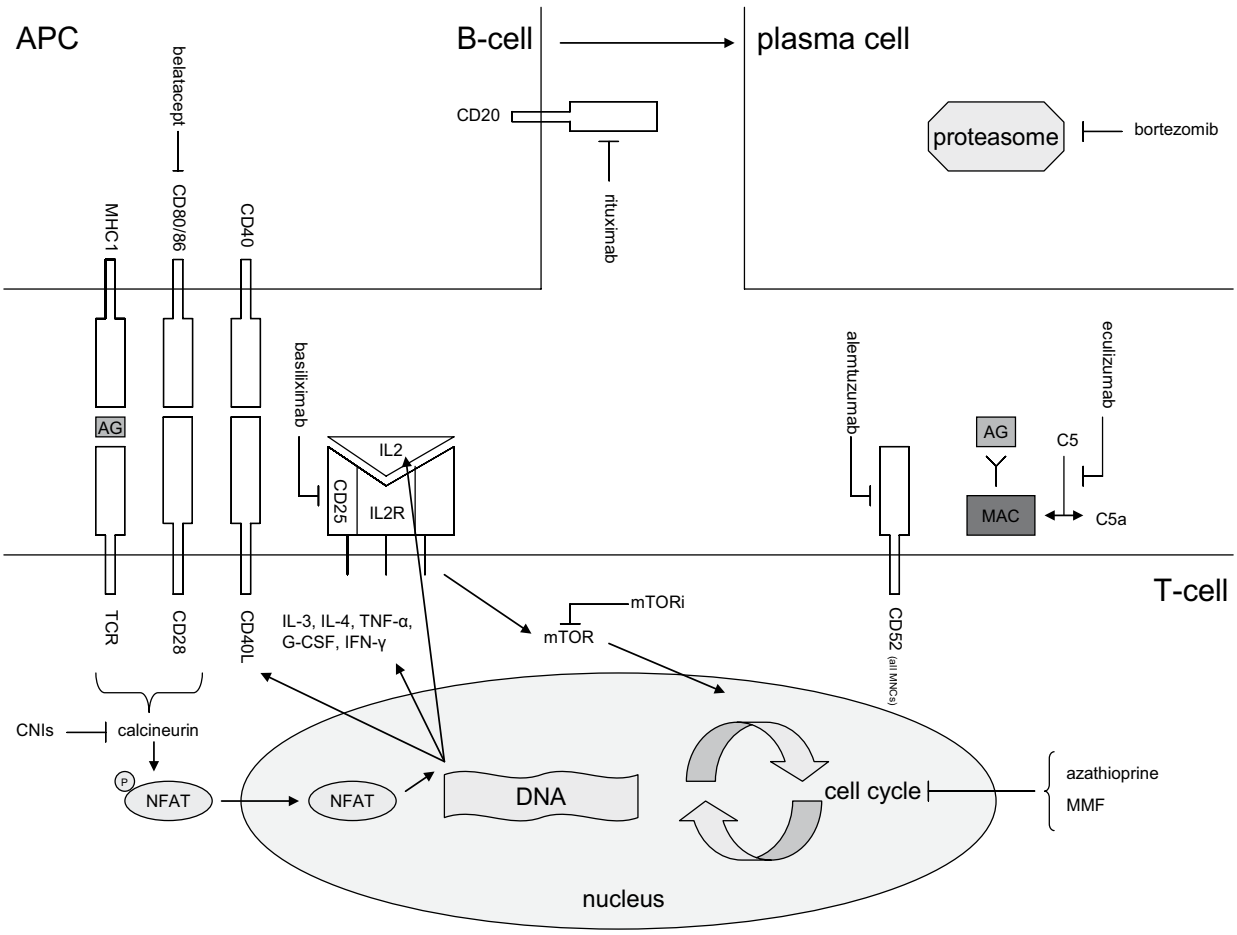
mTOR inhibitors are used for maintenance immunosuppression. A 2006 meta-analysis[46] comparing mTOR inhibitors with antimetabolites and calcineurin inhibitors concluded that mTOR inhibitors lowered the risk of acute rejection and a higher GFR. However, side effects were also more severe, particularly bone marrow suppression and lipid disturbances. The limitation of this meta-analysis was a follow-up of only two years. A randomised-controlled trial published in 2011[47] with a follow-up of eight years revealed different results: maintenance therapy with prednisolone/tacrolimus/MMF was accompanied by lower rates of acute rejection and a higher GFR than either prednisolone/tacrolimus/sirolimus or prednisolone/cyclosporine/sirolimus.

Overall, the place of mTOR inhibitors in immunosuppression after solid organ transplantation is still unclear. mTOR inhibitors are often used in patients experiencing CNI toxicity. A common approach is to start with a combined CNI-plus-mTOR inhibitor regimen, and aim for discontinuation of CNIs at three to six months post-transplantation, thereby avoiding irreversible CNI nephrotoxicity. Two trials for sirolimus[48,49] have shown that this results in improved renal function without significantly increasing acute rejection, whereas the recent ZEUS trial has shown that this holds true for everolimus as well.[50] Finally, in patients developing malignancies after transplantation (e.g. skin cancers, Kaposi sarcomas, post-transplantation lymphoproliferative disease (PTLD)), treatment with mTOR inhibitors seems to be a rational option.

DEPLETING ANTIBODIES

Drugs in this class include antithymocyte globulin (ATG), alemtuzumab and rituximab. ATG is a polyclonal

Figure 2. *Mechanism of action of selected immunosuppressive drugs*



AG = antigen; APC = antigen presenting cell (dendritic cell, macrophage or B-cell); CNIs = calcineurin inhibitors; G-CSF = granulocyte-colony stimulating factor; IL = interleukin; IL2R = IL-2 receptor; MAC = membrane attack complex; MHC = major histocompatibility complex; MMF = mycophenolate mofetil; MNCs = mononucleated cells; mTOR = mammalian target of rapamycin; mTORi = mTOR inhibitor; NFAT = nuclear factor of activated T-cells; TCR = T-cell receptor; TNF-α = tumor necrosis factor α.

immunoglobulin derived from either rabbits or horses that have been immunised with human thymocytes. In addition to T-cell depletion, it induces B-cell apoptosis, interferes with dendritic cell function, modulates adhesion molecules and chemokine receptors and induces regulatory T cells.[51] Administration of ATG induces a cytokine-release syndrome, which includes fever, chills and sometimes hypotension and pulmonary oedema. It also induces a profound lymphopenia that may last beyond one year.[52,53] ATG is used both as induction therapy and in the case of steroid-resistant rejection.

Alemtuzumab is a humanised monoclonal antibody against CD52. CD52 is present on T cells, B cells, NK cells and to a lesser extent on monocytes. As with ATG, alemtuzumab infusion can be followed by a cytokine-release syndrome, but this is much milder than with ATG.[54,55] Autoimmune phenomena have been observed, such as thyroid disease, haemolytic anaemia and thrombocytopenia.[56] Alemtuzumab can be used for induction therapy (see also next section). It is also under investigation for acute rejection. Preliminary studies[57-61] indicate that it may be an equally effective, but less toxic alternative to ATG in steroid-resistant rejection.

Rituximab is a monoclonal antibody against CD20, which is present on almost all B cells, except for plasma cells. In addition to being widely used in patients with haematological and rheumatoid disorders, rituximab is under study for application in antibody-mediated rejection (AMR),[62] desensitisation of HLA-sensitised patients[63] and ABO-incompatible transplantations.[64] It is also effective for PTLD.[65] Some studies have also evaluated rituximab as an induction agent, generally with disappointing results.[66-68] Side effects include infusion-related reactions.

NON-DEPLETING ANTIBODIES

The CD25 monoclonal antibody basiliximab is the main drug in this category. CD25 is the IL-2 receptor alpha chain on T cells and is expressed on activated T cells.[69,70] Side effects are relatively mild; hypersensitivity reactions have been described.[22]

Basiliximab can be used for induction therapy. Several studies have compared induction protocols using ATG, alemtuzumab or basiliximab. A Cochrane review[71] showed that basiliximab and ATG are equivalent in terms of graft loss or acute rejection at six months after transplantation, but that the use of ATG is accompanied by lower acute rejection rates at one year post-transplantation, at the cost of increased malignancies and CMV infections. A recent meta-analysis on alemtuzumab[72] showed that when compared with basiliximab, alemtuzumab results in fewer acute rejections. Alemtuzumab and ATG were equivalent in terms of acute rejection, graft loss, delayed graft function and mortality. Taken together, these results indicate that it is reasonable to reserve the use of ATG for high-risk patients, whereas basiliximab is a good option for low-risk patients, as several studies have shown.[73-75] The place of alemtuzumab in induction protocols remains to be settled, especially now that Genzyme Europe has withdrawn its marketing authorisation for commercial reasons.[76]

OTHER IMMUNOSUPPRESSIVE DRUGS

Belatacept, bortezomib and eculizumab are promising new candidates in this category. Belatacept is a fusion protein composed of the modified extracellular domain of CTLA4 and the Fc domain of human immunoglobulin IgG1. It blocks the CD80/86 co-stimulatory signal that is needed for T-cell activation, providing a new target for maintenance immunosuppression.[76] In low to moderate risk renal transplantation, short-term patient and allograft survival appear comparable with that observed under cyclosporine, with improved renal function despite more frequent and severe early acute rejection. Adverse effects include bone marrow suppression, hypertension, dyslipidaemia[36] and a relatively high frequency of PTLD.[77] Further research is needed to compare its efficacy and safety with other maintenance regimens.

Bortezomib, a proteasome inhibitor frequently used for treating multiple myeloma, is active against mature plasma cells. This sets it apart from many other immunosuppressive drugs, which can deplete immature B cells but not plasma cells. This makes bortezomib a promising candidate in the treatment of antibody-mediated rejection, traditionally associated with poor allograft survival. In one study comparing the addition of bortezomib or rituximab to a standard treatment protocol, 18-month graft survival in the bortezomib group was significantly higher.[78] Several other small studies[79] seem to confirm improved graft survival, especially in early AMR, but larger studies are needed. Neurotoxicity, headache, fatigue and bone marrow suppression are major side effects.

Eculizumab is a humanised monoclonal antibody that blocks the cleavage of human complement component C5 into its pro-inflammatory components.[80] This drug is available for the treatment of paroxysmal nocturnal haemoglobinuria (PNH) and atypical haemolytic uraemic syndrome (aHUS). Together with bortezomib and rituximab, it may be useful in the treatment of AMR. One study showed that eculizumab reduced the incidence of AMR in highly sensitised individuals when administered immediately post-transplantation.[81] A limitation is its price: for PNH, the yearly cost is estimated to be $ 400,000.

FUTURE DEVELOPMENTS

The ultimate goal of transplantation medicine is the induction of tolerance, which would eliminate the need for lifelong use of immunosuppressive medication. Indirect evidence that the human immune system possesses mechanisms to promote tolerance has been available for a long time as reflected by patients who have discontinued their immunosuppressive medication (e.g. due to noncompliance or for medical reasons such as persisting infections or malignancy), but still have functioning transplants.

Recent research has identified specific regulatory immune cells, which are specialised leukocyte populations that are either selected to have regulatory function during their development or acquire immunosuppressive properties in the local microenvironment of the allograft or in the graft-draining lymphoid tissues.[82] Regulatory T cells appear to play a central role, but regulatory B cells, macrophages, dendritic cells, myeloid-derived stromal cells and mesenchymal stromal cells also exist. A common feature of many regulatory cells is their ability to produce IL-10, a cytokine that may create a microenvironment that facilitates regulation and may function to enhance the generation and function of regulatory immune cells.[82]

Every immunosuppressive regimen affects the balance between tolerance and rejection. Induction therapy with depleting antibodies generates a prolonged leucopenia, which is followed by repopulation. This has the potential to tip the balance in favour of immune regulation. Both ATG and alemtuzumab have been shown to induce regulatory T cells.[83,84] Basiliximab, a monoclonal antibody specific for CD25, on the other hand, might have a less beneficial effect on regulatory T cells, as these cells express high levels of CD25. In maintenance immunosuppression, mTOR inhibitors are of particular interest, because rapamycin has been shown to promote expansion of regulatory T cells.[85]

New therapies aiming to promote tolerance can be divided into two groups: those in which regulatory cells are directly infused and those in which regulatory immune cell production is induced. Cellular therapies, in which regulatory immune cells are administered to patients directly, are being studied in the context of graft-versus-host disease (GVHD). Preliminary results in humans show a slight reduction in the incidence of GVHD, without loss of the graft-versus-leukaemia effect or significant safety concerns.[86] A multicentre phase I/II study[87] will investigate the safety of infusing regulatory T cells into renal transplant recipients. The alternative approach, stimulating regulatory immune cell production in humans, was effective in mice suffering from GVHD who were administered IL-2 and rapamycin. Low-dose IL-2 therapy was also used successfully to treat patients with chronic GVHD.[88] It remains to be determined whether the same holds true for recipients of solid organ transplantation.

CONCLUSION

In the past century, enormous steps have been taken in the field of solid organ transplantation. Heart, lung and liver transplantations are life-saving. In renal transplantation, the life expectancy of transplant patients easily exceeds that of dialysis patients. All this has been made possible because of a drastic expansion in the immunosuppressive repertoire. Unfortunately, the side effects of these drugs can be severe, which is one of the reasons that life expectancy of renal transplant patients still falls significantly short of that of the general population. Expanding our understanding of the human immune system will hopefully provide us with newer, smarter drugs that promote immunotolerance without the side effects observed today.

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REVIEW

New treatment options for patients with metastatic prostate cancer

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ABSTRACT

Prostate cancer is one of the most common cancers in men. When metastasised (40% of patients), classic anti-androgen therapy is the first-line treatment. Usually, this treatment becomes ineffective when castration-resistant prostate cancer (CRPC) develops. Thus far, docetaxel was the only chemotherapeutic option that has shown to be able to extend overall survival and improve quality of life in these patients. Recently, cabazitaxel and abiraterone have shown significant survival benefits for patients progressive on or after docetaxel treatment, as did enzalutamide and radium-223. In North America, immune therapy (sipuleucel-T) became available for a subgroup of CRPC patients. These new treatment options will change the treatment paradigm of patients with metastatic castration-resistant prostate cancer. A multidisciplinary approach by both medical oncologists and urologists seems mandatory.

KEYWORDS

Abiraterone, cabazitaxel, CRPC, enzalutamide, prostate cancer

INTRODUCTION

Recently a wide variety of novel treatment options for patients with advanced prostate cancer became available. In this article we want to introduce new drugs that have shown survival advantages in patients with metastatic prostate cancer.

New treatment options are welcome, since one in 11 men will develop prostate cancer during life. Of these patients, 18% have metastatic disease at the time of diagnosis and 40% will develop metastatic disease (2011, cijfersoverkanker.nl).[1,2] Until recently, the treatment options for these patients were sparse.[3]

Before 2011, metastatic prostate cancer was treated with classic androgen ablation therapy. The goal of this therapy was to achieve castrate levels of testosterone (<15 ng/dl), thereby depriving prostate cells of their most important stimulant for growth, function and proliferation *(figure 1)*. The testes are the largest source of most androgens, and adrenal biosynthesis provides an additional 5-10% of circulating androgens levels. Castrate levels of testosterone can be achieved surgically by bilateral orchidectomy, or medically with gonadotropin-releasing hormone (GnRH) agonists or antagonists. Classic anti-androgens inhibit the action of circulating androgens by competitive binding of the androgen receptor of prostate cancer cells.

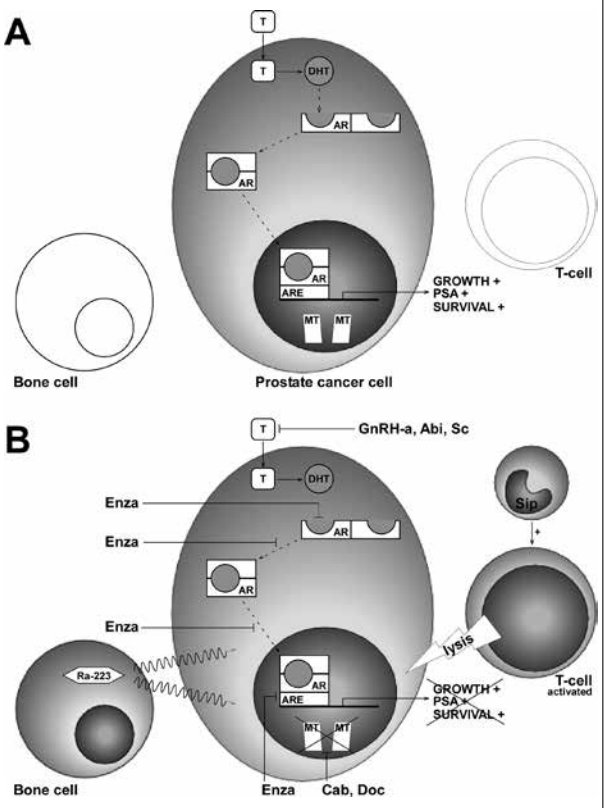
After a median of 2-3 years of therapy, metastatic prostate cancer usually becomes refractory to conventional androgen ablation therapy.[3,4] Prostate-specific antigen (PSA) levels increase again, despite castrate levels of androgens. This phase is called biochemical progressive disease. Clinical manifestations due to bone, lymph node and visceral metastases and local pelvic symptoms usually develop thereafter.

Recent insights, however, show that progression in this stage of the disease is still androgen-dependent due to changes of structure and function of the androgen receptor.[5] There appears to be a gradual shift during prostate cancer progression from dependence of androgens from endocrine sources to dependence of androgens from paracrine, autocrine and intracrine sources.[6] This stage of the disease, where prostate cancer grows despite castrate levels of androgens, is now called castration-resistant prostate cancer (CRPC).[7] The insights into the persistent crucial role of the androgen receptor route led to the

development of new androgen receptor route pathway inhibitors (*figure 1*).

Until 2011, second-line endocrine therapy in patients with progressive metastatic disease consisted of anti-androgen withdrawal or switch, possibly combined with prednisone. Although these treatments have a positive effect on symptoms, none of them extends overall survival.[8] In this phase, chemotherapy may also play a role. In 2004, treatment consisting of docetaxel demonstrated both improved quality of life and overall survival rates when compared with standard treatment consisting of mitoxantrone plus prednisone.[9] Therefore, over the last decade docetaxel plus prednisone became the new standard treatment for metastatic castration-resistant disease. For patients with refractory disease, only palliative systemic or local therapy remains.[10]

Figure 1. *Mechanisms of action of new drugs used in metastasised prostate cancer*



A) Testosterone (T) is the most important external growth stimulus for prostate (cancer) cells. It is internalised by prostate (cancer) cells and converted intracellularly to dihydrotestosterone (DHT). Both T and DHT are ligands of the intracellular androgen receptor (AR). Ligand binding induces a conformational change of the AR after which it transfers to the nucleus. In the nucleus, AR binds to androgen-responsive elements (ARE) in the DNA, thereby inducing gene expression leading to growth and survival of the prostate (cancer) cell. Prostate specific antigen (PSA) is also induced by the AR. Serum level rise is a sign of an active AR route. MT = microtubuli.

B) Blocking the androgen receptor (AR) route is an effective way of depriving the prostate (cancer) cell growth and survival stimuli. GnRH agonist/antagonists (GnRH-a), abiraterone (Abi) and surgical castration (Sc) all lower circulating testosterone levels around prostate (cancer) cells, thereby depleting AR of its ligand. The AR remains inactive. Enzalutamide (Enza) binds the AR ligand site, inhibiting nuclear translocation of the AR, DNA binding, and coactivator recruitment. Cabazitaxel (Cab), like docetaxel (Doc), is a microtubuli (MT) stabilisator. This results into blocking of cell division, thereby inducing cell death. Sipuleucel-T (Sip) induces native T cells to kill prostate cancer cells in an antigen-dependent manner. Radium-223 (Ra-223) targets new bone growth in and around bone metastases. It induces double-strand DNA breaks through alpha radiation over a short distance, thereby inducing cell death.

