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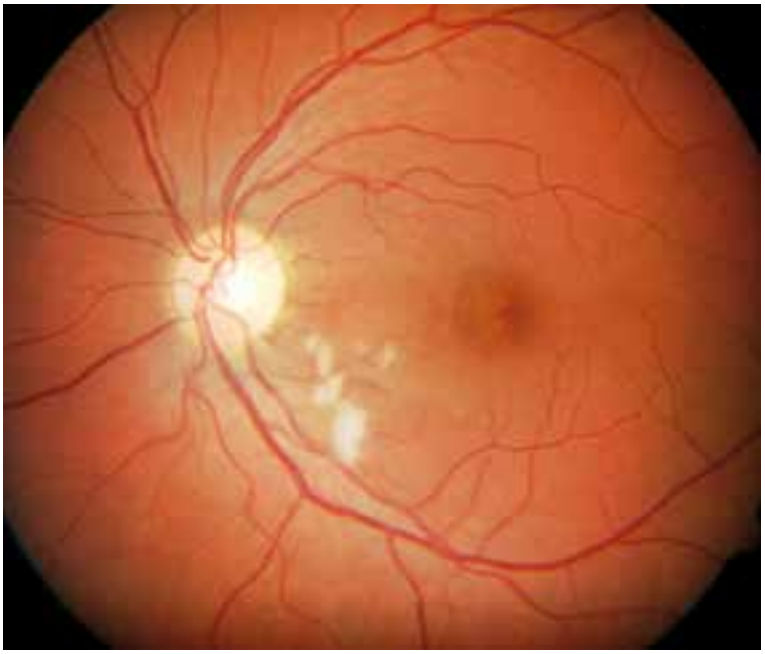

"Pancreatitis and sudden loss of sight: what is your diagnosis?"

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EDITORIAL

Molecular medicine: Promises and patience

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Knowledge of molecular genetics holds an incredible promise for clinical medicine. As many diseases are based on mutations in DNA, either congenital or acquired, specific interference in this DNA or in the downstream products coming from DNA translation may provide better treatment strategies for a myriad of diseases. The most challenging intervention would be to directly change host DNA, which so far has not been feasible in humans. However, interventions aimed at interference with DNA translation have recently been introduced in clinical medicine. In fascinating experiments in patients with Duchenne's muscular dystrophy administration of small strands of ologonucleotides interfered with mRNA splicing and corrected the reading frame by exon skipping of the mutated part of the dystrophin gene, causing a truncated (but biologically active) gene product rather than the inactive mutant protein. Initial clinical studies showed production of the new protein in boys with Duchenne's disease and a modest clinical improvement.[1] Also, gene therapy, i.e. administration of (viral) vectors containing human DNA encoding for a desired protein, has now entered a new phase with proven efficacy and increased safety, for example in rare lipid disorders or haemophilia B.[2,3] In addition, drugs interfering with defective gene products, translated into proteins that are responsible for dysregulation of cell proliferation and development of malignancies, have shown to be effective in oncology and haematology.[4-6] Lastly, identification of genetic mutations or variation may be used as risk stratifying marker in various diseases, including myeloproliferative disease, severe infections, thrombosis, or pancreatitis.[7-10] Taken together, clinical medicine is starting to experience the advantages that knowledge of molecular genetics may bring to improve clinical management.

However, we may have a very long way to go before we can fully translate even the molecular knowledge that has been accumulated so far into clinically applicable interventions.[11] As previously remarked, even for relatively simple genetic disorders, such as sickle cell disease, affecting hundreds of thousands of people worldwide and a monogenetic affection of which the genetic mutation was elucidated more than 50 years ago,[12] this very precise molecular knowledge has so far no effect at all on clinical management. In fact, despite all genetic preciseness patients with painful sickle cell crises are managed with intravenous fluids and painkillers.[13] Similarly, patients with primary haemochromatosis due to precisely defined gain of function mutations in genes involved in iron absorption are managed with blood letting, a therapy that has been with us since the middle ages.[14] Apparently, the gap between the discovery of the genetic base of a disease and the consequences for clinical management is large and it takes a lot of additional research and time before this gap can be bridged. And the given examples all represent monogenetic and relatively simple diseases, let alone the clinical consequences in terms of management of multigenetic disease, such as atherosclerosis and cancer. Nevertheless, it is clear that molecular genetic applications are seeping through into clinical medicine. In this issue of the Netherlands Journal of Medicine, three additional examples of how molecular genetics may innovate clinical medicine are provided.[15-17] Bins *et al.* demonstrate the utility of DNA vaccination, or genetic vaccination. In DNA vaccination immunity is induced by transfecting host cells with DNA that encodes an antigen instead of traditional vaccination by directly injecting antigens in the form of protein or peptide.[15] Once transfected, cells of the host start producing the protein encoded by the DNA leading to an immune response against this protein along similar lines as responses occur against conventional vaccines. The idea is that with DNA vaccination a more appropriate immune response is evoked. Initial studies have shown that DNA vaccination may be a helpful option for specific infectious diseases or for treatment of malignant disease, such as melanoma. Stroes *et al.* provide an overview on the efficacy and safety of treatment with antisense oligonucleotides.[16] Antisense therapy is based on base-pair hybridisation through which antisense oligonucleotides (ASOs) highly