RECENT DEVELOPMENTS: NEW TREATMENT OPTIONS FOR METASTATIC CRPC

In the past two years several new treatment strategies have been developed for patients with metastatic CRCP (mCRPC). Several of these strategies have shown survival advantages in large phase III trials in patients who are refractory after docetaxel treatment. These strategies consist of cytotoxic, anti-androgen, immune, and radiopharmaceutical therapies (*table 1*). All studies were done in patients with a World Health Organisation (WHO) performance status (PS) of mostly 0-1.

NEW CYTOTOXIC THERAPY: CABAZITAXEL

Cabazitaxel, (Jevtana®) like docetaxel, is a microtubuli stabilisator. It is a second-generation taxane which has shown antitumor activity in cell lines resistant to docetaxel. The TROPIC study (Treatment of Hormone Refractory Metastatic Prostate Cancer Previously Treated with a Docetaxel-Containing Regimen) included 755 mCRPC patients with progressive disease after treatment with docetaxel.[11] The median age was 67 years (18% were >70 years), and patients had a beneficial WHO performance status (92% PS 0-1, others: 2). They were treated with tri-weekly cabazitaxel plus prednisone or mitoxantrone plus prednisone.

The median overall survival in the cabazitaxel-treated group was 15.1 months *vs* 12.7 months in the mitoxantrone group (HR 0.70; 95% CI 0.59-0.83; p<0.0001). Median time to tumour progression was longer and pain palliation was equal in both groups. The most common clinically significant grade 3 or higher adverse events were neutropenia (cabazitaxel 82% of patients *vs* mitoxantrone 58% of patients) and diarrhoea (cabazitaxel 6% *vs* mitoxantrone <1% of patients). Eight percent of patients in the cabazitaxel group and 1% in the mitoxantrone group had febrile neutropenia. Eighteen patients (5%) died in the cabazitaxel group and nine patients (2%) in

Table 1. *Phase III trials of drugs showing improved overall survival in mCRPC patients*

Trial	Tested drug	Tested patients	Number of patients	Survival in months (HR)
TROPIC[11]	Cabazitaxel (iv)/prednisone vs mitoxantrone/prednisone	Post-docetaxel	755	15.1 vs 12.7 (0.70)
COU-AA-301[12]	Abiraterone (oral)/prednisone vs placebo/prednisone	Post-docetaxel	1195	14.8 vs 10.9 (0.65)
COU-AA-302[13]	Abiraterone/prednisone vs placebo/prednisone	mCRPC	1088	NR vs 27.2
AFFIRM[15]	Enzalutamide (oral) vs placebo	Post-docetaxel	1199	18.4 vs 13.6 (0.70)
IMPACT[16]	Sipuleucel-T (i.v.) vs controls	mCRPC	512	25.8 vs 21.7 (0.78)
ALSYMPCA[17]	Radium-223 (i.v.)/BSC vs placebo/BSC	Post-docetaxel or unsuitable for docetaxel	921	14.9 vs 11.3 (0.63)

BSC = best supportive care; HR =hazard ratio; mCRPC = metastatic castration-resistant prostate cancer.

the control group because of treatment-related side effects. Eighteen percent of the cabazitaxel group patients *vs* 8% in the mitoxantrone group discontinued study treatment because of side effects. Cabazitaxel is available in Europe for patients with a good PS (0-1) since its approval by the European Medicines Agency (EMA) in March 2011.

NEW ANTI-ANDROGEN THERAPY: ABIRATERONE ACETATE AND ENZALUTAMIDE

Abiraterone acetate

Abiraterone acetate (Zytiga®), an oral prodrug of abiraterone, is a first-in-class selective cytochrome P-450c17 (CYP17) complex inhibitor, thereby making it an androgen synthesis inhibitor. Androgen synthesis in the adrenal glands, testes and prostate cancer cells and their microenvironment is effectively inhibited, thereby further lowering the serum testosterone levels.[6] Inhibition of CYP17 also leads to increased production of mineralocorticosteroids in the adrenal glands, leading to important but treatable side effects. Two trials investigating treatment using abiraterone have been published: one trial in the post-docetaxel setting[12] and another trial in the pre-docetaxel setting.[13]

Abiraterone post-docetaxel

The COU-AA-301 study included 1195 patients with progressive mCRPC with castrate levels of testosterone who had been previously treated with docetaxel.[12] Median age of the patients was 69 years (28% >75 years, PS 0-2). Patients were randomised 2:1 to receive either once a day abiraterone 1000 mg plus prednisone 10 mg or placebo plus prednisone 10 mg, until disease progression. The study was stopped at the first pre-planned interim analysis because of the significant reduction in death in the group of patients treated with abiraterone. The median follow-up was 12.8 months; the median overall survival in the abiraterone group was 14.8 months *vs* 10.9 months in the placebo group (HR 0.65; 95% CI 0.54-0.77; p<0.001). Progression-free survival (5.6 *vs* 3.6 months) and other secondary endpoints (disease progression measured by PSA, radiological response, pain response, time to bone complications) improved significantly in the group of patients treated with abiraterone. Mineralocorticoid side effects (oedema, hypokalaemia and hypertension) were noticed more often in the treatment group (55 *vs* 42% of patients; p<0.001), but were usually mild.

Recently, overall survival analysis data were published after a median follow-up of 20.2 months: the median overall survival was 15.8 *vs* 11.2 months in favour of the abiraterone-treated group (HR 0.74; p<0.0001).[14] At that moment, 16 *vs* 5% of the patients were still alive, suggesting no durable benefit of abiraterone on survival. Disease progression is only deferred. Abiraterone in combination with prednisone after docetaxel treatment is approved by EMA since September 2011.

Abiraterone pre-docetaxel

COU-AA-302 is a randomised controlled trial that compared placebo with abiraterone plus prednisone in asymptomatic or mildly symptomatic, and thus chemo-naive, mCRPC patients (n=1088; median age of patients 70.5 years; 32% ≥75 years; PS 0-1).[13] Very recently, this study was stopped at the second planned interim analysis because one of the co-primary endpoints was reached. Radiographic progression-free survival was 16.5 months in the abiraterone group and 8.3 months in the prednisone-alone group (HR 0.53; 95% CI 0.45-0.62; p<0.001). As for the second co-primary endpoint: there was a strong trend towards improved survival in patients on abiraterone plus prednisone versus prednisone alone (median follow-up 22.2 months: overall survival not reached in abiraterone group *vs* 27.2 months in placebo group). Secondary endpoints (palliative care) showed important improvement: median time to PS decline, cancer-related opiate use and start of cytotoxic chemotherapy were all significantly delayed in the abiraterone group. Based on these results, the EMA approved abiraterone plus prednisone for chemo-naive

asymptomatic or mildly symptomatic chemo-naive mCRPC patients in December 2012.

Enzalutamide

Enzalutamide (XTandi®) is an androgen-receptor-signalling inhibitor (ARSI) that binds the AR ligand site and thereby inhibits nuclear translocation of the AR, DNA binding, and coactivator recruitment. It has no known agonistic effects.[6,15] The AFFIRM trial (A Study Evaluating the Efficacy and Safety of the Investigational Drug MDV3100) enrolled 1199 patients 2:1 to enzalutamide 160 mg daily or placebo.[15] Patients median age was 69 years (25% ≥75 years) and they had a PS of 0-2 (8% PS 2) (supp data). They all had objective progressive disease on or after treatment with docetaxel. At the pre-specified interim analysis the study was stopped because of the reduction in death in the enzalutamide group. The median follow-up was 14.4 months, the median overall survival in the enzalutamide group was 18.4 months *vs* 13.6 months in [the] placebo group (HR 0.63; 95% CI 0.53-0.75; p<0.001). Superiority for enzalutamide was shown for all secondary endpoints, including PSA level response rate, soft tissue response rate, radiographic progression-free survival and time to first skeletal-related event. Although the period of observation of the enzalutamide group was more than twice that of the placebo group, rates of adverse events were similar in the two groups, and the median time to a grade 3 or higher adverse event was 12.6 months in the enzalutamide group, compared with 4.2 months in the placebo group. The most common adverse events in the enzalutamide group included fatigue, diarrhoea, and hot flashes. Convulsions are a dose-dependent toxic effect of enzalutamide and occurred in 0.9% of treated patients. Since August 2012, enzalutamide is approved in North America, and awaiting approval by EMA in Europe.

NEW IMMUNE THERAPY: SIPULEUCEL-T

Immune therapy with the vaccine sipuleucel-T (Provenge®) stimulates the immune system to lyse prostate tumour cells. Over 95% of prostate cancers express prostate acid phosphatase (PAP). The vaccine sipuleucel-T is made of autologous dendritic cells that are stimulated *in vitro* with a fusion protein (PA2024: PAP and granulocyte-macrophage colony stimulating factor). After reinfusion in the patient this vaccine induces native T cells to recognise and kill PAP-expressing prostate cancer cells in an antigen-dependent manner.

The IMPACT (Immunotherapy for Prostate Adenocarcinoma Treatment) study included 512 patients with asymptomatic or minimal symptomatic mCRPC (median age of patients 71 years, PS 0-1, Gleason ≤7, no known visceral metastases).[16] Of the included patients, 18% had used docetaxel therapy before. Patients received a sipuleucel-T infusion or placebo at T=0, 2 and 4 weeks. The median survival was 4.1 months longer in the sipuleucel-T group (25.8 *vs* 21.7 months; HR 0.78; 95% CI 0.61-0.98; p=0.03). Sipuleucel-T is the first immune therapy showing improvement of overall survival in mCRPC patients in a phase 3 randomised controlled trial. This immune therapy has been available in North America since 2010.

NEW RADIOPHARMACEUTICAL THERAPY: RADIUM-223

Radium-223 dichloride (Ra-223, Alpharadin®) is a new bone-seeking (first-in-class) alpha-emitter radionuclide. Radium-223 is a calcium mimetic that naturally targets new bone growth in and around bone metastases. It kills cancer cells through alpha radiation from the decay of radium-223, inducing double-strand DNA breaks in adjacent tumour cells. In the ALSYMPCA (ALpharadin in SYMptomatic Prostate Cancer) trial, radium-223 was tested against placebo in 921 patients with mCRPC and cancer-related bone pain who had previously received or were ineligible for docetaxel (2:1 allocation ratio, mean age of patients 70.5 years; PS 0-2) (clinical trial NCT00699751).[17] The primary endpoint was overall survival. Six intravenous administrations (50 kilo Becquerel/kg body weight) at four weekly intervals were given. At the planned interim analysis, Ra-223 significantly improved overall survival (median overall survival 14.9 *vs* 11.3 months (HR 0.7; 95% CI 0.58-0.83; p=0.001) and time to secondary endpoints (PSA progression and time to first skeletal-related event) as well as quality of life. These data still await publication in a peer-reviewed journal. This drug has not been approved by the FDA yet.

CHANGING TREATMENT PARADIGM FOR METASTATIC CRPC PATIENTS

After an era of little improvement in therapeutic options for mCRPC patients progressive on or after docetaxel treatment, several drugs have recently shown clinically relevant survival advantages and improvement in palliative care, such as delay of bone pain and first skeletal-related event. Since 2011, the cytotoxic drug cabazitaxel and the new anti-androgen abiraterone are available for Dutch mCRPC patients. The new anti-androgen enzalutamide will probably follow soon, as will the radiopharmaceutical radium-223. Immune therapy with sipuleucel-T is available in North America; other vaccines are currently being tested in clinical trials, also in Europe.

Patients with advanced prostate cancer are usually treated by urologists with first-line androgen ablation therapy. With the arrival of additional chemotherapy and new anti-androgens that need close monitoring, the role in treatment of patients with metastatic prostate cancer by medical oncologists becomes more important.[18] Since especially patients with a good WHO performance status have been shown to benefit from recently described treatment options (before or after docetaxel therapy), early referral is important.

Further studies need to investigate what the optimal treatment sequence for patients with mCRPC is. Currently, is seems reasonable to start early with docetaxel in fit patients with progressive mCRPC. For patients who are not candidates for chemotherapy, abiraterone, instead of docetaxel, is an option. Fit patients (PS 0-1) with progressive disease on or after docetaxel could be offered cabazitaxel as second-line therapy. Abiraterone and enzalutamide have shown to be effective after one and two lines of chemotherapy, and can be a third-line therapy.[14,15]

Over the past three years, five differently acting drugs have become available for patients with mCRPC, as a result of the development of further insight into the pathophysiology of advanced prostate cancer. These new treatment options for patients with mCRPC offer a significant extension of overall survival and improvement of palliative care.

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REVIEW

Pre-exposure prophylaxis (PrEP) in HIV-uninfected individuals with high-risk behaviour

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ABSTRACT

The global incidence of human immunodeficiency virus (HIV) infection has decreased by 15% over the past years, but is still too high. Despite current programs to reduce the incidence of HIV infection, further approaches are needed to limit this epidemic. Oral antiretroviral pre-exposure prophylaxis (PrEP) is currently one of the most discussed possible prevention methods. This literature study demonstrates whether orally antiretroviral chemoprophylaxis in HIV-uninfected individuals with high-risk behaviour reduces the transmission of HIV.

We used the PICO method and conducted a search to identify relevant studies. Subjects of the study were HIV-uninfected individuals with high-risk behaviour. Intervention was oral PrEP with tenofovir disoproxil fumarate (TDF) alone or plus emtricitabine (FTC) versus placebo. The primary outcome was the HIV incidence among this high-risk group. Secondary outcomes were adherence to PrEP, frequency and type of adverse effects.

We identified ten studies from which five randomised control trials (RCTs) were included after screening. The results from three out of five trials showed a reduction, but two trials showed no protection in acquiring HIV infection. There were no significant differences in adverse events. The adherence was different among different groups and affected the outcome of the studies.

In conclusion, this prophylaxis might offer protection when used in combination with intense monitoring and guidance in uninfected individuals with a high risk of HIV acquisition. However, there are still many unresolved questions. Drug adherence seems to be a crucial factor in the effectiveness of PrEP. Therefore, individual risk behaviour remains an important determinant for success in the prevention of HIV transmission.

KEYWORDS

Human immunodeficiency virus (HIV), HIV prevention, HIV prophylaxis, pre-exposure prophylaxis, tenofovir – emtricitabine

BACKGROUND

The global incidence of human immunodeficiency virus (HIV) infection has decreased by 15% over the past ten years. However, due to the increased number of treated patients, more people are living with HIV infection.[1] In the Netherlands, the number of newly infected HIV individuals remains stable and is approximately 1100/year. The highest incidence is found in men who have sex with men (MSM).[2]

Behaviour change programs have been considered the key factor to reduce the incidence of HIV in different countries. However, studies show that despite these programs, a considerable number of people are still infected.[1] Therefore, further approaches are needed to limit the expansion of HIV worldwide.[3]

Evidence is present that combined antiretroviral therapy (cART) has a considerable contribution to the prevention of HIV transmission in serodiscordant (one partner HIV-positive and one partner HIV-negative) couples.[4,5] In addition, prompt treatment with antiretroviral drugs is extremely important in prevention of mother-to-child HIV transmission.[6,7] This treatment of the pregnant mother can be considered as a form of pre-exposure prophylaxis (PrEP) of the newborn. Interestingly, introduction of PrEP also seems promising for the future in adults. It means that antiretroviral agents must be taken prior to exposure by uninfected individuals at high risk.[1,8,9] So far,

the available agents used as PrEP are tenofovir disoproxil fumarate (TDF) and the combination tenofovir disoproxil fumarate and emtricitabine (TDF-FTC or Truvada). These drugs interrupt the reverse transcriptase enzyme from transcribing HIV genetic material (RNA) into DNA before the virus's genetic code is inserted into an infected cell's genome.[6,10]

However, there are various concerns regarding this kind of prophylaxis. Firstly, the long-term side effects of this therapy are unknown in uninfected individuals. Secondly, when adherence to PrEP is low and the patient gets an acute HIV infection, he/she will be treated with one or two instead of three active drugs. This can result in the development of a resistant virus. Another concern is that PrEP will increase high-risk behaviour.[11] A fourth limitation can be the high costs of the agents.

With these points in mind, we did a literature study to answer the question whether orally antiretroviral chemoprophylaxis in HIV-uninfected individuals with high-risk behaviour reduces the transmission of HIV infection.

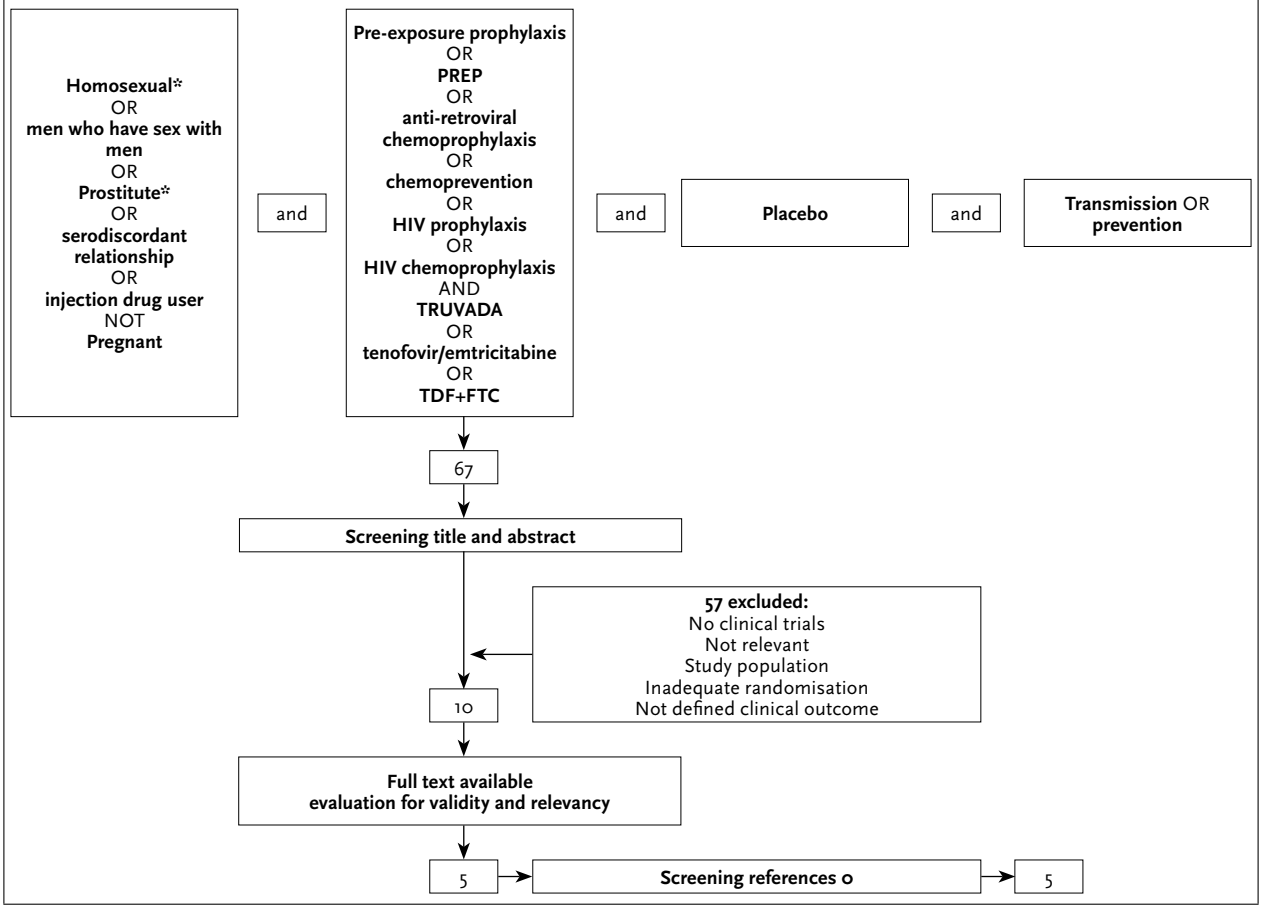
METHODS

We used the PICO (**P**atient/**P**opulation, **I**ntervention, **C**omparison, **O**utcome) method, which allows you to take a more evidence-based approach when searching bibliographic databases, and conducted a comprehensive search to identify all relevant studies.

Patients (P) were HIV-uninfected individuals with high-risk behaviour, namely in serodiscordant relationships, commercial sex workers, intravenous drug users and MSM. Pregnant women were excluded. The intervention (I) was an oral PrEP regime with TDF-FTC. The studies involving topical application of antiretroviral agents such as vaginal gels were excluded. For the comparison (C) an oral PrEP regime was compared with placebo or no treatment. The primary outcome (O) was HIV incidence among this high-risk group. Secondary outcomes were adherence to PrEP, safety and frequency and type of adverse effects or complications.

The titles and abstracts of the search output from the different databases were screened to identify eligibility of

Figure 1. *Flow chart with search terms and results of search strategy*



the studies. Full-text articles were obtained for all citations identified as potentially eligible. Extracted information included the study design, population, intervention details, namely type of drug, comparator, dose, duration and route of administration, and primary and secondary outcomes. We searched the available and upcoming data. Inclusion was limited to randomised controlled trials (RCTs).

RESULTS

Results of the search

A total number of 67 titles and abstracts were eligible. We identified ten relevant studies, from which five RCTs were finally included after critical appraisal according to the Dutch Cochrane Appraisal Form (see *figure 1.* Flowchart).[8,12,13,15-17]

Outcomes: HIV incidence and adherence

Grant *et al.* (iPrEx) studied daily TDF-FTC versus placebo in 2499 MSM aged 18 years and older, conducted in Peru, Ecuador, South Africa, Brazil, Thailand and the United States; they demonstrated in this trial that a once-daily oral tablet of a combination of TDF-FTC reduced HIV incidence among MSM by 44% after a median follow-up of 1.2 years.[8] Adherence was evaluated in two ways. The relative reduction in the risk of HIV infection was 73% with an adherence of 90% measured by pill counts and 92% among the participants with detectable tenofovir concentrations in serum.[8]

Baeten *et al.* showed in the Partners PrEP Study that daily oral PrEP reduced the risk for HIV infection in the HIV-uninfected partner in serodiscordant, heterosexual African couples by 67% with TDF and 75% with TDF-FTC, each compared with placebo, after a median follow-up of about two years.[12] There was no significant difference between the TDF and TDF-FTC groups.[12] Relative to placebo, the effect of TDF in women was 71% and for TDF-FTC 66%; among men, the efficacies were 63% and 84%, respectively. Among the participants in the treated group who acquired HIV, 31% had a detectable tenofovir level in plasma compared with 82% among participants who were not infected. A good blood concentration of tenofovir was associated with an estimated reduction in the relative risk of being infected of 86% with TDF and 90% with TDF-FTC, respectively.

Peterson *et al.* studied the efficacy and safety of TDF versus placebo in a phase 2, randomised, double-blind, placebo-controlled trial. The study was conducted among women between 18 and 35 years, who were at risk of HIV infection by having an average of ≥3 coital acts per week and ≥4 sexual partners per month. This study reported inconclusive data on effectiveness of the treatment, mainly because sites were closed earlier due to non-compliance.[14]

In the TDF2 study, daily oral TDF-FTC for at-risk heterosexual men and women decreased HIV incidence by 62% after two years.[15]

Two trials failed to show efficacy of PrEP in African women. FEMPrEP (Pre-exposure Prophylaxis Trial for HIV Prevention among African Women) studied women at-risk in several African countries, who took oral TDF-FTC or placebo.[16] An independent Data Safety Monitoring Board (DSMB) terminated the study early because of ineffectiveness of the intervention.[16]

Table 1. *Overview of included trials about effect of PrEP on HIV transmission*

Study location [Reference]	Population and number	Design intervention	Relative reduction in HIV incidence	Agent present in blood samples
iPrEx (Peru, Ecuador, South Africa, Brazil, Thailand and the United States) Grant et al.[8]	2499 MSM	RCT Oral FTC/TDF or Placebo	FTC/TDF: **44%** (95% CI 15-63%, p=0.005)	92% protection with blood concentration of TDF-FTC
Partners PrEP (Uganda and Kenya) Baeten et al.[12]	4747 heterosexual men and women with HIV positive partner	RCT TDF, FTC/TDF or placebo	TDF **67%** 95% CI 44-81% p=0.0001 FTC/TDF **75%** 95% CI 55-87%, p<0.0001	86% protection with blood concentration of TDF 90% protection with blood concentration of TDF-FTC
TDF 2 (Botswana) Thigpon et al.[15]	1219 heterosexual men and women	RCT FTC/TDF or placebo	FTC/TDF **63%** 95% CI, 22-83%, p=0.01	79%
FEM PrEP (Kenya, South Africa, Tanzania) Van Damme et al.[16]	202 women	RCT FTC/TDF or placebo	**No HIV** protection	35-38% at single visit; 26% at two consecutive visits
VOICE (South Africa, Uganda, Zimbabwe)[1,13]	5029 women	RCT Oral TDF, FTC/TDF or placebo Vaginal gel or placebo	**No HIV** protection in any arm	<30% samples had tenofovir detected >50% women in each arm had *never* tenofovir detect during any visit

The results of the VOICE (Vaginal and Oral Interventions to Control the Epidemic) study are particularly disappointing, because of the high rates of new HIV infection that occurred in women included in the trial. This was a double-blind, placebo-controlled, five-arm trial of daily oral TDF, oral FTC/TDF, oral placebo, vaginal tenofovir gel or vaginal placebo gel, as PrEP for the prevention of HIV acquisition by HIV-negative women. A total number of 5029 women, who had reported having vaginal sex in the three months prior to enrolment and who were willing to use contraception, were enrolled at sites in South Africa. Two years after the start of the trial the oral TDF arm was stopped by the DSMB, because it appeared to not be effective for HIV prevention.[13] A few months later the vaginal tenofovir gel arm was stopped (and its placebo gel arm) for the same reason. VOICE continued to evaluate the effect of Truvada until the scheduled end of the study, but none proved to be effective.[1,13]

Safety and adverse events profile

Baeten *et al.* reported no significant difference in the frequency of death, and serious adverse events as renal failure among three different groups (TDF, TDF-FTC and placebo).[12] Neutropenia was observed more in the TDF-FTC group than in the other two groups. During the first months of administration, more gastrointestinal problems were reported in the treatment compared with the placebo group.[12] Grant *et al.* presented a similar rate of serious adverse events in two groups. Nausea was reported more frequently during the first month in the treatment group than with placebo.[8] Additionally, Peterson *et al.* documented no increase in clinical or laboratory adverse events.[14]

DISCUSSION

In the present review we show that randomised clinical trials with oral tenofovir-based PrEP reveal inconsistent results for effectiveness. In theory, this novel prophylaxis might offer the opportunity to combat the HIV epidemic in the distant future, but there are still many unresolved practical issues.

In the studies that failed to provide convincing evidence of HIV protection, lack of adherence to the treatment was high. Importantly, the proportion of participants with detectable levels of the study drug was far lower in the terminated studies compared with the studies in which a decreased HIV incidence was found.[15] Therefore, it seems that high adherence is crucial for obtaining clinical benefit from PrEP. This issue already raises the first point of concern regarding the application of this treatment. Access, acceptability, motivating and monitoring adherence among the populations at highest risk is of great importance. Better achievement will mainly rely on programs implemented in community settings, which show high success rates. So it is again not the agent, but the difference in human behaviour which leads to the varying degrees of success. As with behaviour prevention strategies, it underlines the complexities of achieving a new strategy with which many individuals might benefit. In addition, it is important to study how PrEP can be combined with other proven behaviours to accomplish better prevention.

Despite these summarised studies, there are many unresolved questions. In the first place, more research is needed to find the factors affecting adherence. Perhaps interviewing participants of the terminated studies can provide more information on how adherence can be improved. Moreover, a dangerous situation will occur when the adherence to PrEP is low and the patient gets an acute HIV infection. This was the case in five patients in the iPrEx trial.[8] Most patients with an acute infection have a very high HIV load. In the above-mentioned trial,[8] they were treated with two instead of three active drugs. This is the ideal situation for the development of a resistant virus. Because the patient thinks that he/she is not infected (or even safe), this individual has the potential to transmit a drug-resistant virus to another person which may restrict later treatment options.[18]

Furthermore, it would be interesting to study the best method for administration – oral, vaginal or rectal – and the optimal dose frequency: daily or intermittently before a high-risk act. Daily administration might improve protection and would offer better adherence, on the other hand not much is known about the long-term side effects in these healthy persons. Since TDF is a relatively new agent (around ten years on the market) the side effects in the long-term are still unknown, as for example progressive renal insufficiency.[19] This is especially relevant because this treatment will mainly be given to relatively young and healthy persons. Another important issue that will need to be addressed is the long-term cost-effectiveness of PrEP. At this moment 30 tablets of TDF/FTC cost about 550 euros.[20]

The members of the Dutch Association of HIV-treating Physicians (NVHB) discussed all the above-mentioned issues during a meeting in January 2012. Because of all the concerns raised, as mentioned above, they concluded that at this moment no clinical indication for PrEP is present in the Netherlands. However, clinical research about this promising form of prophylaxis is urgently warranted to answer the above-mentioned questions, but also to investigate the implementation possibilities and barriers in the Netherlands.

In conclusion, this prophylaxis might offer protection when used in combination with intense monitoring

and guidance in individuals with a high risk of HIV acquisition. However, there are still many questions which need to be answered. Since behaviour also seems to be an important determinant for success in this strategy, it is still by no means the single effective strategy in preventing HIV transmission.

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REVIEW

Smelling the diagnosis

A review on the use of scent in diagnosing disease

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ABSTRACT

For centuries, our sense of smell has been used as a diagnostic tool in the practice of medicine, be it for recognising gas gangrene on the battle field or diabetic ketoacidosis in the emergency room. In recent decades, many scent detection studies have been performed with human, animal and electronic noses. The ability of humans to diagnose disease by smelling has only rarely been the subject of quantitative studies. Scent detection by animals, on the other hand, has been addressed in several diagnostic studies, which all suggest similar or even superior accuracy compared with standard diagnostic methods. Examples include, amongst many others, the use of dogs for the detection of lung cancer in breath samples, or rats for *Mycobacterium tuberculosis* detection in sputum. Studies using different types of electronic noses in conditions such as pulmonary disease and cancer have also shown promising results with high overall sensitivity and specificity. However, results of different types of noses are not easily generalisable and independent confirmation studies are generally lacking, which should be a focus for future research.

In conclusion, scent detection by animals and electronic noses holds promise for the future and should receive higher priority in terms of research effort and funding.

KEYWORDS

Electronic nose, detection dog, odours, scent detection, smell

INTRODUCTION

As early as 2000 BC, the ancient Greek and Chinese used scent to diagnose infectious diseases such as tuberculosis.[1] Ever since, our sense of smell has been used as a diagnostic tool in the practice of medicine. Well-known examples include fetor hepaticus surrounding patients with liver failure, and the fruity smell of ketones in exhaled breath of patients with diabetic ketoacidosis.

The sense of smell depends on the ability of specialised sensory cells of the nose to perceive volatile compounds. Diseases such as infections and malignancies can be associated with changes in host metabolism, accompanied by production of different metabolic compounds, and thus a different odour. In the late 1980s, a dog handler became increasingly suspicious of a mole after her dog constantly kept sniffing at the lesion on her leg and eventually even tried to bite it off.[2] The consulted dermatologist subsequently diagnosed a melanoma. Since then, several studies have addressed animal scent detection as a diagnostic technique. Attempts to mimic the biological olfactory system resulted in several types of electronic noses (Enoses),[3] which are also increasingly used in the medical field.

In this clinical review, we discuss different types and applications of scent detection and their potential as diagnostic tools in modern medicine.

METHODS

Two systematic literature searches were performed. One included scent detection by animals and humans, the other focused at scent detection by Enose. We followed the PRISMA statement as a guideline for the systematic search.[4] Search terms such as “volatile organic compound”, “detection dog”, “scent detection”, “electronic nose” and “olfactory detection” were used in the following databases: Medline, Embase and Web of Science. For a detailed description of the search strategies, see *appendix I*. We use the term ‘Enose in the broadest sense of the word, including applications such

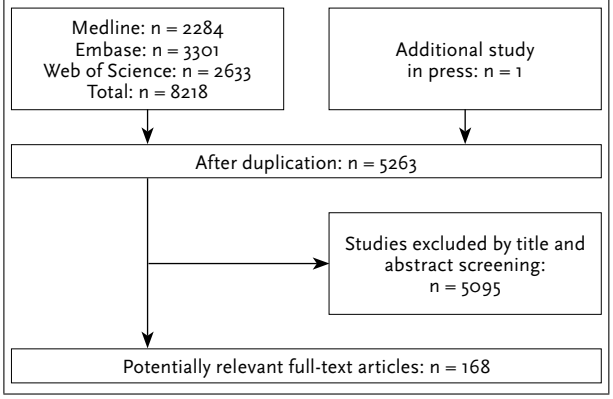
as chemical gas sensors, gas chromatography, optical sensor systems, infrared spectroscopy, and mass spectrometry. The electronic search was supplemented by hand searching of references cited in available literature.

Studies were included if human, animal, or electronic noses were used for diagnostic analyses of patient material (e.g. breath, faeces, urine, and tissue) and written in English. Duplicates and case reports were excluded. Using the remaining potentially relevant research articles, we then aimed to give a narrative review of the key studies in scent detection per medical field.

RESULTS

Figure 1 shows a flow diagram of the literature search. A total of 168 studies were included, of which the key studies in scent detection per medical field are reviewed here.

Figure 1. *Flow diagram of the literature search*



Cancer

Scent detection for the diagnosis of cancer has the benefit of being non-invasive and could therefore have great potential as a screening tool. As mentioned in the introduction, the first time an animal was described to detect a disease was in fact a case of cancer (melanoma).[2] Enoses have been used for a few decades now, but their application in diagnosing cancer is rather new. Here we describe several types of cancer for which animals and Enoses were used as a diagnostic tool. The main findings are summarised in *table 1*. No studies on scent detection of cancer by humans have been reported.

Lung cancer

Trained dogs perform well in detecting lung carcinoma in breath samples. Recently, in one of the largest animal scent detection studies to date, breath samples of 220 participants (healthy individuals, patients with lung cancer, and patients with chronic obstructive pulmonary disease – COPD) were presented to sniffer dogs. Lung cancer was identified with an overall sensitivity of 71% and a specificity of 93%, independent of the presence of COPD or tobacco smoke.[5] Studies in which exhaled breath is analysed by Enose were first conducted in 1971.[6] In 1985, it proved possible to use this type of breath analysis as a non-invasive marker of lung cancer.[7] Since then, many reports studying VOCs in lung cancer have appeared, showing a fair overall sensitivity (71-85%) and good specificity (92-100%).[8-11] Moreover, both dogs and Enoses are able to discriminate between lung cancer and COPD.[5,12]

Ovarian cancer

Dogs performed extremely well in identifying ovarian carcinoma in both blood and tissue samples, reaching a

Table 1. *Characteristics of key scent detection studies by dogs and electronic noses (Enoses) for different types of cancer*

Cancer	Type of nose	Type of sample	Sensitivity / Specificity (95% CI when available) or success rate	Sample size (diseased/healthy)
Lung[5]	Dog	Breath	71% (51-88%) / 93% (87-98%)	60 / 160
Lung[8]	Enose	Breath	71% / 100%	65 / 31
Lung[9]	Enose	Breath	85% / 100%	56 / 36
Lung[10]	Enose	Breath	94% success rate	35 / 25
Lung[11]	Enose	Breath	71% (42-92%) / 92% (82-97%)	14 / 62
Ovarian[13]	Dog	Tissue and blood	Tissue : 99% / 97% Blood : 100% / 98%	40 / 200
Ovarian[14]	Enose	Tissue	84% / 87%	15 / 15
Breast[17]	Dog	Breath	88% (75-100%) / 98% (90-99%)	6 / 17
Breast[16]	Enose	Breath	94% / 74%	51 / 147
Breast[18]	Enose	Breath	75% / 85%	54 / 204
Bladder[19]	Dog	Urine	41% success rate (23-58%)	9 / 54
Bladder[20]	Enose	Urine	100% / 100%	25 / 18
Colorectal[21]	Dog	Breath and faeces	Breath: 91%/ 99% Faeces: 97% / 99%	Breath: 33 / 132 Faeces: 37 /148
Melanoma[26]	Dog	Tissue	75-86% success rate	7 / 98
Melanoma[27]	Enose	Tissue	70% / 90%	10 / 47

sensitivity of 100% and a specificity of 98%.[13] The same research group tested an Enose for the detection of ovarian carcinoma in tissue samples. In keeping with the lower sensitivity of Enoses compared with the dog's nose, the Enose study suggested a somewhat lower overall sensitivity and specificity, of 84% and 87% respectively, compared with the dog's performance.[14]

Breast cancer

Breast cancer is the most prevalent malignancy amongst women in the Western world.[15]

Both dogs and Enoses have been tested for the detection of breast carcinoma in breath samples. The study using an Enose identified five volatile organic compounds (VOCs) in exhaled breath that could predict the presence or absence of breast cancer.[16] A few years later, a study including detection dogs was performed, where sensitivity and specificity of dog detection was 88% and 98%, and Enose reached 94% and 74%, respectively.[16,17] A more recent Enose study analysed 258 breath samples and found a sensitivity of 75% and a specificity of 85%, supporting the notion that Enoses do not reach the same diagnostic accuracy as dogs.[18]

Bladder cancer

Bladder cancer was the first disease for which the diagnostic accuracy of animal scent detection was systematically analysed. In this study, dogs were trained to recognise bladder cancer in urine samples; the subsequent formal evaluation study showed a diagnostic success rate of 41%, whereas based on chance a success rate of only 14% was anticipated.[19] Another study found that an Enose was also able to discriminate urine samples of healthy patients from those of patients with bladder cancer, with a diagnostic accuracy of 100%.[20]

Colorectal cancer

Dogs have also been trained to identify colorectal carcinoma. In 350 stool and breath samples, the dogs' diagnostic accuracy was very high, with a sensitivity of 91% and 97% in breath and faecal samples, respectively, and a specificity of 99% for both sample types.[21] In comparison, the sensitivity of the haemoccult test ranges from 25-44%.[22-24] Only one sizeable study for the detection of colorectal carcinoma using an Enose has been performed. The Enose was able to discriminate breath samples of patients with colorectal carcinoma (n= 26) from samples of healthy controls (n= 22) by means of characteristic VOC patterns, but a diagnostic accuracy analysis was not included in this work.[25]

Melanoma

After the first anecdotal report of a dog detecting melanoma,[2] a study using a dog as a diagnostic tool for this type of cancer was performed. This was the first study in which dogs were trained to sniff actual patients in the clinic, rather than a sample of patient material (e.g. faeces, urine, breath, etc.). Melanoma samples were hidden in bandages on volunteers and the dogs were correct in their assessment in 75-86% of the cases.[26] Three years later, an Enose study addressed the ability to detect melanoma in tissue samples (n=57), and found a sensitivity and specificity of 70% and 90%, respectively.[27]

Infections

The odour of infectious diseases has fascinated mankind for many years. For example, the typical smell of gas gangrene, a severe skin and soft tissue infection caused by *Clostridium perfringens,* was described as early as in the Middle Ages.[28] Throughout history, infectious diseases have played a major role in battles and wars. In both the First and Second World War, many soldiers suffered from gas gangrene, to which 50% succumbed. Since no other diagnostic tools were available, physicians solely relied on their senses, particularly smell.