specifically bind to its complementary mRNA target. Subsequent selective cleavage of the target mRNA leads to a corresponding reduction in target protein. Indeed, several studies, including human studies, have shown that ASOs can potently and selectively inhibit the synthesis of a protein of interest. In the article by Stroes *et al*. the application of antisense drugs in the management of lipid disorders is reviewed, whereas other clinical applications of antisense that are currently being developed are in the area of antithrombotic interventions, oncology and diabetes.[18,19] Lastly, De Graaff *et al*. present an article on the clinical applicability of pharmacogenetics.[17] Indeed, our knowledge on genetic variation as a predictor of drug efficacy but also occurrence of major drug-induced adverse events is rapidly increasing. However, it is not always clear whether this knowledge is clinically relevant. Graaff *et al*. provide data on CYP450 and HLA genotypes relevant to the 100 most commonly used drugs. They discuss the availability and costs of pharmacogenetic testing, show a calculation of the 'number needed to genotype' and, based on these data, they propose a decision model for pharmacogenetic testing by clinicians.

Based on all these new developments, it may be concluded that the steep increase in knowledge on molecular genetics has increasing impact on practical clinical medicine. It also demonstrates that fundamental research is crucial for further development of our insight into normal biology and disease but translational research to bring these results to practical solutions for patients is just as critical and may require major investment and a lot of patience.

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REVIEW

Recent advances towards the clinical application of DNA vaccines

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ABSTRACT

DNA vaccination is an attractive method for therapeutic vaccination against intracellular pathogens and cancer. This review provides an introduction into the DNA vaccination field and discusses the pre-clinical successes and most interesting clinical achievements thus far. Furthermore, general attributes, mechanism of action and safety of DNA vaccination will be discussed. Since clinical results with DNA vaccination so far show room for improvement, possibilities to improve the delivery and immunogenicity of DNA vaccines are reviewed. In the coming years, these new developments should show whether DNA vaccination is able to induce clinically relevant responses in patients.

KEYWORDS

DNA vaccination, cancer vaccination, infection diseases

WHAT IS DNA VACCINATION?

DNA vaccination, or genetic vaccination, is the common name for vaccination methods that induce immunity by transfecting host cells with DNA that encodes an antigen, rather than by injecting antigens in the form of protein or peptide. Once transfected, cells of the host start producing the protein encoded by the DNA leading to an immune response against this protein along similar lines as responses that occur against conventional vaccines. Nonetheless DNA vaccines have clear advantages that we will address in the next paragraphs.

Currently, no DNA vaccines are registered for use in humans. Although some DNA vaccines are registered for veterinary use (e.g. a prophylactic West Nile virus DNA vaccine for horses[1,2] and a therapeutic DNA vaccine for melanoma in dogs[3,4]) and plenty of reports show efficacy of DNA vaccination in non-human primates, evidence for efficacy in humans is scarce. However, there is an ongoing research effort aimed at putting the DNA vaccination platform to human use. Based on these studies the advantageous attributes of DNA vaccines have become clear.

GENERAL ATTRIBUTES OF DNA VACCINES AND ADVANTAGES OVER ALTERNATIVE VACCINATION PLATFORMS

In contrast to the complicated processes needed for vaccines such as attenuated viruses or subunit protein vaccines, plasmid DNA (pDNA) is easy to design and construct. Moreover, it is cheap and also relatively easy to manufacture. Furthermore, pDNA is fairly stable at room temperature[5] again in contrast to attenuated viral vaccines, whose storage and global delivery are complicated by the need to keep the vaccines cold. Another attractive feature of DNA vaccines is that, since the antigen is made in situ, it will inherently get post-translationally modified in a similar way as during infection with the cognate pathogen. Another important attribute of DNA vaccination is that the protection induced by DNA vaccines tends to skew towards cellular immunity, which is believed to be crucial for successful vaccination against intracellular pathogens (e.g. viruses) and cancers.[6,7]