Bedside diagnosis by smelling is still applied. For example, wound infections caused by *Pseudomonas aeruginosa* are characterised in textbooks and by clinicians as having a 'fruity' odour, and bacterial vaginosis has its distinctive 'fishy' smell. In recent years, studies have attempted to assess the superior smelling characteristics of animals, and newly developed scent detection tools have made earlier recognition of specific infectious diseases possible. *Table 2* shows the characteristics of the key studies.

Pulmonary infections

The ancient Greeks and Chinese had an interesting method of detecting *Mycobacterium tuberculosis*. The doctor set fire to the patient's sputum and diagnosed tuberculosis by recognising the specific smell in the fumes.[29] Nowadays, sputum is examined under a microscope (e.g. with an acid-fast stain), but this method has only limited sensitivity. Polymerase chain reaction is more sensitive, but also more expensive. Culturing is a sensitive method of detecting tuberculosis, bur generally takes at least three weeks.[30] Could scent detection offer a solution?

After an interesting study on rats being able to detect landmines,[31] the same research group studied the accuracy of trained rats for detecting tuberculosis. It turned out that rats can detect these bacteria in sputum samples with an accuracy of 74% and process 1680 samples a day, whereas a lab clinician has a limited capacity of 40 samples a day.[32] A more recent study on rats detecting TB showed a sensitivity of 68% and a specificity of 87%.[33] Bees may be able to detect tuberculosis as well.[34]

A study using Enoses suggested that *M. tuberculosis* can be detected in sputum with an accuracy of 85%.[35] *P. aeruginosa* can be detected in exhaled breath by Enose with a sensitivity exceeding 90% and a specificity of 88%.[36]

Table 2. *Characteristics of key scent detection studies by human, animal, and electronic nose (Enose) for different infectious diseases*

Infection	Type of nose	Type of sample	Sensitivity / Specificity (95%CI when available) or Success rate	Sample size (diseased/ healthy)
Mycobacterium tuberculosis[32]	Rat	Sputum	80% / 72%	28 / 111
Mycobacterium tuberculosis[33]	Rat	Sputum	68% / 87%	162 / 748
Mycobacterium tuberculosis[35]	Enose	Breath	84% / 65%	65 / 161
Pseudomonas aeruginosa[36]	Enose	Breath	90% / 88%	32 / 40
Rotavirus[37]	Human	Faeces	38% / 88%	26 / 42
Clostridium difficile[38]	Human	Faeces	55% (33-77%) / 83% (76-90%)	37 / 81
Clostridium difficile[39]	Dog	Faeces and hospitalised patients	Faeces: 100% / 100 % (91-100%) Patients: 83% (65-94%) /97% (95-99%)	Faeces: 50 / 50 Patients: 30 / 270
Clostridium difficile[40]	Enose	Faeces	95% success rate	22 / 30

We found no studies addressing scent detection in other types of pulmonary infection, particularly not for common pathogens such as *S. pneumoniae*.

Intestinal infections

In 1987, the human nose was tested in distinguishing diarrhoea caused by rotavirus infection from diarrhoea caused by other organisms (i.e. adenovirus, *E. coli, Campylobacter,* or no isolated organism). Nurses were asked to classify stool samples by smell. Specificity was good (88%), but sensitivity was very low (38%).[37]

Clostridium difficile infections (CDI) are a common cause of diarrhoea in hospitals and other healthcare facilities. Humans are able to recognise *C. difficile* diarrhoea by its smell. Trained nurses reach a sensitivity and specificity of 55% and 83%, respectively.[38] Recently, a dog proved capable of detecting *C. difficile* both in faecal samples and at the patients' bedside on hospital wards. Sensitivity and specificity for stool samples were 100% and 94-100%, respectively. Sensitivity and specificity for identifying CDI patients on the hospital ward were 83-93% and 97-98%, respectively.[39]

When tested by Enose, faeces of CDI patients has a significantly different VOC pattern from faeces of asymptomatic volunteers, patients with *Campylobacter jejuni* infection, and patients with ulcerative colitis.[40] Furthermore, the Enose is able to discriminate between different aerobic bacteria such as *Helicobacter pylori, Escherichia coli,* and *Enterococcus* species on the basis of differences in volatile compounds.[41]

Metabolic and other diseases

Normal human metabolism generates countless VOCs that can generate a specific odour. Pathological processes influence the VOC composition by producing different VOCs, or by metabolic consumption of VOC substrates that are normally present. Notorious examples include the smell of acetone on the breath of patients with diabetic ketoacidosis and the 'musty' smelling breath of patients with hepatic encephalopathy.

There are several rare metabolic diseases that are accompanied by such a distinct smell that they owe their name to it; e.g. trimethylaminuria (also known as 'fish odour disease') is due to abnormal excretion of trimethylamine in breath, urine, sweat, saliva and vaginal secretions. The odour consists of sulphur and nitrogen compounds (amines) and resembles the smell of decaying fish. Another example is maple syrup urine disease, or MSUD. It is caused by a deficient enzyme, branched-chain alpha-keto acid dehydrogenase. Patients have been reported to smell like caramel, maple syrup, or to spread a 'malty' odour.

Although no formal diagnostic studies have been done, there are case reports that suggest that dogs are able to detect hypoglycaemia. In these cases, the dog acts in a stereotypical way to alarm the handler before he or she suffers from hypoglycaemic symptoms. It is unclear what triggers the dog's reaction, but the detection of specific VOCs has been proposed as the most plausible explanation.[42] A similar phenomenon was described in the 1980s when a woman with epilepsy reported that her dog could predict her seizures. Since then, there has been great interest in 'seizure dogs', but their reliability remains unknown due to the lack of formal studies. Seizure alert dog owners have reported improvements in seizure rates which they attributed to their dogs.[43,44]

Table 3 shows the characteristics of scent detection studies by Enoses in the group of metabolic and other diseases. No studies were found testing humans or animals.

Metabolic diseases

Enoses have found a significantly different VOC pattern in breath from people with diabetes and healthy controls (sensitivity 90%, specificity 92%).[45] Besides that, a breath marker for oxidative stress has been described that could potentially identify diabetic patients at increased risk for complications.[46]

The characteristic smell of patients with liver failure, fetor hepaticus, is caused by increased levels of sulphur-

Table 3. *Characteristics of key scent detection studies by electronic nose (Enose) in metabolic – and other diseases*

Disease	Type of nose	Type of sample	Sensitivity / Specificity	Sample size (diseased/healthy)
Diabetes[45]	Enose	Breath	90% / 92%	21 / 26
Liver cirrhosis[48]	Enose	Breath	100% / 70%	52 / 50
Asthma[49]	Enose	Breath	-	20 / 20
Asthma & COPD[50]	Enose	Breath	85% / 90%	60 / 40
COPD[51]	Enose	Breath	-	12 / 16

containing compounds.[47] Breath analyses by Enose reportedly discriminate patients with liver cirrhosis from healthy individuals with a sensitivity of 100% and specificity of 70% (n=102).[48]

Other diseases

Both asthma and COPD are common respiratory diseases characterised by airway obstruction. Patients can be differentiated from each other and from healthy controls by breath analysis using Enose.[49,50] Moreover, a recent study in COPD patients suggested that different stages of disease severity can also be identified by Enose.[51]

Finally, breath analysis by Enose has reportedly been able to recognise schizophrenia, in which pentane and carbon sulphide seem to be increased.[52]

CONCLUSION AND DISCUSSION

Physicians have always used their sense of smell as a diagnostic tool, be it for wound infections on the battle field or the patient with diabetic ketoacidosis in the emergency room. The human nose is still a valuable instrument in times when bedside diagnostic skills are losing ground to modern analytical techniques. The ability of humans to diagnose disease by smelling has only very rarely been the subject of quantitative studies. Still, our senses come free of charge, and are among the most readily available diagnostic tools we have. As over years of practice we become experienced clinicians, we literally develop 'a nose' for the medical profession.

The smelling ability of animals holds promise as a detection tool. The studies reviewed here suggest that animals are often as accurate as or even superior to standard diagnostic methods. For example, trained rats are at least as sensitive as the conventional Ziehl-Neelsen stain for detecting *M. tuberculosis* in sputum; moreover, they are able to process over 40 times more samples per day than a lab clinician.[32]

The potential of animals appears to be underestimated, understudied and, consequently, underused in the medical field. Several studies discussed in this review show promising and sometimes even spectacular results. In the six cancer studies with dogs reviewed here, for example, median sensitivity and specificity were 94% and 98%, respectively. Although no direct comparison studies have been performed, dogs appear to outperform Enoses, since median sensitivity and specificity of the Enoses in the seven cancer studies was only 75% and 92%, respectively. It is surprising and unfortunate that independent follow-up studies are generally lacking. One of the explanations could be that the use of animals in healthcare is unconventional and physicians might consider it to be unhygienic. Also, each animal needs special training, which requires specific expertise and can be time-consuming. For instance, the training of detection dogs can take months before they are ready for practice; rats on the other hand can be trained very quickly.[31] After this training phase, animals need individual performance assessment, and regular practice to maintain their skills.

Enose studies have mainly focused on lung diseases and malignancies such as ovarian, bladder, and lung cancer. The overall sensitivity and specificity of Enoses is high in the published studies, but again few confirmation studies are available. Enoses are not widely implemented in daily practice. There are many types of Enoses with a large variety of underlying techniques; results from one type of Enose are not (easily) generalisable to another. Also, Enoses are relatively expensive, but they could prove cost-effective in the long-term.

It remains to be seen, however, if Enoses will ever be able to match the smelling capacity of animals. Dogs, for example, require an average VOC concentration of less than 0.001 part per million.[53] Enoses on the other hand have a detection threshold of 5 to 0.1 parts per million (ppm),[54] although like animals different types of Enoses have different affinity for different volatiles. In comparison, humans have a detection threshold, on average, ranging from 0 to 80 ppm, again depending on of the type of substance. For example, ammonia can not be perceived by humans until it reaches 50 ppm.[55] Taken together, many animals smell up to 100 times better than humans and Enoses, and it may well be worth making appropriate use of this superior technology.[56]

Lately, the main focus of scent detection studies has been on pulmonary diseases (COPD, asthma and lung cancer). For other malignancies, such as colorectal cancer, imperfect (faecal occult blood) or invasive (colonoscopy) screening methods are currently used. Scent detection by animals or Enose could be of considerable value here. Diagnosis of several infectious diseases including tuberculosis could be improved by rapid and accurate animal-assisted screening, particularly in low-resource settings. Scent surveillance by animals or Enoses for transmissible diseases such as *Clostridium difficile*

infections could prevent and contain outbreaks. What are mainly needed are confirmatory studies, as the collective literature, although promising and occasionally spectacular, mainly consists of isolated studies. In conclusion, scent detection holds promise for the future and should receive higher priority in terms of research effort and funding.

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APPENDIX I:

Search performed on scent detection by animals & human and electronic noses (March 2012)

PUBMED – SEARCH STRATEGY

Period: 1966 to March 2012

Animals & Human

("Dogs"[Mesh] OR Dog*[tiab] OR canine*[tiab] OR detection dog*[tiab] OR sniffer dog*[tiab] OR "Nurses"[Mesh] OR nurse*[tiab] OR "Physicians"[Mesh] OR Physician*[tiab] OR "Humans"[Mesh] OR Human*[tiab] OR animal*[tiab] OR "Animals"[Mesh])

AND

("Carcinoma"[Mesh] OR carcinoma*[tiab] OR "Disease"[Mesh] OR disease*[tiab] OR "Infection"[Mesh] OR infection*[tiab])

AND

("Smell"[Mesh] OR smell*[tiab] OR "Odors"[Mesh] OR odor*[tiab] OR "Pheromones"[Mesh] OR pheromone*[tiab] OR "Volatile Organic Compounds"[Mesh] OR volatile organic compound*[tiab] OR volatile*[tiab])

AND

(scent detection[tiab] OR olfactory detection[tiab] OR detection*[tiab])

Enoses

(electronic nose*[tiab] OR bioelectronic nose*[tiab] OR substance class specific sensor*[tiab] OR infrared spectroscop*[tiab] OR infrared spectrometr*[tiab] OR "Spectrophotometry, Infrared"[Mesh] OR gas chromatograph*[tiab] OR "Chromatography, Gas"[Mesh] OR mass spectrometr*[tiab] OR ion mobility spectrometr*[tiab] OR "Mass Spectrometry"[Mesh] OR optical sensor*[tiab])

AND

("Volatile Organic Compounds/analysis"[Mesh] OR volatil*[tiab]))

AND

(humans[mesh] OR human[tiab] OR humans[tiab])

EMBASE – SEARCH STRATEGY

Period: 1980 to March 2012

Animals & Humans

('detection dog':ab,ti OR 'sniffer dog':ab,ti OR 'dog'/de OR canine*:ab,ti OR 'animal'/de OR 'physician'/exp OR physician*:ab,ti OR 'nurse'/exp OR nurse*:ab,ti OR 'rat'/exp OR rat*:ab,ti OR dog*:ab,ti OR animal*:ab,ti OR 'human'/exp OR human*:ab,ti)

AND

('scent detection':ab,ti OR 'olfactory detection':ab,ti OR detection*:ab,ti)

AND

(scent:ab,ti OR 'odor'/de OR odor*:ab,ti OR 'pheromone'/de OR pheromone:ab,ti OR smell:ab,ti OR 'volatile organic compound'/de OR volatile organic compound*:ab,ti)

AND

('carcinoma'/exp OR 'diseases'/exp OR 'infection'/exp OR carcinoma*:ab,ti OR infection*:ab,ti OR disease*:ab,ti)

Enoses

((electronic NEAR/3 nose*):ab,ti OR (bioelectronic NEAR/3 nose*):ab,ti
OR (('infrared spectroscopy'/de OR (infrared NEAR/3 spectroscop*):ab,ti OR ('infra red':ab,ti AND spectroscop*:ab,ti) OR 'infrared spectrometry'/de OR 'infrared spectrophotometry'/de OR (infrared NEAR/3 photospectroscop*):ab,ti OR (infrared NEAR/3 spectrophotometr*):ab,ti OR (infrared NEAR/3 spectrometr*):ab,ti OR (infrared NEAR/3 photospectrometr*):ab,ti OR 'gas chromatography'/exp OR (gas NEAR/3 chromatograph*):ab,ti OR 'mass spectrometry'/exp OR (mass NEAR/3 spectrometr*):ab,ti OR 'ion mobility spectrometry'/de OR (ion:ab,ti AND (mobility NEAR/3 spectrometr*):ab,ti) OR (optical NEAR/3 sensor*):ab,ti)
AND
('volatile organic compound'/exp OR volatil*:ab,ti))).
AND
('human'/exp OR human*:ab,ti)

WEB OF SCIENCE – SEARCH STRATEGY

Period: 1988 to March 2012
Animals & Human
(Carcinoma* OR Infection* OR Disease*)
AND
(Olfactory detection OR scent detection)
AND
(Sniffer dog OR detection dog OR dog* OR canine* OR human* OR physician* OR nurse*)
AND
(Scent* OR smell* OR odor* OR pheromone* OR volatile organic compound*)

Enoses

(((Infrared near/3 spectroscop* OR infra red AND spectroscop*)
OR (Infrared near/3 spectrophotomet* OR infra red AND spectrophotomet*) OR (infrared near/3 spectromet* OR infra red AND spectromet*) OR (gas near/3 chromotograph* OR mass near/3 spectromet*) OR (mobility near/3 spectromet* OR optical near/3 sensor*) OR (bioelectronic near/3 nose* OR electronic near/3 nose*)
AND (volatile*)) AND (human*))
OR
(((Infrared near/3 spectroscop* OR infra red AND spectroscop*)
OR (Infrared near/3 spectrophotomet* OR infra red AND spectrophotomet*) OR (infrared near/3 spectromet* OR infra red AND spectromet*) OR (gas near/3 chromotograph* OR mass near/3 spectromet*) OR (mobility near/3 spectromet* OR optical near/3 sensor*) OR (bioelectronic near/3 nose* OR electronic near/3 nose*)
AND volatile*) AND (disease*))
OR
(((Infrared near/3 spectroscop* OR infra red AND spectroscop*)
OR (Infrared near/3 spectrophotomet* OR infra red AND spectrophotomet*) OR (infrared near/3 spectromet* OR infra red AND spectromet*) OR (gas near/3 chromotograph* OR mass near/3 spectromet*) OR (mobility near/3 spectromet* OR optical near/3 sensor*) OR (bioelectronic near/3 nose* OR electronic near/3 nose*)
AND volatile*) AND (cancer* OR onco* OR respirator* OR pathol*))

Netherlands
The Journal of Medicine

ORIGINAL ARTICLE

Routine duodenal biopsy to screen for coeliac disease is not effective

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ABSTRACT

Background: Routine duodenal biopsies during upper gastrointestinal endoscopy (UGE) have been suggested to be useful in detecting coeliac disease (CD). However results from previous studies are not conclusive. The aim of this study is to investigate the diagnostic yield and cost-effectiveness of routine duodenal biopsy during UGE.
Methods: In this retrospective single-centre study, we studied 6442 patients undergoing first-time UGE at the Rijnstate Hospital, Arnhem, the Netherlands, from January 2009 to December 2010. All UGE reports were analysed for indication, duodenal intubation, and endoscopic aspect of duodenal mucosa. Endomysium and tissue transglutaminase antibody titre, when present, were scored as positive or negative. CD was defined as Marsh 3a or higher. Costs of duodenal biopsies and pathology analysis were calculated. Comparisons were done with T-tests for continuous data and Chi-square tests for categorical data.
Results: Forty-one patients had newly diagnosed CD; 34 of these 41 patients had definite indications for biopsy prior to UGE, e.g. positive serology or symptoms. Thus, routine duodenal biopsies identified seven patients as having CD, who otherwise would not have been biopsied. The number needed to biopsy was therefore 577, spending more than € 30,000 per case.
Conclusions: We do not recommend routine duodenal biopsy to screen for coeliac disease because of the high number needed to biopsy as well as high costs.

KEYWORDS

Coeliac disease, duodenal biopsy, screening

INTRODUCTION

Coeliac disease (CD) is defined as a permanent intolerance to gluten. In genetically susceptible individuals, the ingestion of gluten initiates a specific T-cell driven immune response that ultimately leads to gluten-sensitive enteropathy, which resolves with elimination of gluten from the diet.[1,2]
Small bowel histopathology according to the Marsh classification, consisting of lymphocytic enteritis, hyperplasia of crypts and atrophy of the villi, remains the gold standard in the diagnosis of CD, at least in adults.[3,4] The diagnosis may be supported and for that matter in the future even (partially) replaced by testing for the presence of coeliac-specific autoantibodies (endomysium and tissue transglutaminase) and immunogenetic markers (HLA DQ 2 and/or 8).[5] This strategy is already accepted in recent paediatric guidelines.[6]
Large screening studies in Western countries, based on serological markers, indicate that up to 1 in 100 people are affected.[7,8] Beginning in Europe and expanding throughout the world, studies systematically show great discrepancies between screening prevalence and actual prevalence of the disease.[9] Already in the 1990s, the concept of the coeliac iceberg was introduced, referring to the large majority of CD patients that remain unrecognised 'underneath the surface'.[10-12] Due to improved knowledge, scientific research and education of healthcare workers, the awareness for diagnosing CD has improved. Coeliac disease is now a more common disease throughout the world.[7,8] Incidence rates have been rising since, but still the largest part of the iceberg remains to be brought to the surface.
Although the burden of asymptomatic CD is rather unknown, population screening has even been suggested.[13,14] As CD may present with a diffuse spectrum of (mild) symptoms, it has been suggested that routine duodenal biopsies taken at upper gastrointestinal

endoscopy (UGE) for various, non-specific indications such as iron deficiency anaemia, dyspepsia, (upper) abdominal symptoms and in patients who are known to have any other autoimmune disease, may help to identify un-recognised CD patients. Several studies have addressed this issue, but conclusions run both ways, claiming routine biopsies as either useful[15-18] or not effective.[19,20]

To our knowledge no studies have evaluated the cost-effectiveness of this procedure.

In the endoscopic detection of CD a variety of features are described: reduced or absent folds, scalloping of folds, mosaic pattern of the mucosa and mucosal fissures or cracks.[21-24] However, the sensitivity for detecting villous atrophy during standard UGE on endoscopic interpretation alone is poor (59%), in part because partial villous atrophy may elude visual detection.[25]

The aim of this study is to investigate the diagnostic yield and cost-effectiveness of routine duodenal biopsy during UGE in the identification of CD.

METHODS

Study design and patient population

In this retrospective single-centre study, we studied all patients undergoing UGE at the Rijnstate Hospital, Arnhem, the Netherlands, from January 2009 to December 2010. All endoscopies were performed by one of seven gastroenterology staff members or three gastroenterology residents. The majority of the procedures were performed with, at the time of the investigation, the latest selection of endoscopes on the market (GIF-Q180, GIF-H180 endoscopes on CV-180 Excera II processors Olympus Medical Systems Corp, Tokyo, Japan). The GIF-H180 endoscope gives the opportunity of real-time high-definition images. Depending on the indication, some endoscopies were performed with intervention-type endoscopes such as the GIF-1TQ160 endoscope on a CV-160 Excera processor (Olympus Medical Systems Corp, Tokyo, Japan). The Rijnstate Hospital has a long interest in CD research and for most endoscopists routine duodenal biopsies are the standard of care at first UGE. Approval of the medical ethics committee was therefore not necessary. All patient identifiers were coded and could not be traced back to the patient.

Data collection

All UGE study reports from the endoscopy database, Endobase (Olympus Medical Systems Corp, Tokyo, Japan), containing full report text, age and sex, in the study period were entered into the research database. Second, the pathology reports from duodenal biopsies, when present, were retrieved from the pathology database and matched with the UGE study reports. Then, the endomysium and tissue transglutaminase antibody titres, when present, were retrieved from the laboratory database and matched with the data in the research database. For patients with signs of villous atrophy in pathology specimens or CD-specific antibodies, as well as for patients either with clinical suspicion for CD or in follow-up, haemoglobin levels in the period surrounding biopsy, ± 3 months, were retrieved from the laboratory database.

Only one study report per patient was taken into analysis. This was either the first investigation, or the first investigation with a report of duodenal biopsy during the study period.

Data analysis

All UGE reports were analysed for indication, duodenal intubation, and endoscopic aspect of duodenal mucosa.

Next, all duodenal biopsy pathology reports were analysed for mentioning any signs of villous atrophy. CD was defined as Marsh 3a or higher. All endomysium and tissue transglutaminase antibody titres, when present, were scored as positive or negative. Anaemia was diagnosed when decreased haemoglobin levels were found, according to local reference values. Costs of duodenal biopsies and pathology analysis were calculated as if they were actually billed.

Comparisons were done with T-tests for continuous data and Chi-square tests for categorical data. Statistical analyses were performed using SPSS 20 for Mac (SPSS inc, Chicago, Illinois, USA)

RESULTS

During the study period 8350 UGE were performed in 6442 patients; 4085 patients had duodenal biopsies analysed at our pathology lab (*figure 1*).

Figure 1. *Upper gastrointestinal endoscopies (UGE) during study period 2009-2010*

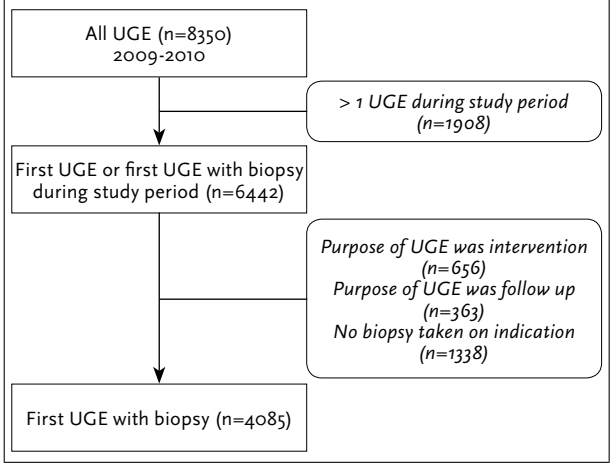

Table 1. *Patient characteristics and indications of UGE**

	Biopsy (n=4085)		No biopsy (n=2357)		P
Characteristic	Mean	SD	Mean	SD	
Age	54.6	18.5	62.4	17.4	<0.001
Sex	N	%	N	%	
Male	1705	41.7	1244	52.8	
Female	2380	58.3	1113	47.2	
Indication	N	%	N	%	
Anaemia	454	11.1	158	6.7	<0.001
Diarrhoea	225	5.5	36	1.5	<0.001
Weight loss	244	6.0	54	2.3	<0.001
Dyspepsia	1421	34.8	358	15.2	<0.001
Clinical suspicion of coeliac disease	133	3.3	6	0.3	<0.001
Coeliac disease follow-up	81	2.0	6	0.3	<0.001
Lactose intolerance	2	0.04	2	0.1	0.577
Other	1605	39.3	831	35.3	0.001
Intervention endoscopy	109	2.7	656	27.8	<0.001
Follow-up endoscopy	0		363	15.4	<0.001

***More than one indication possible. Comparison is based on T-tests for continuous variables and Chi-square tests for categorical variables. UGE = upper gastrointestinal endoscopy.**

Table 2. *Diagnosis of coeliac disease (number of UGE, n=4085)**

Indication	UGE with biopsy	CD n (%)
Anaemia	454	0
Diarrhoea	225	3 (1.3)
Weight loss	244	2 (0.82)
Dyspepsia	1421	2 (0.14)
Clinical suspicion of coeliac disease	133	31 (23.3)
Coeliac follow-up	82	10 (12.2)
Lactose intolerance	2	0
Other	1605	3 (0.19) 2 heartburn 1 dysphagia

***More than one indication possible, the diagnosis of coeliac disease has only been attributed to one indication.**

Table 3. *The diagnostic yield of coeliac disease (CD) specific serology* (number of patients, n=4085)*

Serology*	
Positive	61
CD follow-up	19
Suspected CD	42
Villous atrophy in patients with suspected CD and positive serology	
Marsh 3A or higher	35
Marsh 2	2
Marsh 0	5

***Tissue transglutaminase and/or endomysium antibodies.**

Table 4. *Characteristics of new coeliac disease (CD) patients (n=41)*

Characteristic		SD
Age*	37.9	27.6
Sex		
Male	14	
Female	27	
Indication	n	%
Anaemia (no other signs of CD)	0	0
Diarrhoea (no other signs of CD)	3	7.3
Weight loss	2	4.9
Dyspepsia	2	4.9
Clinical suspicion of coeliac disease i.e. positive serology† or typical symptoms‡	31	75.6
Heartburn	2	4.9
Dysphagia	1	2.4
Total	41	100
Serology*		
Positive	35	85.4
Negative	3	7.3
No serology	3	7.3
Total	41	100
Anaemia at diagnosis§	11	26.8

***Age in years. †Tissue transglutaminase and/or endomysium antibodies. ‡Diarrhoea, steatorrhoea, abdominal complaints, weight loss etc. §Anaemia according to age and sex adjusted levels.**

The patient characteristics and indications for UGE are summarised in *table 1*. Pathology results revealed histological abnormalities graded as Marsh 3a or higher, i.e. compatible with CD, in 51 patients: this is 1.25% of all UGE with duodenal biopsy. Forty-one patients (1.00%) were 'newly' diagnosed, ten patients were in follow-up. The number of diagnoses of CD per indication are summarised in *table 2*. CD-specific serology was positive in 61 patients, 42 patients without prior history of CD and 19 patients in follow-up for CD. Out of 41 newly diagnosed CD patients, 26 had positive serology prior to the endoscopy. Thirty-five out of 42 had duodenal biopsy specimens revealing Marsh 3a or higher and thus 7/42 patients with CD antibodies had no CD-related enteropathy (*table 3*). CD-specific serology was positive in 35 of 41 newly diagnosed CD patients, 3 out of 41 had no serology tests performed, 3 were seronegative (*table 4*). Anaemia was present in 11 out of 41 newly diagnosed CD patients (*table 4*) and 6 out of 10 known CD patients with follow-up biopsy still graded as Marsh 3a or higher, of which one patient was diagnosed with enteropathy associated T-cell lymphoma.

Endoscopic abnormalities of the duodenal mucosa were seen in a total of 305 patients. CD-specific abnormalities, i.e. indicators of atrophy, were seen in 96 patients. The endoscopic abnormalities observed during UGE and their predictive values are summarised in *table 5*.

Table 5. *Abnormalities of the duodenal mucosa and the predictive value for coeliac disease (CD)* (number of patients, n=4085)*

	Frequency of endoscopic abnormality N	Incidence of CD in patients with endoscopic abnormality n(%)
Absent folds	10	7 (70.0)
Reduced folds	31	16 (51.6)
Mosaic pattern	20	2 (10.0)
Crackles or fissures	8	6 (75.0)
Scalloping	21	0
Abnormal appearance of villi	90	1 (1.1)
Vascular [lesions]	31	0
Other non-specific abnormalities	209	0

***More than one abnormality possible.**

Table 6. *Cost analysis and number needed to biopsy on UGE per indication (number of patients, n=4085)*

Indication	NNTB	Costs/diagnosis*
Anaemia (no other signs of CD)	Unlimited	Unlimited
Diarrhoea (no other signs of CD)	75	€4719.75
Weight loss	122	€7677.46
Dyspepsia	710.5	€44,711.77
Clinical suspicion of coeliac disease i.e. positive serology or symptom complex	4.3	€269.99
Other	535	€33,667.55

***Only standard pathology analysis charges and biopsy costs of €62.93 calculated. In case of abnormalities additional costs may be charged. UGE = upper gastrointestinal endoscopy; NNTB = number needed to biopsy; CD= coeliac disease.**

No complications due to duodenal biopsies occurred during the study period, according to the complication records.

The costs of performing routine duodenal biopsies, on first endoscopy, in our setting largely depend on the need for biopsies of other tissue(s) during the same procedure. Pathology labs can only bill one specific analysis per application. The costs, categorised per indication and calculated as if no other biopsies were taken, are shown in *table 6*. In our setting, as described before, the additional costs were negligible as over 99% of patients with duodenal biopsies had other tissue(s) (gastric antrum and/or corpus) biopsied as well.

DISCUSSION

Over the last decades several studies have addressed the efficacy of routine duodenal biopsy during UGE in different subgroups, populations and in different indications with contradicting outcomes.[10-13] Furthermore, to our knowledge none of these studies addressed the cost-effectiveness of this procedure.

We retrospectively studied the outcome of routine duodenal biopsy during UGE in a large Dutch hospital with a long history of specific interest in CD research. Out of 6442 endoscopies performed in patients, 4085 had duodenal biopsies taken during UGE. The remaining 2357 patients had no biopsy because of intervention, another explanation for the symptoms or follow-up.

CD was newly diagnosed in 41 out of 4085 patients (1.00%). However, 26 of the 41 newly diagnosed patients had positive serology before UGE, 5 patients had typical symptoms for CD and 3 patients had diarrhoea, all definite indications for biopsy. Thus, routine duodenal biopsies identified 7 patients to have CD, who otherwise would not have been biopsied. This implicates that over 577 patients needed to be biopsied in order to find one CD patient. Compared with previously published studies the incidence of newly diagnosed CD in this study is slightly lower.[10-13] A possible explanation for this can be a higher prevalence of detected CD and a lower prevalence of non-detected CD in our study population. This might be explained by the hospitals specific interest and thus higher prevalence of detected CD (data not published) and could possibly lead to a shift towards lesser symptomatology in the population of non-detected individuals with CD. Another explanation for the lower incidence compared with other studies is the pathology diagnosis of CD, in this study defined as Marsh 3a or higher. Other studies diagnosed less progressed lesions as CD, where in another study the criteria of diagnosis were not specified.[16,18,20]

The predictive value of CD-specific serology (endomysium and tissue transglutaminase antibodies) found in this study is lower than described in the literature.[26-28] The tissue transglutaminase antibodies test used in our hospital is manufactured by Phadia AB, Uppsala, Sweden, and commercially available. All patients with positive serology and negative biopsies had on average over six biopsy samples. Anti-tissue transglutaminase antibody levels were on average only five times the upper limit of normal, a possible explanation for false positivity.

Endoscopic abnormalities of duodenal mucosa more or less specific for the diagnosis of CD can be a guide towards diagnosis, though the sensitivity remains insufficient to rely on. An earlier study reported a sensitivity of 59% for the detection of villous atrophy during standard endoscopy.[25] Endoscopic features for detecting villous atrophy had predictive values between 51-75% in our study, except for the mosaic pattern of the mucosa with a value of 10%.

Payments for specific procedures, in this case duodenal biopsy, can vary per country, region and even insurance company. Local settings and payment agreements, as well as the prevalence of the disease in the hospital's target population, can also attribute to the cost-effectiveness or ineffectiveness. We tried to estimate the 'fictive' costs of routine duodenal biopsies, as if all pathology analyses had actually been billed, in our setting which is largely comparable to other larger hospitals in the Netherlands. The large majority of CD patients will be identified by taking biopsies on indications as positive serology, anaemia, chronic diarrhoea, family history and other autoimmune diseases and endoscopic features that correlate with CD. For the remaining small group, taking biopsies in over 577 individuals and spending more than € 30,000 to identify one patient, seems at least disputable. Besides CD, other diagnoses such as Crohn's disease, Giardiasis and Whipple's disease can arise from duodenal biopsies. However, these conditions, due to their symptomatology and in the case of Crohn's disease endoscopic features, were not found in the group of patients without specific symptomatology. The question is when a diagnosis of CD will be cost-effective. Except for two North American studies that cannot simply be extrapolated to our setting, to our knowledge no literature is available on this topic.[29,30] Furthermore the benefit of being diagnosed on both quality of life and general health needs to be taken into consideration.

Therefore we conclude that, although duodenal biopsies seem a safe screening tool, random biopsies cannot be propagated in general during UGE.

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CASE REPORT

Disappearance of epilepsy after resection of catecholamine secreting extra-adrenal paragangliomas: a case report

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ABSTRACT

Epileptic seizures have been associated with increased catecholamine levels, however, direct proof is lacking. We report a case with catecholamine secreting extra-adrenal paragangliomas and a continuous state of epilepsy not responding to therapy. The epileptic seizures resolved after resection of the paragangliomas and normalization of catecholamine excretion.

KEYWORDS

Paraganglioma, epilepsy, noradrenaline

INTRODUCTION

Epilepsy is a disease with a broad array of causes.[1] The association of seizures and catecholamine-producing paraganglioma is rare, mainly described in paediatric cases and few adult cases.[2-5]

We report a patient with catecholamine-secreting extra-adrenal paragangliomas and a continuous state of epilepsy, not responding to therapy. The epileptic seizures resolved after resection of the paragangliomas and normalisation of catecholamine excretion. Hypertensive encephalopathy was not considered to play a causative role as blood pressure levels were well measured and overall in the normal range. The patient was able to discontinue his anticonvulsant therapy. Epileptic seizures and paraganglioma have been associated before, but this is the first case report showing a direct association with increased catecholamine levels.

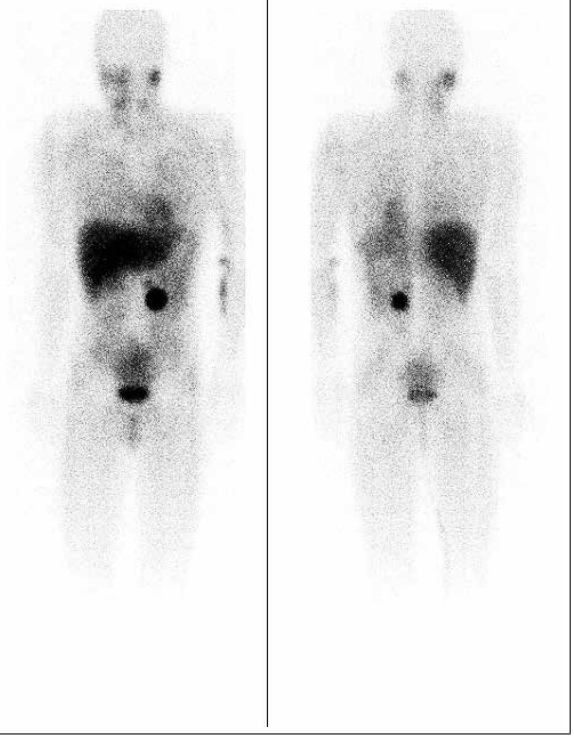
CASE REPORT

A 32-year-old male with an unremarkable medical history presented to the emergency department after an episode of aphasia, altered consciousness and confusion. He had recently experienced mild headaches. He was not using any medication or drugs. Physical examination at admission revealed no abnormalities; his blood pressure was 165/72 mmHg. After determination of this blood pressure, fundoscopy for papilloedema was not performed. He deteriorated, became aphasic and lost comprehension. Laboratory tests only revealed a slightly elevated white blood cell count of 10.2 x 10^9/l. A subsequently performed CT cerebrum showed no abnormalities, while lumbar puncture revealed an elevated lymphocyte count of 304/field. Under suspicion of viral encephalitis he was admitted to the neurology department and treated with broad-spectrum antibiotics and acyclovir. Elaborate tests for viral and bacterial infectious agents (cultures of blood and cerebrospinal fluid included) and autoimmune disease were negative. Electroencephalogram (EEG) showed complex partial seizures originating from the frontotemporal lobe on the left side. Despite anticonvulsant therapy he deteriorated again, with recurrence of the above-described symptoms. Repeated EEG showed frequent steep rhythmic delta activity in the left frontotemporal region with bradycardia: the episodes were due to complex, partial epileptic seizures. Subsequent MRI showed enhanced uptake of gadolinium in the frontal part of the meninges. Despite quadruple anticonvulsant therapy, the epilepsy was not adequately controlled, so he was transferred to the ICU for sedation and ventilation. Blood pressure levels during admission in the ICU in a sedated setting fluctuated within an

overall normal range. Maximum systolic pressure was 160 mmHg with a highest mean arterial pressure of 104 mmHg; these peak levels occurred in a short time interval. His therapy-resistant epilepsy was thought to be atypical and possibly due to paraneoplastic or limbic encephalitis. Therefore a CT scan of the head and neck, thorax, abdomen and pelvis was performed and showed three vascular lesions in the neck and two in the abdomen, both para-aortic. Twenty-four hour urinary excretion of norepinephrine (NE) and epinephrine collected in a sedated and ventilated setting was increased at 1.04 µmol/24 h (reference 0.04-0.47) and 0.19 µmol/24 h (reference 0-0.16), respectively; urinary excretion of metanephrines was normal. 123I-MIBG imaging showed increased uptake in the two abdominal extra-adrenal lesions *(figure 1)*. These hypervascular MIBG positive lesions were suggestive of multifocal paraganglioma. The patient was referred to the university hospital for laparoscopic removal of the two abdominal extra-adrenal paragangliomas, after preoperative alpha and beta blockade and intravenous hydration.

Removal was uneventful. Histology showed two localisations of a paraganglioma. Genetic testing showed an SDHD (Asp92Tyr) mutation on chromosome 11; SDHB immunostaining of paraganglioma tissue was compatible with the SDHD mutation. After surgery, NE excretion normalised and the anticonvulsant therapy could be discontinued. Almost two years after surgery the patient has not experienced any further epileptic activity.