Apart from these minor advantageous attributes, one feature of DNA vaccines stands out in comparison with vaccines based on vector systems such as modified vaccinia Ankara (MVA) and adenoviruses. This concerns

so-called 'vector-specific immunity': the phenomenon that pre-existing immunity against the vector decreases the efficacy of the vaccine. For example, in a study using an MVA-based vector, patients with pre-existing immunity against smallpox responded significantly less to vaccination than individuals without pre-existing immunity against the vector.[8] It should be noted that a bias was present in this study though, as older patients were overrepresented in the group with pre-existing vector-specific immunity (smallpox immunisation was terminated in 1980) and ageing is associated with decreasing immune responses. However, in a study using an adenoviral-based HIV vaccine an attenuating effect of pre-existing adenoviral immunity was observed as well.[9] A variant to this phenomenon is the situation in which, during consecutive boosts, the immunity directed against irrelevant components of a vaccine hampers that against the relevant antigen, resulting in loss of efficacy.[10]

Besides loss of efficacy, vector-specific immunity may result in more serious side effects as illustrated in the STEP trial. This clinical trial used a modified adenovirus (strain Ad5) encoding HIV antigens[11] and included individuals with both high and low titres of pre-existing antibodies directed against Ad5. The study was stopped for futility (i.e. it was obvious that vaccine efficacy would not be demonstrable). However, after extended follow-up, vaccinees with pre-existing anti-Ad5 antibodies showed higher infection rates with HIV within 18 months after vaccination than those in the placebo group.[12] In individuals with low anti-Ad titres, no difference in HIV infection rate was observed. It has to be noted that despite substantial efforts, no causal link has been established between Ad5 seropositivity and HIV infection (PLoS One. 2012; 7(4): e33969). Nevertheless, these results highlight the need to increase our understanding of the role of anti-vector immunity in adenoviral- and MVA-based vaccination.

Contrary to adenoviral- and MVA-based vaccines, DNA vaccines consist simply of naked pDNA. Sometimes it is formulated with a synthetic carrier, but it never contains other antigens. Vaccination only leads to the production of those proteins that are specifically desired for the immune response. Hence, vector-specific immune responses do not arise. This facilitates regimens based on multiple consecutive boosts. Based on the fact that the majority of the current human vaccines require two or more administrations, it is unlikely that a single administration will suffice for novel vaccines against difficult targets such as HIV, mycobacteria or cancer. For such targets, a scenario in which multiple boosts are required during a lifetime for optimal protection seems more realistic. Even more so if protection depends on cellular immunity, as in the process of ageing cellular immunity wanes considerably.[13] Currently, only DNA, RNA, subunit and (long) peptide vaccines provide optimal 'boostability' in this respect since these platforms do not expose the vaccinee to potentially immunogenic moieties other than the antigen of choice.

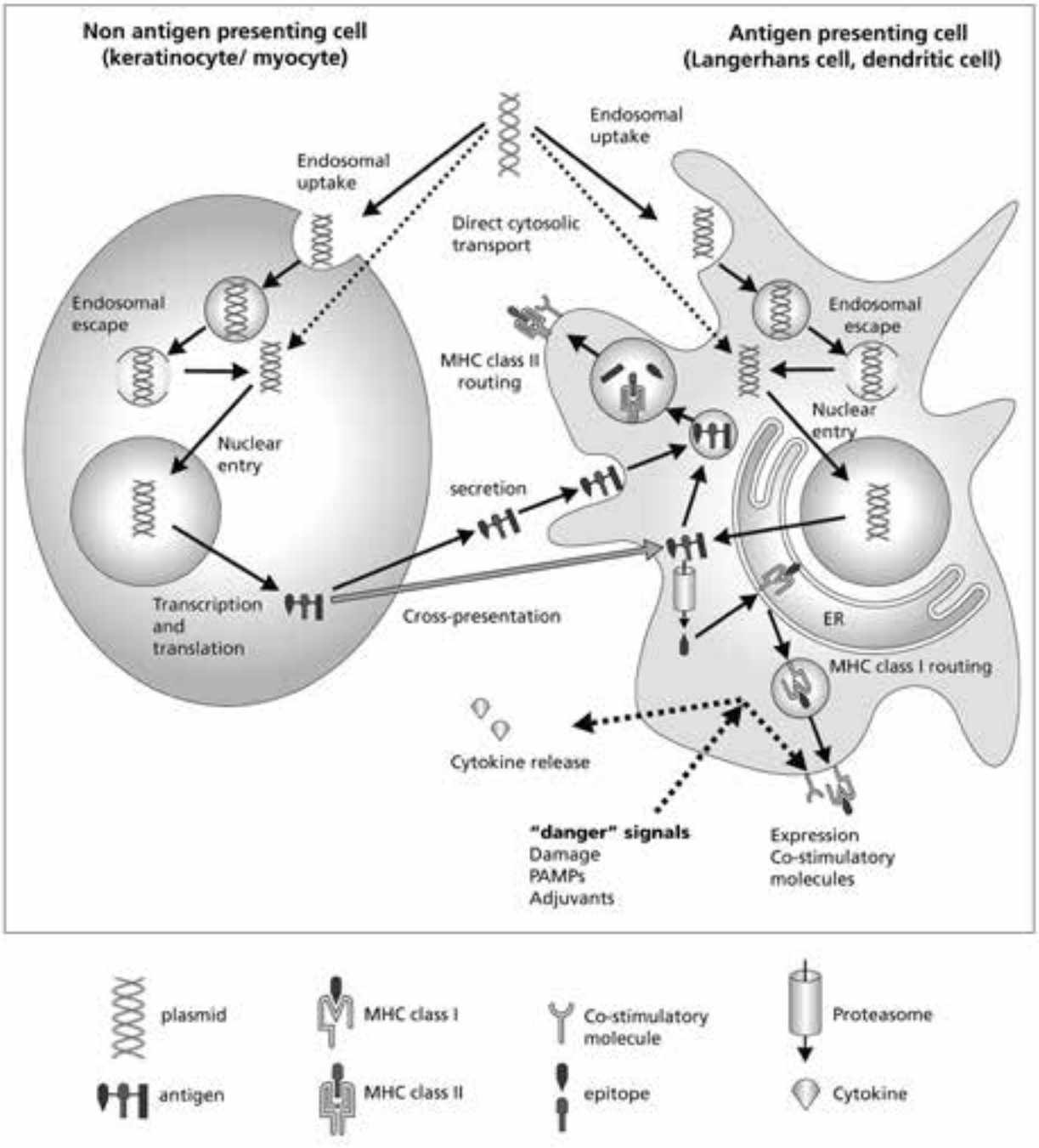
MECHANISM

More than 20 years after its introduction, the mechanism of B- and T-cell induction after DNA vaccination is still only partly understood. Initial reports suggested a mechanism where the injected DNA transfected professional antigen presenting cells (pAPCs) present at the injection site, leading to their maturation, migration to the draining lymph node (DLN) and subsequent priming of T and B cells.[14] However, this notion was contradicted by reports showing that DNA vaccines encoding antigens driven by myocyte-specific promoters were equally immunogenic as those driven by ubiquitous promoters.[15] Since myocytes can neither migrate to the DLN nor prime T cells (they do not express co-stimulatory molecules) it quickly became clear that pAPCs can ingest the antigen produced by myocytes and then prime the ensuing immune response. Surprisingly, apart from priming B and $CD4^+$ T cells as part of a humoral response, these pAPCs also primed a strong cellular response consisting of $CD8^+$ T cells.

Why is this surprising? Exogenous antigen ingested by pAPCs localises to endocytic vesicles that fuse with the lysosome, leading to degradation of the contents. The antigenic fragments then get loaded onto MHC-II molecules to form an MHC-II–peptide complex that is targeted back to the cell membrane in order to stimulate cognate $CD4^+$ T cells in the DLN (which in turn provide help for B-cell priming). The fact that these vesicles are known not to contain MHC-I molecules and hence do not generate MHC-I–peptide complexes for $CD8^+$ T cell stimulation made this 'priming pathway' hard to swallow as the pathway responsible for $CD8^+$ T-cell priming. However, in recent years various molecular mechanisms have been described that explain how antigens ingested by pAPCs are presented on MHC-I, leading to CD8 T cell stimulation. These mechanisms are referred to as 'cross-presentation'. The mechanism in which pAPCs get transfected themselves (the standard pathway for generation of MHC-I–peptide complexes in any cell) instead of ingesting the antigen, is referred to as 'direct presentation'. Currently, it is clear that both pathways contribute to T-cell priming by DNA vaccines (See *figure 1* for a schematic diagram of antigen expression and presentation upon DNA vaccination).

In DNA vaccination the amount of antigen produced is very limited due to the low transfection efficiency of host cells by the pDNA. This limitation makes the quantity of antigen that is expressed by the transfected tissue the

Figure 1. *Schematic diagram of antigen expression and presentation upon DNA vaccination. DNA is taken up by cells via endocytosis or via direct cytosolic uptake. After endosomal escape, cytosolic trafficking and nuclear entry, the pDNA can be transcribed into mRNA, followed by intracellular translation of the antigen. For T-lymphocyte activation, antigens have to be presented in the context of MHC class I or MHC class II molecules in the presence of co-stimulatory molecules (such as CD80/86 and CD40). Since professional antigen presenting cells (pAPCs) are the only cell type that have both classes of MHC, can express co-stimulatory molecules and can migrate to the lymphatic system, their role in antigen presentation and T-lymphocyte activation is crucial. Via extracellular release by non-pAPCs or cell death, antigens can enter the MHC II pathway. Antigens produced by direct transfection of pAPCs are presented by MHC I. In addition, antigen intracellularly produced by non-pAPCs can enter the MHC class I pathway in pAPCs by a process called cross-presentation. (Reprinted from reference 58, with permission from Informa Healthcare)*



Achilles heel of the method. Therefore, it is crucial that the little antigen that is produced is maximally available for antigen presentation by pAPCs. As efficient presentation along each route requires different antigenic properties, it is very important to know whether priming occurs optimally via direct or cross presentation. At the moment this is unclear. Neither do we know what circumstances favour each pathway.