Figure 1. *MIBG scan showing active paragangliomas*



This case shows a continuous state of epilepsy not responding to therapy in a patient with extra-adrenal catecholamine secreting paraganglioma. The epileptic seizures resolved after resection of the secreting paragangliomas and normalisation of catecholamine excretion. Since the head and neck paragangliomas were not resected, it was hypothesised that the increased level of catecholamines, especially NE, was associated with the seizures. Contradictory to this, it is well documented that the neurotransmitter NE has anticonvulsant properties.[6] Anticonvulsant drugs either deactivate neurons through the inhibitory neurotransmitter GABA or decrease the NE level in cerebrospinal fluid.[2,7]

However, NE also has proconvulsant properties under some conditions.[2] NE has an 'activating' role in the brain and can produce neural activity that may induce epilepsy, as reflected by the increased incidence of epilepsy after stressors.[8] The mechanism of action by which NE achieves its proconvulsant effects remains unclear. One hypothesis is that NE enhances cellular membrane potential by binding to its adrenoreceptors, consistent with the idea that NE increases the general excitability of neurons.[2] Due to an NE-secreting paraganglioma, NE levels increase systemically. The accompanying hypertension may lead to increased permeability of the blood-brain barrier to NE.[10] Another hypothesis postulates not direct activation of the cell membrane potential by NE but through a second messenger process, facilitating convulsions originating in those neurons.[2] Adrenoceptors are G protein coupled receptors that produce changes intracellularly at the level of cyclic AMP, after binding to NE.[2] A third possibility is activation of neurons participating in circuits that facilitate convulsions in non-adrenergic receptor bearing cells.[2] In addition vascular causes cannot be excluded. Elevated catecholamines can lead to cerebral ischaemia through vasospasm and provoke epileptic activity.[9]

When combining these proconvulsant and anticonvulsant hypotheses, we hypothesise that the elevation of NE might have played a role in the induction and maintenance of the continuous epileptic state in our patient. MRI scans of the brain were repeatedly normal. In the absence of hypertension, posterior reversible encephalopathy syndrome (PRES) was not considered. A paraneoplastic phenomenon was considered, despite negative tests for antibodies. But since removal of the metabolically active paragangliomas resolved his symptoms and the anticonvulsant medication could be stopped, this diagnosis was also rejected.

In conclusion, we believe this is the first case report in an adult patient showing a direct association between active paraganglioma leading to increased NE levels and epileptic seizures.

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Intense muscle aches after cleaning a boat on the Amsterdam canals

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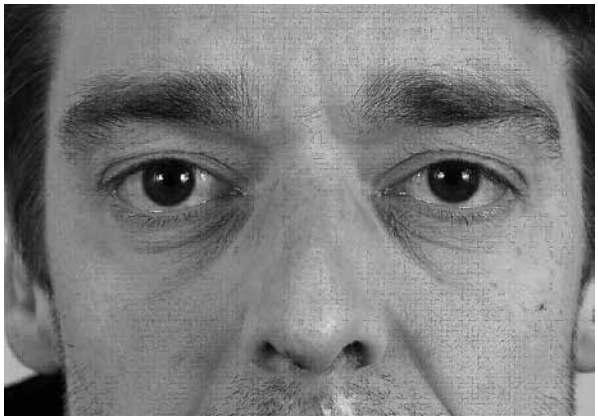
A 37-year-old man presented to our emergency department complaining of intense muscle aches and malaise. The muscle aches had begun five days after he had cleaned his boat on the Amsterdam canals in the beginning of January and had persisted for another five days, despite taking acetaminophen and ibuprofen. The patient smoked one pack of cigarettes per day and reported an alcohol intake of six units per day and occasional MDMA and cannabis use. On physical examination, he was haemodynamically stable, had a temperature of 37.4°C and was jaundiced. Further examination, including a neurological exam, revealed no abnormalities.

Laboratory results were as follows: haemoglobin 6.1mmol/l, leukocytes 14.0 x 10^9/l with a normal differentiation, thrombocytes 47 x 10^9/l, creatinine 199 µmol/l (later increasing to 457 µmol/l), creatine kinase 19.180 IU/l, lactate dehydrogenase 830 IU/l, aspartate aminotransferase 669 IU/l, alanine aminotransferase 187 IU/l, bilirubin 166 µmol/l (conjugated bilirubin 164 µmol/l, later increasing to 450 µmol/l), alkaline phosphatise 129 IU/L, and gamma glutamyltransferase 38 IU/l. Urinalysis revealed >0 leukocytes per field and >50 erythrocytes per field. Urine culture and blood cultures were negative. Serology was negative for acute infection with HIV, EBV, CMV, hepatitis A, B, C and E virus and hantavirus. A chest X-ray and abdominal ultrasound were both normal. The ECG showed atrial fibrillation with repolarisation abnormalities, without abnormalities on transthoracic echocardiogram.

Figure 1.



WHAT IS YOUR DIAGNOSIS?

See page 320 for the answer to this photo quiz.

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PHOTO QUIZ

An enlarged heart with hyperdense consolidation

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CASE REPORT

A 47-year-old man presented with progressive dyspnoea and dry cough. He had a history of dilated cardiomyopathy and ventricular tachyarrhythmia. The patient had been treated with placement of an implantable cardioverter-defibrillator and amiodarone (200 mg/day for two years). On chest auscultation, fine crackles were audible at the right lung base. Blood count and routine serum biochemistry test results were normal except for a slightly elevated alanine transaminase level (60 U/l). Chest X-ray and computed tomography (CT) demonstrated an enlarged heart, right pleural effusion, and hyperdense consolidation in the right lung (*figure 1*). The liver parenchyma was also dense.

WHAT IS YOUR DIAGNOSIS?

See page 321 for the answer to this photo quiz.

Figure 1. *Axial (A) and coronal (B) computed tomographic images demonstrating increased heart volume, right pleural effusion, and peripheral hyperdense consolidation in the right lower lobe of the lung. Note also the hyperdensity of the liver (the liver is denser than the heart) and the presence of a cardioverter-defibrillator*

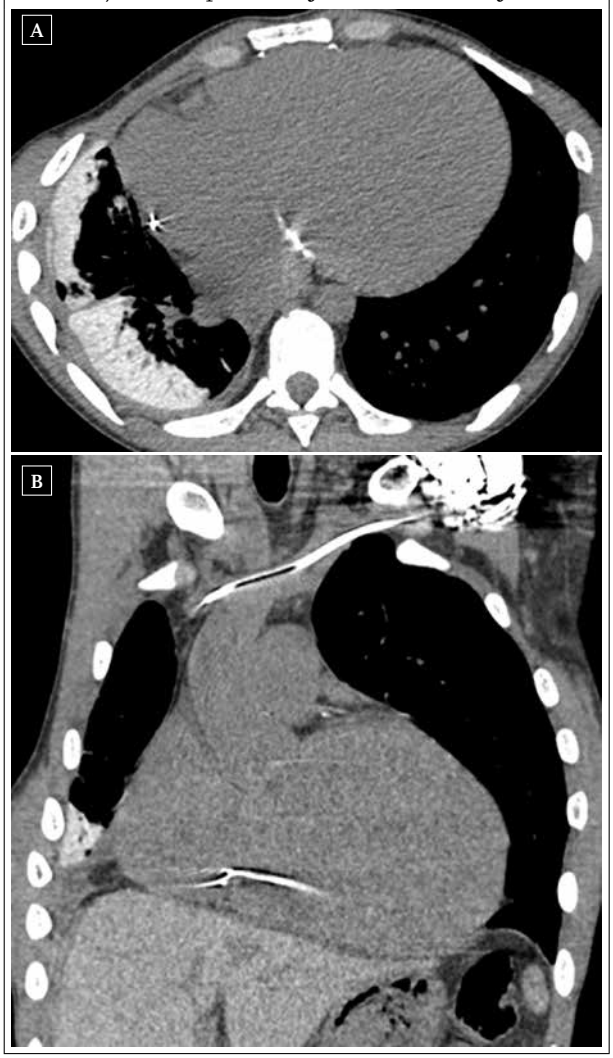

PHOTO QUIZ

A rare cause of spontaneous perirenal haemorrhage in a patient with ANCA-associated vasculitis

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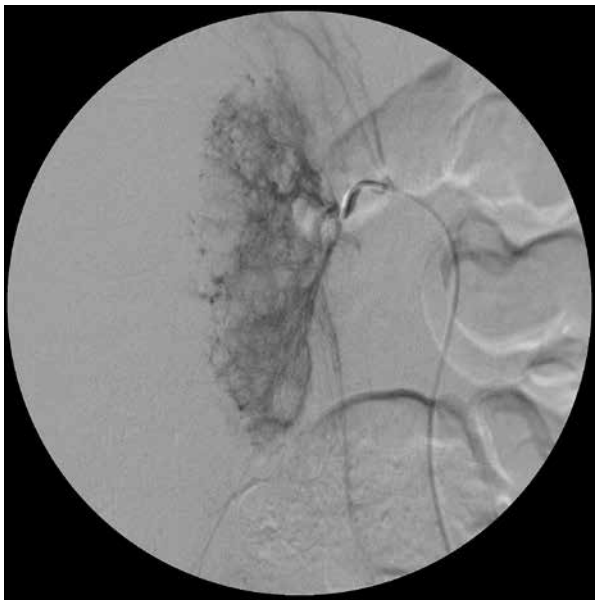
CASE REPORT

A 51-year-old female presented with lower abdominal and flank pain. Her medical history revealed ANCA (PR-3) associated vasculitis with extracapillary necrotising glomerulonephritis that started 15 years ago. After several episodes of treatment and relapses, she developed end-stage renal failure four years later and started haemodialysis. Four years ago she had a spontaneous renal haemorrhage of her left contracted kidney that resolved with conservative therapy.

Physical examination showed a patient in circulatory shock with some tenderness in the lower abdomen. All laboratory results were normal except for a haemoglobin level of 3.4 mmol/l.

Ultrasonographic evaluation revealed a right-sided retroperitoneal haematoma. Rapid blood transfusion and fluid resuscitation was not sufficient to stabilise the patient. Therefore, emergency intervention angiography was performed to determine the source of the bleeding and to evaluate treatment options (*figure 1*).

Figure 1. *Selective catherisation of the right renal artery*



WHAT IS YOUR DIAGNOSIS?

See page 322 for the answer to this photo quiz.

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PHOTO QUIZ

Fingertip necrosis and cervical lymphadenopathy

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CASE REPORT

In January 2013, an 83-year-old man was admitted to the hospital for fingertip necrosis of both hands that had developed over three weeks. His medical history was remarkable for 70 pack-years of cigarette smoking until 1995, when excision for a vocal cord epidermoid carcinoma was performed. He denied use of any medication or illicit drugs. At examination, the patient was fatigued and had lost 5 kg. The tips of his 2nd and 4th fingers of the right hand and of the 4th of the left hand were necrotic (*figure 1*). Peripheral pulses were palpable. He did not report Raynaud's phenomenon. The heart exam was normal. An asymptomatic left cervical lymphadenopathy was found, hard and fixed, 6 cm in diameter. Routine blood cell count and serum creatinine were unremarkable. Thrombophilia work-up revealed a lupus anticoagulant and IgM anticardiolipin antibodies at 44 U (positive >15). The plasma level of platelet microparticles of the anionic phosphatidylserine was elevated at 7844/µl (normal 393-937) indicating *in vivo* platelet activation. Platelet aggregation induced by arachidonic acid and adenosine diphosphate was increased. Antinuclear antibodies were positive at 1/1280 with anti-SSA/Ro 60 kD antibodies. Cryoglobulinaemia was present at a very low concentration but could not be identified. Duplex ultrasonography of the upper extremity arteries was normal including palmar metacarpal arteries. Electrocardiogram showed a sinus rhythm. Transthoracic echocardiogram revealed no abnormalities; insertion of the transoesophageal echocardiography probe was unsuccessfully attempted. Aortic computed tomography scan was normal. 18-F fluorodeoxyglucose (FDG)-positron emission tomography detected accumulation of the tracer located to the left cervical lymphadenopathy and to right cervical lymph nodes. Fine needle aspiration of the left cervical lymphadenopathy was consistent with a metastatic localisation of epidermoid carcinoma. The search for the primary tumour was negative. Treatment with iloprost, heparin and aspirin was ineffective.

Figure 1. *Fingertip necrosis of both hands*

WHAT IS YOUR DIAGNOSIS?

See page 323 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 316)

INTENSE MUSCLE ACHES AFTER CLEANING A BOAT ON THE AMSTERDAM CANALS

DIAGNOSIS

After ruling out a toxic cause of rhabdomyolysis, we considered leptospirosis, because of the combination of rhabdomyolysis, acute kidney injury and severe conjugated hyperbilirubinaemia. Serology was obtained on the day of admission and was negative for IgM (ELISA); the agglutination test was weakly positive. Since seroconversion usually occurs after 7-10 days of symptoms, serology was repeated after one week. An IgM-titre of 1:640 was detected with a positive agglutination test, most strongly for serogroup Icterohaemorrhagiae (titre 1:1280). After two months, IgM was still positive with declining titres in the agglutination test. Polymerase chain reaction was negative and culture results were still pending at the time of submission. Based on serology, the infection was caused by the serogroup Icterohaemorrhagiae. The patient was treated with ceftriaxone 2g iv once daily for one week and has recovered completely.

Leptospirosis is a zoonosis caused by the spirochete *Leptospira interrogans*, which can be further subdivided into various serogroups such as the Icterohaemorrhagiae. It infects both wild and domestic animals, especially rodents. Humans can get infected through contact with water contaminated by animal urine. The incubation time ranges from 4-14 days and is classically followed by a biphasic illness. The first phase is a nonspecific flu-like illness, which after a brief asymptomatic period is followed by a variable immune-mediated phase, which in severe cases may include acute kidney injury, rhabdomyolysis, severe conjugated hyperbilirubinaemia, myocarditis and diffuse haemorrhage due to capillary leakage.[1]

What is unique about this case is that it describes a leptospira infection in a temperate region during winter, whereas the majority of cases of leptospirosis occur in the tropics, with water temperatures high enough to enable the organism to survive for an extended period of time. A possible explanation is that the water temperature inside the patient's boat cabin may have been significantly higher than outside. Due to the cold temperature outside, rats may have used his boat as shelter.

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ANSWER TO PHOTO QUIZ (PAGE 317)

AN ENLARGED HEART WITH HYPERDENSE CONSOLIDATION

DIAGNOSIS

These tomographic changes were consistent with amiodarone-induced pulmonary toxicity (APT). Amiodarone was stopped, and an alternative antiarrhythmic agent was used. The patient's symptoms improved, and follow-up CT demonstrated regression of the consolidation.

Amiodarone is one of the most commonly used antiarrhythmic medications worldwide, frequently employed in the treatment of supraventricular and ventricular arrhythmias. This drug is an iodine-containing compound that tends to accumulate in several organs, including the liver and lung parenchyma. It has been associated with significant adverse effects, the most serious of which is APT.

The spectrum of APT ranges from mild chronic or subacute to rapidly progressing acute lung disease, including acute respiratory distress syndrome with high mortality. Symptoms can include progressive dyspnoea, dry cough, fever, malaise, and pleuritic chest pain. The most common CT findings include septal thickening, interstitial fibrosis, and consolidations. The drug's high iodine content enables the detection of amiodarone deposits in the lung by CT as high-attenuation focal or multiple parenchymal opacities. The association of dense lung air-space consolidations with high density of the liver and/or spleen is characteristic of amiodarone impregnation.

The diagnosis of APT is based on exclusion because the signs and symptoms are non-specific, and no laboratory test allows the diagnosis. APT should be suspected in any patient taking amiodarone who has new or worsening symptoms and/or new infiltrates on a chest X-ray. Early recognition of APT is important because discontinuation of amiodarone could prevent its progression. The prognosis is usually good in cases of chronic or subacute disease. Open lung biopsy should be avoided because of the tendency for APT to worsen after thoracic surgery, and because affected patients usually have impaired cardiac and pulmonary functions.

ANSWER TO PHOTO QUIZ (PAGE 318)

A RARE CAUSE OF SPONTANEOUS PERIRENAL HAEMORRHAGE IN A PATIENT WITH ANCA-ASSOCIATED VASCULITIS

DIAGNOSIS

A diagnosis of multiple renal aneurysms (the several small black dots in the right kidney) was made based on the selective catheterisation of the right renal artery, with clear extravasation of contrast media from one of these aneurysms (right upper pole). Selective catheterisation of the involved segmental arteries was followed by successful obliteration by embolisation.

Spontaneous perirenal haemorrhage is a rare but dramatic clinical problem with different aetiologies.[1,2] The predisposing conditions responsible for this clinical entity include malignant and benign neoplasm, renal artery aneurysm, renal vein thrombosis, polycystic kidney disease, arteriovenous malformations and more rarely infectious disease and sickle-cell trait. Renal vasculitis is also mentioned as a cause, dominantly described in patients with classical polyarteriitis nodosa (PAN).[1,2] In PAN, small and medium sized arteries are involved. The pathological basis of aneurysm formation is active fibrinoid necrosis of the arterial media, followed by extension of the process into the intima and adventitia. Subsequently, an inflammatory response invades the layers of the vessel wall. If areas of segmental necrosis involve the elastic wall to a sufficient degree, an aneurysm may develop.

In most publications and textbooks, renal aneurysm formation has solely been attributed to PAN. Before the era of ANCA, in patients with suspicion of renal vasculitis, the absence or presence of renal aneurysms by diagnostic angiography was even the main clue to discriminate between Wegener's granulomatosis and PAN, especially in cases where classical granuloma could not be detected. In previous reports, only two patients have been described with supposed Wegener's granulomatosis and spontaneous renal haemorrhage.[1,3] Only in one case was association with similar multiple renal aneurysm formation, as in this case, confirmed by angiography. This patient had the classical triad of biopsy-proven granulomas in the nose with pulmonary and renal involvement.[3] Therefore, this case appears to be the second case that shows us that renal aneurysm formation is not exclusively seen in PAN, but also in ANCA-associated vasculitis. In patients with ANCA-associated renal disease, we should be aware of renal aneurysm formation when spontaneous renal haemorrhage is detected.

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ANSWER TO PHOTO QUIZ (PAGE 319)

FINGERTIP NECROSIS AND CERVICAL LYMPHADENOPATHY

DIAGNOSIS

A diagnosis of acral vascular paraneoplastic phenomenon was made.

DISCUSSION

Acral vascular paraneoplastic syndrome consists of Raynaud's phenomenon, acrocyanosis and gangrene developing in patients with malignancy and improving with the treatment of the underlying condition.[1,2] Indeed, in a review of the literature in 2002, 68 cases were identified associated with adenocarcinoma in 41% of patents (mainly lung and ovary cancers), but also with haematological diseases in 18% (such as lymphoma, leukaemia and plasmocytoma). Only a few patients had head and neck epidermoid carcinoma. Most patients had advanced cancer and the acral vacular syndrome preceded diagnosis of the malignancy. Pathogenesis is still disputed. Indeed, a vasoconstrictive substance produced by the tumour cells, microfragments of the tumour and hyperstimulation of the sympathetic nervous system have been advocated,[1] but also activation of coagulation and of platelets,[3] reported commonly in patients with malignancies and observed in our patient. Of note, multiple autoimmune abnormalities observed in our patient including antinuclear antibodies, cryoglobulins, and anticardiolipin antibodies, could play a role as described in the literature.[1,4] Interestingly, in our patient treatment combining cetuximab and carboplatin resulted in dramatic regression of the left cervical lymphadenopathy and of fingertip necrosis within three months. Indeed, an early diagnosis of the malignancy is mandatory since cancer treatment is the only intervention that can result in dramatic improvement of the lesions.