Besides the availability of antigen, both cross and direct presentations require maturation signals for the pAPCs in order to present the antigen. During infection with a pathogen these signals are provided by non-self 'molecular patterns' on the pathogen that are perceived by receptors on the pAPCs. These 'pathogen-associated molecular patterns' (PAMPs) ligate 'pattern-recognition receptors' (PRRs) on pAPCs leading to their maturation and migration to the DLN. Examples of common PAMPs are double-stranded RNA, components of the cell wall of gram-negative bacteria (LPS) and DNA sequences containing unmethylated cytosine-guanine DNA sequences ('CpG' sequences). These molecules are all scarce or absent in the host and abundant in viruses and bacteria. Hence they have evolved to provide the first 'danger signal' to be perceived by the host immune system upon infection. This signal appears to be indispensable for mounting a B- or T-immune response.

In vaccines that do not contain live attenuated pathogens, this 'danger signal' is provided by so-called 'adjuvants'. These are substances that are added to the formulation in order to trigger the PRRs of pAPCs, thereby providing the danger signal leading to their maturation and migration to the DLN. Contrary to this, DNA vaccines have long been thought to have adjuvancy by their own merit. Due to its bacterial origin, *pDNA* is abundant in unmethylated CpG sequences, which ligate a PRR on the pAPC that is named 'Toll-like receptor 9' (TLR9).

Surprisingly, DNA vaccination studies in TLR9 deficient (-/-) and proficient (+/+) mice have not pointed out a clear role for TLR9 and CpG motifs. Babiuk *et al.*[16] found that TLR9+/+ and TLR9-/-mice mounted immune responses of similar potency. Most reports show that activation via TLR9 is not necessary for the generation of immune responses by DNA vaccines,[17,18] but does increase their potency to some extent. An explanation that accounts for this is that although CpG-mediated activation of DCs plays a role, other danger signals are generated during transfection of host tissue, making the TLR9 signal less critical. For example, in keratinocytes the presence of pDNA in the cytoplasm can be detected by other molecular sensors such as DAI (DNA-dependent activator of IFN-regulatory factors) and AIM-2 (absent in melanoma-2),[19] resulting in the activation of a cell stress signalling complex named the inflammasome and leading to the production of immunogenic cytokines such as IL-1 and IL-18.

INTERESTING TRIAL RESULTS

A number of human clinical trials of DNA vaccines have been performed or are ongoing for infectious diseases, as well as cancer and autoimmune diseases (reviewed in references 20 and 21). We will briefly summarise some of the results below.

After an initial series of disappointing clinical trials in the early 1990s, the first moderately successful DNA vaccination result in humans was obtained with a malaria-specific DNA vaccine.[22] This trial demonstrated the emergence of vaccine specific T cells in the peripheral blood of 11 out of 20 malaria-naive volunteers after three intramuscular pDNA injections. The study did not assess clinical benefit. Similarly, gene gun administration of HBV DNA was able to induce antibodies in 12 out of 16 patients who had not responded to the licensed (recombinant protein based) vaccine.[23] Although protection against HBV was not assessed, the induced antibody titres were considered protective based on data obtained with conventional HBV vaccines.

Various other trials have provided proof of principle for the capacity of DNA vaccines to induce humoral and cellular immune responses in humans.[23-26] However, the immune responses measured were not as robust as anticipated from the preclinical studies in any of these trials. For example, HIV-infected patients with high viral counts mounted a modest T-cell response against HIV Nef after DNA vaccination with a DNA vaccine encoding several HIV antigens,[27] without any effect on viral counts.

In an effort to combine the qualities of DNA vaccines with those of adenoviral- or MVA-based vaccines, so-called 'heterologous prime-boost' regimens have been developed. In these regimens the strong but broad (partly vector-specific) immunity induced by MVA- or adenoviral-based vaccines gets focused on the relevant antigens by DNA vaccination, which is very specific but less potent. Usually, in these regimens the DNA vaccine comes first as a 'prime', followed by the vector-based vaccine as a 'boost'. Particularly for HIV, where T cells may be the key to protection, many such trials have been performed and are ongoing, utilising boosts with modified adenoviruses[28-30] or MVA.[8,31-36]

DNA priming followed by MVA boosting has been studied clinically[37-39] for malaria vaccination with moderate success. One study that included a live malarial challenge following immunisation demonstrated that a DNA prime encoding the ME-TRAP antigen followed by an (ME-TRAP recombinant) MVA boost resulted in partial protection from challenge with live parasite.[37]

Currently, worldwide 27 clinical trials involving DNA vaccination are ongoing (*table 1*, based on www.clinicaltrials.gov). Notably, 15 of the 27 clinical trials are directed against tumour antigens, of which five against virus (HPV) derived tumour antigens. This illustrates that over the

Table 1. *Number of trials worldwide involving DNA vaccination that are actively recruiting patients*

Disease	Number of trials currently open
HIV	8
CIN / Cervical cancer	4
Hepatitis B	1
Hepatitis C	1
HPV-related head/neck cancer	1
Influenza	1
Leukaemia	1
Lymphoma	2
Breast cancer	1
Prostate cancer	3
NET of skin (Merkel cell carcinoma)	1
Ovarian cancer	1
Pancreatic cancer	1
Allergy for Japanese Red Cedar	1

CIN = cervical intraepithelial neoplasia; HIV = human immunodeficiency virus.

last 20 years the focus of DNA vaccine development has shifted to tumour antigens, probably due to better funding opportunities in that field. In the Netherlands no clinical DNA vaccination studies are currently recruiting. However, in the first half of 2013 a phase I clinical trial will open at the Netherlands Cancer Institute/Antoni van Leeuwenhoek hospital for HPV-related cancer patients.

SAFETY IN PATIENTS

In general, DNA vaccines are considered safe for both patient and environment. Studies have reported good tolerability of DNA vaccines in humans and local reactivity at the injection site is the most commonly reported side effect in clinical trials thus far.[26,40-42] Nonetheless, a major concern of using DNA vaccines in the clinic is the potential risk that genetic information of the plasmid is integrated in the host genome of somatic cells. Genomic integration can occur during random or homologous recombinations and might lead to the activation of oncogenes or the inactivation of tumour suppressor genes, potentially resulting in neoplastic transformation. This remote risk is worth considering when DNA vaccines are applied for therapeutic vaccination in young patients.

To provide a context in the assessment of this risk, it is generally accepted to compare the integration rate of pDNA with the spontaneous mutation frequency of autologous genes. Although this spontaneous mutation rate varies largely between genes and individuals (based on external factors such as smoking or UV exposure) 2×10^{-6} spontaneous gene-inactivating mutations per gene is generally accepted as the standard value. This number is adapted from a study by Cole *et al.* in which the mutation frequency of three genes in circulating cells was determined in several hundred volunteers.[43]

To our knowledge, genomic integration of DNA vaccines in humans has never been studied in a similar setting, probably because of the difficulty to obtain a biopsy from the administration site. Nevertheless, several studies have analysed the integration of pDNA in animal models such as mice, rabbits and guinea pigs.[44-49] Although genomic integration could be confirmed in these animal studies, integration rates were always several-fold lower than the spontaneous integration rate. Furthermore, the probability that a random integration occurs at a growth-regulatory gene (thus initiating oncogenesis) is even lower, since many integration events will be innocuous. Multiplying the low levels of integration observed with the low probability of interfering with growth-regulatory genes results in an extremely low risk of oncogenesis. Using mRNA instead of plasmid could theoretically annihilate the risk of genomic integration.[50] Nevertheless, the high production costs and lower stability of mRNA constitute a limitation for the broad application of RNA vaccination.

Vertical transmission of vaccine-derived pDNA into germline cells is another potential risk of DNA vaccines. However, this only occurs when pDNA is injected directly into the gonads[51] and not when DNA vaccines are administered in other tissues. This means that vertical transmission is not a relevant risk when DNA vaccines are administered via the common intramuscular and intradermal delivery routes.

DELIVERY OF DNA VACCINES

As discussed above, the clinical responses upon DNA vaccination thus far are rather disappointing. To overcome this, the DNA vaccination field is putting a lot of focus on optimisation of the delivery methods, carrier molecules and genetic optimisation of the construct used. Two administration routes are commonly used for the administration of DNA vaccines: intramuscular (IM) and intradermal (ID). Upon IM administration, the encoded antigen will primarily be produced in myocytes that can potentially transfer their antigen to pAPCs for cross presentation. This administration route will result in the highest levels of antigen expression but may not be the most immunogenic, since the frequency of pAPCs in muscle tissue is rather low. Although ID delivery of DNA vaccines does not lead to the amount of protein production that is obtained upon IM injection, it is potentially much more immunogenic, since the skin is the natural port d'entrée of pathogens and full with pAPCs ready to take up and present antigens.