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SPECIAL ARTICLE

An observational cohort study on geriatric patient profile in an emergency department in the Netherlands

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ABSTRACT

Background: Currently, Dutch emergency care systems focus on rapid emergency department (ED) patient management with short completion times, which may not meet specific geriatric care needs.
Methods: Six-week observational study in patients aged ≥70 years, attending the ED of VU University Medical Center (VUmc, Amsterdam, the Netherlands) during weekday peak presentation times (10 AM - 10 PM).
Results: During six weeks, a total of 183 patients aged ≥70 years attended the ED, of which 117 (63.9%) presented during weekday peak hours. One hundred patients with a median age of 81 (min-max; 70-97 years) were prospectively observed. The majority presented with fall-related complaints (30%), multiple comorbidities (≥3 in 50.0%) and polymedication (≥5 in 53.7%). Mean ED length of stay was 175.8 (range 20-399) minutes (n=98). Of the patients discharged to their usual residence prior to the ED visit (n=58), 36.2% returned to our ED within 30 days; one in five of these patients had initially presented with a fall.
Conclusion: In this study, fall-related injuries were the most frequent presenting complaint during weekday peak presentation times in 70-plus patients. Of these, one in five discharged from the ED returned within 30 days. Our emergency care system may not adequately cover comprehensive ED geriatric assessment, or provide sufficient outpatient care after ED home discharge. We believe that EPs should be more aware of the complex problems encountered in acute geriatric patients and address follow-up care pathways such as geriatric outpatient services, more often in frail elderly patients discharged home.

KEYWORDS

Emergency department, acute care, geriatric, fall-risk, assessment

INTRODUCTION

Older patients represent an increasing proportion of emergency department (ED) populations.[1-3] Due to multiple comorbidities, polymedication and atypical presenting symptoms,[4] emergency care for the older patient is complicated and multifaceted. The current focus in Dutch emergency care is on rapid patient management with short (four-hour) completion times,[5,6] with care being delivered by certified emergency physicians, junior doctors, residents or consultants of different medical specialities.[7] Because of complex care needs, acute geriatric care requires a more integrated approach. As such, multiple studies on patterns of geriatric emergency care use have been conducted.[4,8-12] Due to international differences in organisational models of emergency care, these studies may not apply to the Dutch situation.
With this prospective observational study, we planned to explore the needs and care delivery in older patients presenting to the ED of a university hospital in the Netherlands, in order to pinpoint components deserving special attention.

MATERIALS AND METHODS

We performed a six-week exploratory prospective observational study from 21 November 2011 to 2 January 2012 at the ED of VU University Medical Center (VUmc) in Amsterdam, the Netherlands. VUmc is a 733-bed university medical centre with a top level ED, providing care for approximately 32,000 patients per year. During the period this study was conducted, the ED was staffed with certified emergency and acute (internal medicine) physicians, junior doctors, residents and specialist consultants. The only inclusion criteria were age ≥70 years, and the capability of the patient to provide informed consent. The nature of

the study was explained to the patients and subsequently written informed consent was obtained according to the principles of the Declaration of Helsinki. Critically ill patients presenting at the 'critical care room'[13] were not eligible for inclusion.
Study approval was provided by the local research ethics committee.

Data collection
All study patient data were collected by a single observer, a final-year medical student (QT) with clinical experience within the field of geriatric medicine, who was trained and supervised by a geriatric consultant (OJV) and a researcher involved in geriatric research with considerable clinical experience (EJMS). The observer was present in the ED on weekdays between 10 AM and 10 PM which have previously been shown to be the peak presentation times at the ED of VUmc.[5] Patient characteristics, including age, gender, comorbidity, outpatient medication use, residence before admission, cognitive function and presence of delirium in the ED, and information on adequacy of acute care, including presenting symptoms, referring specialist, consulting specialist(s), ED length of stay, ED disposition, and unplanned 30-day return visit, were extracted from paper-based and electronic medical patient records (computer system 'iSOFT Mirador') by the observer. Polymedication was defined as using five or more prescription drugs in patients for whom current medications could be checked against a current outpatient medication list obtained by the emergency physician via the loco regional pharmacy. Data on cognitive function and delirium were extracted from the patient's medical records, as well as prospectively collected by the observer with standardised observer-rated screening instruments (see next section). If the observer had any doubts about the cognitive assessment, a supervisor was consulted. ED length of stay was defined as the time spent in the ED from the moment of presentation until the moment of ED discharge, data which were extracted from the electronic patient registration system ('Medical Office'). 'ED home discharge' was defined as discharge back to the patient's residence prior to ED presentation. The VUmc electronic medical patient record of each participant was checked 30 days after initial ED presentation to evaluate unplanned repeat ED visits.

Standardised screening instruments for cognitive function and delirium
In order to objectively evaluate cognitive function and delirium, five validated assessment tools were used: 6-item Cognitive Impairment Test (6-CIT),[14] VMS delirium risk questions,[15] Confusion Assessment Method (CAM)[16] and delirium criteria according to the Diagnostic and Statistical Manual of Mental Disorders – fourth edition (DSM-IV criteria).[17]

Presence of cognitive impairment (CI) was directly assessed by the observer using the 6-CIT with a score ≥11/28 indicating CI.[14,18] Patients were screened for delirium risk with the VMS delirium-risk questions (score ≥1 indicating increased risk of developing in-hospital delirium).[15] To assess whether delirium symptoms were present in the ED, a shortened Dutch version of the CAM was used.[19] The CAM is a standardised tool which includes four features that enable non-psychiatrically trained clinicians to identify delirium and distinguish those symptoms from other cognitive disorders. Delirium was diagnosed according to the DSM-IV criteria.[17] The results of these tests were blinded to the physician responsible for the patient's treatment.

Statistical analysis
All statistical analyses were performed with Microsoft Excel 2003 and IBM® SPSS Statistics 20. Descriptive statistics were used to calculate median and means for patient characteristics data. The independent samples t-test was used for group statistics to calculate differences in ED length of stay for patients evaluated by one and two or more medical specialists. Two-tailed p values <0.05 were considered to indicate a statistically significant difference between those two groups.

RESULTS

Mean monthly visits and subsequent hospital admissions over the past 20 years of patients aged ≥70 years at VUmc are represented in *figure 1*. During this six-week study period, 183 patients aged ≥70 attended the ED, of which 66 presented outside weekday peak presentation times (10 PM to 10 AM). Of the remaining 117 patients, 14 presented to the critical care room, and three refused to participate, resulting in the inclusion of 100 patients for this observational study (*figure 2*).

Patient characteristics and presenting problems (*table 1*)
The median age of the participants was 81 (min-max; 70-97) years, 35% were men. Prior to ED presentation, 86% were living independently at home, and the majority were referred by their general practitioner (44%). The mean number of comorbidities per patient with a known medical history (n=94) was 3.04 (SD ±1.9; range 0-8). Fall-related injuries were the most common presenting symptom (30%).

Polymedication (*table 2*)
The treating ED physicians had access to a current outpatient medication list in 82 participants. The mean number of prescribed medications per patient was 5.3 (SD ±3.3; range 0-14). Polymedication (≥5 prescribed medications) was present in 53.7%.

Figure 1. *Mean monthly ED visits and subsequent hospital admissions of patients aged ≥70 years at VU University Medical Center (VUmc), Amsterdam, the Netherlands 1992-2011*

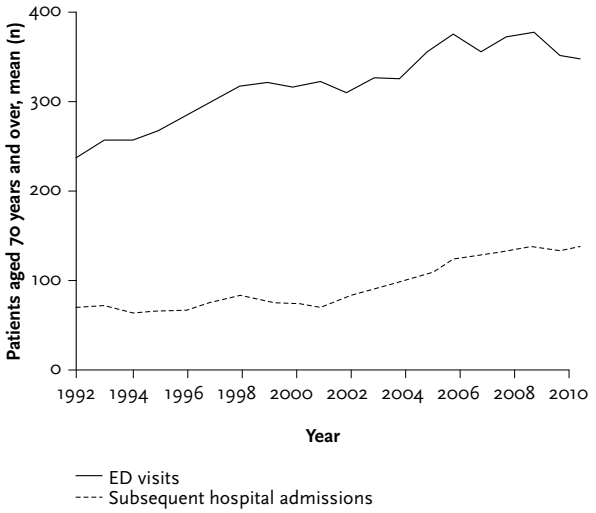

Figure 2. *Study flowchart*

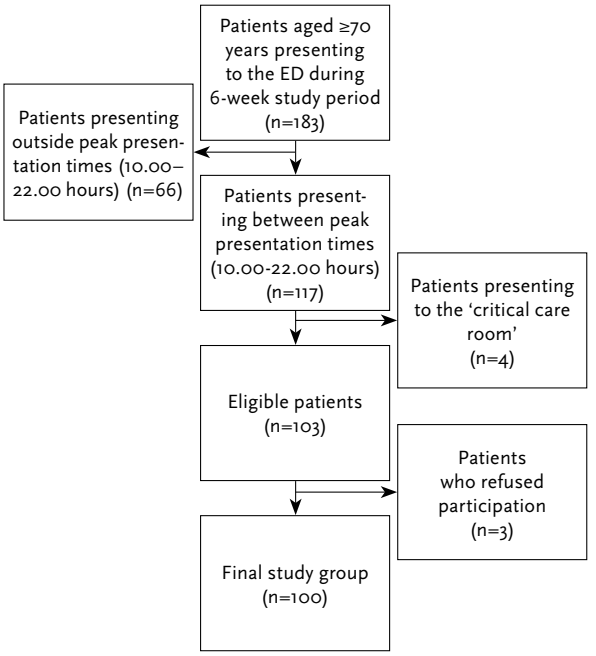

Table 1. *Study population characteristics and presenting problems*[¥]

Median age, years (min-max)	81 (70-97)
Male, %	35
Residence	
Independent at home	86
Assisted living	7
Nursing home	4
Rehabilitation centre	3
Comorbidities* (n=94), n	
Mean per patient (±SD; range)	3.04 (±1.9; 0-8)
≥3 comorbidities	47
Cardiovascular	85
Malignancy	30
Cerebrovascular	29
Endocrine disorder	20
Neuropsychiatric	16
COPD	14
Renal failure	12
Musculoskeletal	7
ED referral	
General practitioner	44
Self-referral	30
Emergency services	24
Nursing home physician	2
Presenting problems	
Falls	30
Shortness of breath	13
Neurological symptoms	10
General malaise	9
Abdominal pain	8
Cognitive or functional decline	6
Fever	6
Other±	18

[¥]Percentage (%) in the total study population (n=100), unless noted otherwise; *Multiple comorbidities per patient. Most frequent were: cardiovascular including (chronic) heart failure, hypertension, intermittent claudication, abdominal aortic aneurysm, and deep vein thrombosis; cerebrovascular including transient ischaemic attack (TIA), and cerebrovascular attack (stroke); endocrine disorders including diabetes and thyroid disease; neuropsychiatric including epilepsy, dementia, and psychological problems; ±including anaemia, melaena, mastitis, epistaxis, rectal bleeding, renal failure, pain/swelling/redness extremity, urine retention, minor injuries not related to falls.

Cognitive impairment and delirium (*table 3*)

In 16% of the participants CI was documented in the medical history, and included subtypes of dementia, Alzheimer's disease and Korsakoff's syndrome. Prospective evaluation of cognitive problems with the observer-rated 6-CIT (n=98) indicated signs of CI in 28.6%. The presence of delirium in the ED was monitored with the CAM, and delirium diagnosis was conclusive according to the DSM-IV criteria in 9%.

ED care delivery and disposition (*table 4*)

Mean ED length of stay in this cohort was 181.3 (SD ±100; range 20-720) minutes, and was registered in all but one patient (n=99). One patient stayed in the ED overnight (720 minutes) because of nursing home transfer problems. Excluding this patient, mean ED length of stay was 175.8 (SD ±84.1; range 20-399) minutes (n=98). Compared with clinical evaluation by one medical specialist (n=83),

Table 2. *Polymedication*

Mean prescribed medications*, n (±SD; range)	5.3 (±3.3; 0-14)
≥ 5 medications, n (%)	44 (53.7)
< 5 medications, n (%)	38 (46.3)

***Based on the availability of current outpatient medication lists via the loco regional pharmacy (n=82).**

Table 3. *Cognitive impairment and delirium¥*

Documented in medical record, n	16
Observer rated with the 6-CIT (n=98), n (%)	28 (28.6)
Delirium	9

¥Prevalence in the total study population (n=100), unless otherwise noted.

Table 4. *ED care delivery and disposition**

Mean ED LOS# (n=98), minutes (±SD; range)	175.8 (±84.1; 20-399)
Consulting specialists per patient§ (n=98)	
One, n (%)	83 (84.7)
Two or more, n (%)	15 (15.3)
ED diagnosis (n=100)	
Care problem	2
Cerebrovascular event	5
Decompensated heart failure	5
Fracture after fall	17
Infection	17
Other$	54
ED disposition	
Home discharge**	53
Hospital admission^	42
Nursing home	3
Rehabilitation centre	2
30-day ED return visit after ED discharge (n=58), n (%)	21 (36.2)
Initial presentation with falls, n (%)	4 (19.0)

***Percentage (%) in the total study population (n=100), unless noted otherwise; #ED length of stay (LOS) was not registered in one patient, and one other patient had to stay in the ED overnight because of nursing home transfer problems. Both were left out of the analysis; §including emergency physicians, internists neurologists, cardiologists, orthopaedists, surgeons, gastroenterologists, urologists, geriatricians, pulmonologists, oncologists, nephrologists, ear nose throat physicians, rheumatologists; $including contusion, COPD exacerbation, distorsion, neutropenic fever, hypoglycaemia, anaemia of unknown origin, cholecystolithiasis, diverticulitis, gastrointestinal bleeding, constipation, renal failure, aneurysm, atrial fibrillation, gastro-enteritis, medication side effect, haematoma, mastitis, benign paroxysmal positional vertigo, myelum metastases, hypothermia, hypercalcaemia, dislocated double J stent, empyema, commotion cerebri, epistaxis, skin injury, thrombocytopenia, peripheral vascular disease, lung tumour, cerebral vasculitis, infection after surgery (foot and knee), leg pain without a probable cause; **defined as discharge to usual residence prior to ED presentation; ^including 5 patients who were transferred and admitted to another hospital due to bed shortage at VUmc.**

consecutive patient evaluation by two or more consulting specialists (n=15) prolonged ED length of stay: 164.4 (SD ±78.9) and 238.9 (SD ±86.7) minutes (p=0.015) respectively. The overall admission rate was 42%. Nearly one in five (19%) participants with an unscheduled 30-day return visit to our ED after primary discharge (36.2%) initially presented with falls.

DISCUSSION

This exploratory prospective observational study was conducted to retrieve insight into the characteristics of geriatric patients presenting to the ED of a university hospital in the Netherlands. Some of the trends seen in our Dutch study population correspond to previous findings in studies conducted in the United States, Canada, United Kingdom and Belgium:[8,20-25] older patient ED visits and subsequent hospital admission rates are increasing, older patients are at risk for ED repeat visits, fall-related injuries represent a frequent reason for ED visits, multiple comorbidities and polymedication are the rule rather than the exception, and both CI and delirium are more prevalent than they are documented.

Illustrated by data retrieved from our hospital administrative database, ED visit rates for patients aged ≥70 years have steadily increased over the last two decades (*figure 1*). Due to a global increase in ED patient visits with a continuous threat of overcrowding, emergency care has focussed on rapid patient management with a four-hour maximum completion time target.[5,6,26,27] For the geriatric emergency population, this target may not benefit the quality of acute care delivered to them. A study conducted in the UK, evaluating the quality of ED care under the four-hour target between 2003 and 2006, showed that ED return visits and return visits ending in hospital admission increased in the elderly population (age ≥65 years), while visits of older patients were generally stable (annual change -0.19%; 95% CI -0.44% to 0.06%).[28,29] We found that 36.2% of the patients discharged to their usual residence prior to ED visit (n=58) returned to our ED within 30 days. Nearly one in five of these patients initially presented with fall-related complaints. Since patients were included during weekday peak presentation times and we merely evaluated unscheduled 30-day return visits to our ED, we believe our results may therefore underestimate the true extent of fall-related injuries in emergency care. Previous studies have shown that falls in the older adult are an important frailty indicator, and patients who have fallen in the past year are likely to fall again (likelihood ratio range, 2.3-2.8).[30] Even more important than the substantial health care costs,[31,32] falls are associated with adverse patient outcome.[33,34] Although different interventions such as fall risk and comprehensive geriatric assessment positively

affect the outcome for older patients after ED discharge,[35-37] general compliance to these guidelines is inadequate.[38,39] In 2008, the Hospital Patient Safety Program was initiated nationally in all Dutch hospitals to manage patient safety systematically according to ten different themes,[40] one of which addresses frailty in older adults. Its goal is to screen hospitalised patients aged 70 years and over on four frailty aspects (i.e. delirium, fall-risk, malnutrition and physical impairment), and to initiate preventative interventions when risk factors are identified. The relatively high rate of 30-day ED return visits in our study population triggered us to think about how we can improve follow-up for this particular group. Currently, facilities to profile and assess geriatric patients in an outpatient setting are available, but the contact between the EPs and these outpatient facilities are, in our opinion, suboptimal. What we would like to see happening is that EPs are trained in recognising geriatric patients likely to require additional care after ED home discharge, and that these patients are more often directly referred to a geriatric outpatient clinic. EPs and geriatric specialists should together develop a practical screening method to qualify patients in the acute care setting who may benefit from such a follow-up care pathway after ED home discharge. Also, directly approaching the patient's general practitioner to discuss at-home support services may also contribute to the quality of follow-up care after ED discharge, and with that, reduce the number of repeated ED visits or (unnecessary) hospital admissions.

The prevalence of CI (28.6%) and delirium (9%) in our exploratory study was similar to previous reports.[41] Although cognitive – in addition to or together with functional – decline prior to a geriatric adult ED visit is sometimes the only disease indicator, CI is not routinely screened by emergency room physicians.[42,43] Observations of our participants' medical records in addition to prospective cognitive function testing by the observer with the 6-CIT showed that CI was far more prevalent than it was documented: 28.6% (n=98) and 16% (n=100), respectively. Older patients with CI on admission are more prone to functional decline during the hospital stay.[44] The implications of cognitive dysfunction on elderly patient outcome underscore the importance of recognition and assessment of cognitive dysfunction in the ED. Although ED delirium prevalence was 9% in our survey, we only found enquiries on delirium symptoms in the participant's medical record when the referral question was (possible) delirium (n=3; 33.3%). Several publications have shown that although delirium is an important illness indicator and relatively prevalent among older ED patients, it is frequently not recognised as such.[45-48] Patients with unrecognised delirium may be sent home with untreated underlying medical conditions, leading to ED return visits and potentially increased morbidity or mortality.[49,50] In addition, delirious patients discharged home from the ED are more likely to miscomprehend discharge instructions,[51] leading to poor therapy adherence. Prior studies have reported in-hospital delirium incidence from 10-31% in comparable populations,[52-56] with in-hospital delirium contributing to prolonged hospital length of stay.[56,57] The lack of awareness, non-availability of practical screening tools and the typical fluctuation of delirium symptoms during the day, among other factors, make it difficult for acute physicians to observe delirium symptoms in the sometimes highly demanding ED. The substantial economic burden and negative patient outcome associated with delirium,[47,53,58-61] however, support the need to improve awareness of delirium among acute care physicians.

Several limitations of our exploratory study should be discussed. Because informed consent was required for this study, patients presenting to the critical care room could not be enrolled. A single trained observer was assigned to the data collection of this study, because of which we choose to merely include patients during peak presentation times (10 AM-10 PM) on weekdays. As such, a certain fraction of the older adults attending the ED during the six-week study period was missed (n=66, 36%), and unintended selection bias may have occurred. This was a single-centre exploratory prospective observational study, and one might question whether our results really reflect daily clinical practice in Dutch EDs. On the other hand, our results did generally correspond to previous published data. The aim of this observational study was therefore to pinpoint (unrecognised) components of geriatric emergency care in our hospital, and to create awareness about the frailty aspects in older patients in the Dutch emergency setting that require special attention. Despite the fact that our study population was relatively small and results were mainly observational in nature, it certainly informs us on some very relevant problems and systematic pitfalls which we believe must be tackled to optimise acute care for these particular patients.