In the past decade a large number of technical devices have been developed for IM and ID delivery of DNA vaccines. The 'gene gun', also referred to as biolistic particle delivery system, is a commonly used tool. This so-called 'particle-mediated epidermal delivery' (PMED) method requires the pDNA to be coated onto cold particles in order to be shot into the skin.[52] In a similar fashion DNA can been shot in surgically exposed muscle tissue.

Furthermore, electroporation (EP) is used as a strategy to increase the transfection of DNA vaccines upon IM or ID administration. EP uses short electrical pulses to destabilise cell membranes. Under optimal conditions, this will lead to the formation of transient pores, which allows the entrance of macromolecules such as DNA into the cell. It is thought that electro-permeabilisation is followed by electrophoretic displacement of the negatively charged DNA molecule into the cytoplasm of the cell. Several research groups and companies are developing EP-based devices for the delivery of DNA vaccines and some of these devices have already been tested in the clinic.[26,42] Jet injection, ultrasound and micro needles are other mechanical delivery methods that are currently under development for the delivery of DNA vaccines.[53-55]

Our group has developed a technique called DNA tattooing for the intradermal administration of DNA vaccines.[56] We have shown that this strategy is highly immunogenic in mice and non-human primates.[57] The potency of DNA tattooing is probably mediated by the abundance of danger signals that are generated in the damaged skin upon mechanical disruption by the tattoo needles. Clinical trials that are currently running should prove whether DNA tattooing is also immunogenic in patients.

In addition to technical delivery devices, naked pDNA is often formulated into a synthetic carrier molecule/ nanoparticle composed of lipids or polymers, in order to increase pDNA stability and cellular uptake (reviewed in reference 58).

EFFORTS TO IMPROVE DNA VACCINES

Another common way to improve DNA vaccines is to increase the immunogenicity of the encoded antigen. Roughly two methods can be distinguished, 1) the addition of genetic adjuvants and 2) the modification of the gene encoding the antigen itself. A genetic adjuvant is a protein with adjuvant properties that is encoded by the pDNA together with the antigen and hence co-expressed with the antigen, bolstering the immune response towards this antigen. Examples are GM-CSF[59-61] HGMB1[62] and IL-15.[61] Most often these adjuvants are encoded for by a separate plasmid that is admixed with the DNA vaccine. A more sophisticated way is to combine the genetic adjuvant and the antigen in one plasmid (i.e. in a bicistronic cassette). This last method ensures that any transfected cells express both the antigen and the adjuvant.

Many different modification strategies have been suggested in order to optimise the immunogenicity of the antigen itself. We shall briefly discuss three common approaches, i.e. codon optimisation,[63] addition of signal sequences[64,65] and genetic fusion to an entire protein or protein domain, referred to as a carrier protein. Codon optimisation means that the gene encoding the antigen is rewritten for optimal transcription and translation in the species that the vaccine is meant for. Within the redundancy of the genetic code the optimal tRNA for any amino acid varies from species to species. Especially when native prokaryotic or viral genes are used in DNA vaccines, codon optimisation can considerably augment its transcription in the eukaryotic cells of the vaccinated host.[66]

The addition of signal sequences can target the antigen to different subcellular compartments, thereby improving the immunogenicity of DNA vaccines.[64,65,67] Moreover, by the addition of motifs with affinity for receptors on pAPCs that are involved in antigen uptake, antigens may be targeted

Table 2. *Selected examples of carrier proteins known to improve the immunogenicity of HPV16 E7 or E6 encoding DNA vaccines*

Carrier protein	Antigen	Proposed mode of action	Reference
Mycobacterium tuberculosis HSP-70	E7	Provision of $CD4^+$ T-cell help, increased antigen uptake by DC	Chen et al., 2000
Heat shock protein 60	E6, E7	Increased antigen uptake by DC	Huang et al., 2007
Calreticulin	E6, E7	Targeting of antigen into the antigen presentation pathway	Cheng et al., 2001; Peng et al., 2004
Extracellular domain of Flt3 ligand	E7	Altered subcellular localisation/ increased antigen uptake by DC	Hung et al., 2001b
HSV VP22	E7	Antigen spreading, improved antigen stability	Michel et al., 2002
E. coli β-glucoronidase	E7	Enhanced stability/ altered subcellular localisation	Smahel et al., 2004
Pseudomonas aeruginosa exotoxin A (domain II)	E7	Enhanced cross presentation	Hung et al., 2001a
Invariant chain with PADRE epitope insertion	E6	Provision of $CD4^+$ T cell help	Wu et al., 2011
IP-10	E7	Enhanced antigen presentation, chemoattraction	Kang et al., 2011
TTFC	E6, E7	Provision of $CD4^+$ help, increased antigen stability	Oosterhuis et al., 2011; Stevenson et al., 2004

to pAPCs with the intention to make their presentation more efficient.[68]