CONCLUSION

In conclusion, our exploratory results demonstrated that falls were the most frequent presenting complaint during weekday peak presentation times in 70-plus patients. One in five patients presenting with falls returned to our ED within 30 days after initial home discharge. In addition, cognitive impairment and delirium were under-recognised. These results suggest that our emergency care system may not adequately cover comprehensive ED geriatric assessment, or provide sufficient outpatient care after ED discharge. Current European acute care systems focus on rapid patient management with care being delivered by many specialities. As a result, care delivery is fragmented, leading to poor care coordination. Reducing ED length

of stay by rapidly moving patients out of the ED may not result in optimal care delivery for the more frail geriatric patient population. We believe that EPs must be more aware of the complex problems encountered in acute geriatric patients and address follow-up care pathways such as geriatric outpatient services more often in frail elderly patients discharged home.

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SPECIAL ARTICLE

Gerontology and Geriatrics in Dutch medical education

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ABSTRACT

Background: The world population is ageing and healthcare services require trained staff who can address the needs of older patients. In this study we determined how current medical education prepares Dutch students of medicine in the field of Gerontology and Geriatrics (G&G).
Methods: Using a checklist of the essentials of G&G, we assessed Dutch medical education on three levels. On the national level we analysed the latest National Blueprint for higher medical education (Raamplan artsopleiding 2009). On the faculty level we reviewed medical curricula on the basis of interviews with program directors and inspection of course materials. On the student level we assessed the topics addressed in the questions of the cross-institutional progress test (CIPT).
Results: The National Blueprint contains few specific G&G objectives. Obligatory G&G courses in medical schools on average amount to 2.2% of the total curriculum measured as European Credit Transfer System units (ECTS). Only two out of eight medical schools have practical training during the Master phase in the form of a clerkship in G&G. In the CIPT, on average 1.5% of questions cover G&G.
Conclusion: Geriatric education in the Netherlands does not seem to be in line with current demographic trends. The National Blueprint falls short of providing sufficiently detailed objectives for education on the care of older people. The geriatric content offered by medical schools is varied and incomplete, and students are only marginally tested on their knowledge of G&G in the CIPT.

KEYWORDS

Medical education, the Netherlands, Gerontology & Geriatrics

INTRODUCTION

All around the world, the number of older people will grow.[1] In the Netherlands alone, the population of over 65 year olds will increase from 2.6 million (16% of total population) to 4.4 million (25%) in 2035.[2] Older people are frequent users of healthcare: in 2011, 84% of people aged ≥65 years visited their general practitioner (on average 6.7 times a year per person) and 61% visited a medical specialist (on average 3.5 times a year). There were 18 hospital admissions per 100 over 65 year olds (amounting to 33% of all admissions), and 77% of over 65 year olds used one or more prescribed drugs.[2,3] We therefore expect all future medical doctors to be confronted with an increase – both absolute and relative – in older patients. Due to the frequent occurrence of multimorbidity, older patients require a different approach. Since all medical doctors, both generalists and specialists, will be confronted with the growing number of elderly patients, it is essential that all medical students receive a good basic training in the principles of Gerontology and Geriatrics (G&G).
Previous studies have shown that not all medical schools pay attention to their G&G education.[4-8] In both the UK and USA, undergraduate education in G&G was not offered at all medical schools. In a UK inquiry, G&G courses were offered at 22 of the 23 responding schools (on a total of 31), in a US inquiry 105 of 121 responding schools (on a total of 144) were found to have a 'distinct academic geriatric program'.[4,5] In the UK inquiry, moreover, only 53% of the responding schools had a geriatrics department to promote the presence of geriatrics in the curriculum.[4,9] Furthermore, geriatrics was taught as a separate subject in only two out of 23 schools.[4] Teaching geriatrics as a separate course was found to be a more effective learning experience in a study performed by Duque *et al.*[10] Members of the British Geriatrics Society Education and Training Committee and heads of departments of geriatric medicine

in the UK also consider teaching G&G in a separate course preferable.[4] A recent survey conducted among teachers and students of Nursing in the Netherlands found that students not only lack knowledge of geriatrics but also do not wish to work with older people in their future career.[11] For the medical curricula in the Netherlands, no formal investigation on G&G has ever been conducted.

Therefore, we conducted a survey of the current state of G&G education in all medical curricula in the Netherlands. We compared our results with the international literature and make recommendations to prepare the next generation of doctors for their future practice.

MATERIALS AND METHODS

A task group, including all three professors of geriatric medicine of the Netherlands, different ageing experts and experts in medical education drew up a checklist (*appendix A*) of essential topics in G&G.[12] This checklist resembles the guidelines set out in the British Geriatrics Society (BGS) curriculum and adds several topics agreed upon by the expert panel.[13] Using the checklist as a frame of reference, we analysed G&G education on three different levels: the national level, the faculty level and the student level.

On the national level, we evaluated which G&G topics were included in the latest National Blueprint (Raamplan artsopleiding 2009), established by the Federation of Dutch University Medical Centres (NFU) .

On the faculty level, we discussed our checklist with the responsible educational officers of all medical schools. The officers then provided us with course materials that in their respective opinions covered topics on our checklist. To get a complete as possible overview of all topics concerning G&G we included all courses in our study that 1) included topics mentioned on the checklist; 2) included topics designated in the course materials as concerning G&G; 3) included topics that the educational officers of the respective faculties considered G&G.

We examined the available course materials in depth and produced both a qualitative and a quantitative description of the G&G education at every medical school. For the quantitative description we assessed the number of ECTS (European Credit Transfer System units): 1 ECTS equals 28 hours of study load. The number of ECTS for courses partly devoted to G&G was calculated from the percentage of the course devoted to G&G. Stand-alone lectures were not included in the quantitative approach, because these were small in number and their size in ECTS hard to estimate. We made a distinction between both practical and theoretical courses and whether these were offered in the Bachelor or Master phase of the curriculum. The Dutch undergraduate medical curriculum is divided into a Bachelor and a Master phase of three years each. Each year consists of 60 ECTS, so that the entire undergraduate curriculum consists of 360 ECTS. Generally speaking, the Bachelor phase is the theoretical part of the education (e.g. lectures in auditoria and self-study assignments), while the Master phase offers more practical courses (clinical and scientific internships/clerkships). The distinction between theoretical and practical is important because practical experience is essential to transferring certain knowledge, skills and attitudes. Our initial results were submitted to the responsible educational officers of the faculties for verification and feedback.

Finally, on the student level, we measured the number of questions students had to answer on G&G in the cross-institutional progress test (CIPT)[14] which is taken by students of five of the eight medical schools. In this case, we selected G&G questions if these related to topics on the checklist or when the old age of the patient(s) described in the question was decisive in determining the correct answer.

RESULTS

National level

The most recent National Blueprint (Raamplan artsopleiding) was released in 2009. This Blueprint describes, among other things, the competence domains (or CanMEDS-2005 roles) that medical doctors should master. It describes issues in health and disease with which a doctor should be familiar; the requirements of the Bachelor diploma or first years of medical school; basic subjects in the study of medicine; and a number of skills in the practice of medicine. The National Blueprint does not provide an extensive list of G&G topics, but instead only describes several broad terms that the students need to master. On several occasions it is mentioned that students should take age or life phase into account. Elsewhere it is stated that 'it is assumed that particularisation for age and sex is self-evident.' *Appendix B* shows the complete list of all parts of the guideline where G&G is explicitly mentioned.

Level of the medical school

The G&G courses offered in the medical curricula vary considerably in format (integrated or separate), size, and content. *Figure 1* shows the obligatory education on G&G in the different curricula divided in practical and theoretical courses and in Bachelor and Master courses. Six schools offered practical courses.

In the Master phase, four medical schools offered theoretical courses that mostly resembled the theoretical courses offered in the Bachelor phase by other schools. Two faculties offered practical courses in the form of a clerkship in G&G. In a Dutch clerkship the medical student provides

supervised assistance to a resident in patient care. On a total of 360 ECTS for every curriculum, on average G&G courses amounted to 7.9 ECTS or 2.2%.

All medical schools provided the opportunity to follow elective courses in G&G. The main electives in geriatric education are a scientific internship, a geriatric clerkship, and a semi-resident clerkship. *Figure 2* shows the amount of ECTS that could potentially be spent on G&G for each medical school, again subdivided for Bachelor/Master and practical/theoretical courses. Compared with the obligatory courses, the elective courses show greater homogeneity and greater amounts of ECTS (on average 53) were devoted to G&G. However, each year, only a limited number of students (<10%) are able to participate in a G&G elective course.

Student level

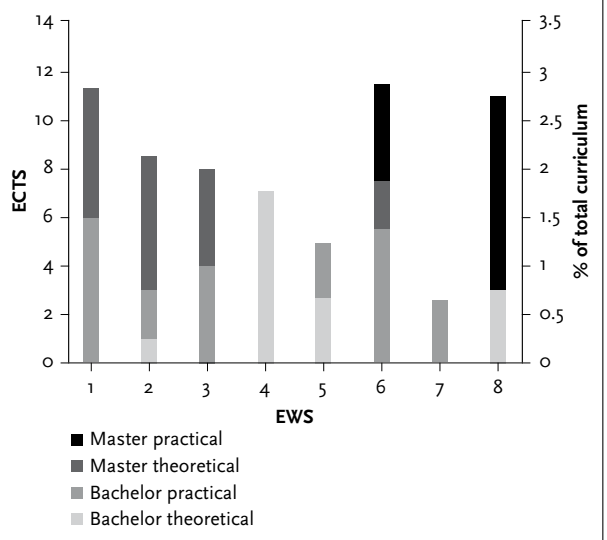
In order to assess the extent to which students are tested on their knowledge of G&G, we analysed the questions of the CIPT.[14] This exam is taken four times a year by all students of five out of eight medical schools and serves as a curriculum transcending test on which students should perform better in each consecutive year. Its questions are divided into mostly specialist categories: respiratory system; blood and lymph system; cardiac system etc. G&G is not among these categories, although there is one category 'life phases/ general'. We analysed five recent exams: those of February 2010, May 2010, September 2010, December 2010, and May 2011. On average three out of 200 questions (1.5%) dealt with G&G.

DISCUSSION

Geriatric education in the Netherlands does not seem to be in line with current demographic trends. The National Blueprint falls short of providing sufficiently detailed prescriptions for the care of older people. The geriatric content offered by medical schools, probably as a consequence,[6] is varied and incomplete, and students are only marginally tested for their knowledge of G&G. The task group that set up the checklist used to analyse geriatric education was made up of professors of geriatric medicine, ageing experts and experts in medical education from different universities. While having in this way assembled a notable amount of knowledge and experience, other panels including nursing home physicians, general practitioners and internal medicine specialists might have constructed different checklists. However, we believe these lists would have shown considerable resemblance to the current checklist and it would not influence our conclusions.

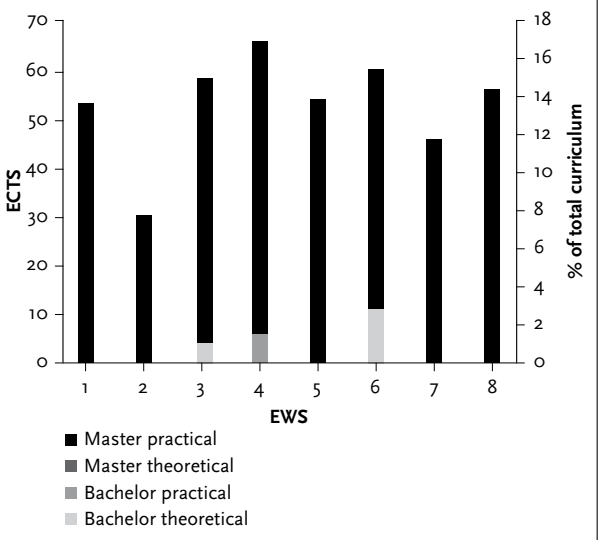
By virtue of the small size of the Netherlands, we were able to analyse the curricula of all medical schools through both interviews and close examination of course materials. Most geriatric content was delivered in separate courses which allowed us to quantify the contribution of G&G in the curriculum, and through the aforementioned examination of the course materials we were, in most cases, even able to assign weight to the integrated contents.

Figure 1. *G&G education in obligatory courses for all eight Dutch medical schools*



ECTS (European Credit Transfer System units) devoted to G&G education in obligatory courses (left y-axis) and as a percentage of the total undergraduate curriculum (right y-axis) for all eight medical schools in the Netherlands (x-axis).

Figure 2. *G&G education in elective courses for all eight Dutch medical schools*



ECTS (European Credit Transfer System units) devoted to G&G education in facultative courses (left y-axis) and as a percentage of the total undergraduate curriculum (right y-axis) for all eight medical schools of the Netherlands (x-axis).

In view of the source material for this study, we will have inevitably overlooked some forms of G&G teaching offered by medical schools, the most important among which may be the skills and attitudes conferred to students by their supervisors in direct contact with older patients. We have examined what students are tested for based on the CIPT. For future research, therefore, it would be interesting to also look at the exams of the individual medical schools.

Our results are in line with previous findings.[4,6-8] In the United Kingdom, for instance, the national guidelines from the General Medical Council on training of medical students have been criticised for being too generic and not specific enough on G&G.[15] The British Geriatrics Society (BGS) has devised a list of more detailed learning outcomes for medical students,[13] aiming to improve training in the essentials of G&G. However, UK medical schools fall far short of BGS expectations; a survey undertaken by Gordon *et al.*[16] showed that of 21 of the suggested BGS learning outcomes in G&G, only eight were taught by all medical schools and none of these were examined by all medical schools.

In our opinion, G&G education has to be secured on three levels: the national level, the faculty level, and the individual student level. We recommend to establish detailed national guidelines for the instruction of geriatric medicine to undergraduate students. An alternative could be to leave more room at the level of the medical school, but then another way should be implemented to secure that adequate attention is given to G&G. Concerning the form of education, we recommend that all students follow a clerkship in G&G.[17,18]

Given the special approach, skills and considerations in geriatrics and the large part of their future work devoted to this, we recommend that all medical students receive formal training by geriatricians.[19] The elderly patients treated by the department of internal medicine are interesting case studies, as these often suffer from a multitude of diseases. Specialists in internal medicine could also play an important role in the education of students in the principles of G&G.

Finally, the contents of these curricula should be adequately reflected in the exams that test students for their knowledge of G&G. Students will direct their study efforts towards what is required at examination, so that the contents of the exam determine to a great extent how much knowledge is transmitted.[20]

We realise that geriatrics is not the only medical speciality that wishes to increase its share in the medical curricula. However, we feel that there is a clear and unjust discrepancy between the fact that people aged ≥65 now account for one third of hospital admissions,[2,3] and that (on average) only 2.2% of the medical curriculum teaches about G&G. Moreover, in light of the ongoing demographic changes, this discrepancy is likely to increase if appropriate action is not taken.

As ageing populations are a global phenomenon, we would also like to prompt other countries to undertake a survey of G&G in their medical curricula.

ACKNOWLEDGEMENTS

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APPENDIX A

Checklist of G&G topics

Knowledge

Demography of ageing:
Demographic transition
Prevalence mortality/disease

Epidemiology of ageing:
Epidemiological transition
Causality, causal pie model Rothman

Psychology of ageing:
Disability paradox

Sociology of ageing:
Life course perspective
Networks
Intergenerational relations

Ageing international:
Increase in life expectancy in international perspective

Work and ageing:
Disease and disability
Retirement and ageing

Organisation and structure of elderly care:
Models of care
Policy, capacity
Costs of elderly care

Understanding

Health and disease from an evolutionary perspective (ultimate explanations):
Evolutionary theories of ageing
- Life history regulation
- Decrease of natural selection with age (selection shadow)
- Mutation accumulation in the gene pool
- Trade offs
- Antagonistic pleiotropy
- Disposable soma theory

Gene-environment interactions, plasticity
Health and disease from an evolutionary perspective (Stearns)
Clustering of disease

Biological mechanisms of ageing (proximate explanations):
Stress and damage (exogenous, endogenous)
Cellular reactions to stress (recovery, senescence, apoptosis)
Physiological reactions to stress (neuro-endocrine regulation)
Homeostasis (retain norm) and allostasis (adapt norm)

Practice

Prevention/ healthy ageing:
Nutrition
Physical exercise
Changing risk management in the elderly
- Hypertension, dyslipaedemia, overweight

Quality of life

Diagnostics:
Medical history
Geriatric assessment
Geriatric giants
Clinical (often atypical) presentation of elderly
Additional diagnostics

Complex care

Therapy:
Multimorbidity
Polypharmacy
Individual therapy
Goals of treatment
Abstention from treatment
Rehabilitation

Organisation and structure of elderly care:
Direction
Not one medical specialist, but a medical team
Referral, who is responsible?

Research in the elderly:
Inclusion/ exclusion
Guidelines elderly care
Lab values in the elderly
Evidence-based medicine for elderly

Ageism:
Elderly abuse

APPENDIX B

Geriatrics and Gerontology in National Blueprint (Raamplan artsopleiding) 2009

Chapter 6, Competences of the doctor at graduation
Medical expert, ¶ 6.2.1:
The newly graduated doctor has the ability to
-apply skills regarding (amongst others) diagnosis and therapy taking gender, age, and life phase into account.
Communicator, ¶ 6.2.2:
The newly graduated doctor has the ability to
-adequately deal with diverse patient groups like (...)elderly (...)
-have a conversation with the patient taking the patient's age into account

Chapter 7, Issues in health and disease
¶ 7.1: The doctor should, in determining the diagnostic and therapeutic policy, take (....) contextual factors into account. This goes especially for the significance of life phases, both young age and old age.
¶ 7.2.4: Medicalisation

Chapter 8, Bachelor of Medicine: goals and profile
¶ 8.3.1: The Bachelor of Medicine has knowledge and understanding of
-the genesis and development, growth, sexual maturation and ageing and dying of an organism
-the physiological mechanism of degeneration, wear and ageing and their structural and (patho) physiological consequences

Chapter 9, Basic subjects in medical education
¶ 9.2.2: The newly graduated doctor has knowledge and understanding with regard to
-the genesis and development, growth, sexual maturation and ageing and dying of an organism
-the physiological mechanism of degeneration, wear and ageing and their structural and (patho) physiological consequences
Keywords:
-Molecular and cellular aspects of ageing
-Physiological aspects of tissue and organ ageing and the functioning of an organism
-Pathophysiology of dying and the death of an organism
-Epidemiology of diseases related to old age and death
-The backgrounds to syndromes of premature ageing
¶ 9.3.2: The newly graduated doctor has knowledge and understanding of the normal psychological and social characteristics of man.
Keywords:
- Development in the lifecycle (baby, child (....) elderly)
The newly graduated doctor has knowledge and understanding of the structure of the Dutch society
Keywords:
-Long-term changes (for example the ageing population, immigration)

Appendix 3. List of skills
In principle, every skill is mentioned (only) once. Even skills that have specific applications in for example children/elderly (...). It is assumed that particularisation for age and sex is self-evident.

APPENDIX C

Members of the task group

Members of the task group:
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APPENDIX C

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INFORMATION FOR AUTHORS

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The title should be informative and not exceed 90 characters, including spaces. Avoid use of extraneous words such as 'study', 'investigation' as well as priority claims (new, novel, first). Give a running title of less than 50 characters. If data from the manuscript have been presented at a meeting, list the name, date and location of the meeting and reference and previously published abstracts in the bibliography. Give a word count (including references, excluding tables and legends) at the bottom of this page.

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

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

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

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

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