Fusion of the antigen to a carrier protein is another trick that is often employed in the design of DNA vaccines. To illustrate this, *table 2* summarises popular carrier proteins used for fusions with HPV-16 E6 and E7. Although many different mechanisms have been postulated to explain the positive effect of such fusions on the immunogenicity of an antigen, we propose three mechanisms to be most important in this respect. Firstly, genetic fusions often affect the half-life of the antigen.[69-71] We have shown that antigen half-life is a critical determinant of DNA vaccine immunogenicity.[72] Allegedly this is because antigens that accumulate in the transfected cell are more efficiently cross-presented. Secondly, these carriers are often foreign proteins that are likely to contain $CD4^+$ helper epitopes. Since DNA vaccine-induced $CD8^+$ T-cell responses are strictly dependent on $CD4^+$ T-cell help (as illustrated by the fact that MHC class II deficient mice do not mount detectable T-cell responses upon DNA vaccination[73]) at least part of the potentiating effect of any foreign carrier protein can be explained by the addition of $CD4^+$ T-cell help. Thirdly, a carrier protein can affect the subcellular localisation of the antigen. Many of the commonly used carrier proteins (such as calreticulin and invariant chain) are likely to impact on the subcellular localisation of the antigen and hence may act via this mechanism. In this regard, localisation of E6 and E7 to the endoplasmic reticulum (ER) of a cell can increase the T-cell responses measured in peripheral blood by an unknown mechanism.[74,75]

We have recently developed so-called modular DNA vaccines[74] and demonstrate that the addition of ER localisation/retention signals combined with a set of minimal $CD4^+$ T-cell epitopes can tremendously improve the immunogenicity of HPV16 E6 and E7 encoding DNA vaccines. The key advantage of this approach is that besides the antigen only minimal additional sequences are added, thereby preventing off-target immune responses. In conclusion it can be said that many different strategies can be applied to improve the immunogenicity of antigens encoded in DNA vaccines. As the molecular mechanisms are being unravelled, the opportunities to rationally improve DNA vaccines become manifold.

CONCLUSIONS

As discussed, DNA vaccines form an interesting platform for therapeutic vaccination against viral infections and cancers. Since mice to men translation appears to be extremely complex in DNA vaccination, future widespread clinical application depends on the successful development of new delivery techniques, adjuvants and the genetic optimisation of the plasmids used. Hopefully these improvements will eventually lead to DNA vaccine products that are immunogenic enough to be applied as a standalone modality in the clinic.

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REVIEW

Antisense oligonucleotides in the treatment of lipid disorders: Pitfalls and promises

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ABSTRACT

Dyslipidaemia is one of the pivotal risk factors for cardiovascular disease (CVD), and lipid-lowering therapy is therefore the cornerstone in cardiovascular risk management. With the currently available treatment options the relative risk reduction in CVD is approximately 30%, leaving a large residual risk. This calls for the development of additional therapeutic moieties and antisense oligonucleotides (ASOs) have proven to be such a new and effective treatment. ASOs are short single strands of DNA that intracellularly bind mRNA of specific proteins. This induces the degradation of the mRNA through which the protein cannot be produced. Based on knowledge of lipid metabolism several targets of ASO therapy can be identified. This review offers a summary of current developments in ASO therapy regarding lipid disorders.

KEYWORDS

Antisense oligonucleotides, mipomersen, lipid disorders, PCSK9

INTRODUCTION

Cardiovascular disease (CVD) is an important cause of mortality and morbidity in Western countries. Hypercholesterolaemia is an important risk factor for CVD, which is exemplified by the finding that patients with familial hypercholesterolaemia (FH) are at highly increased risk of CVD. In line, firm reduction of low-density lipoprotein cholesterol (LDL-C) results in reduction of CVD risk. Statins (HMG CoA reductase inhibitors), which strongly diminish LDL-C, are therefore widely accepted as the first-line treatment in patients with increased CVD risk. Despite optimal statin therapy, however, a substantial number of these patients will eventually experience a cardiovascular event (e.g. myocardial infarction or stroke). Apparently, the 30-50% reduction of LDL-C by statins is not able to abolish the risk of CVD. This is partly due to the fact that maximal dosages of statins, whether or not in combination with other lipid-lowering drugs, are not capable of lowering LDL-C to target levels in some of the patients. Moreover, increasing the dose often results in cumulative side effects which hinder dose augmentation.

Hence, there is a need for the identification of novel therapeutic moieties that lower LDL-C on top of statin therapy. Antisense oligonucleotides (ASOs) are considered a potential novel lipid-lowering therapy, which acts via a different mechanism than in statins. In this review we describe the mechanism of action of ASOs and provide an overview of ASOs with potential therapeutic value in the treatment of lipid disorders.

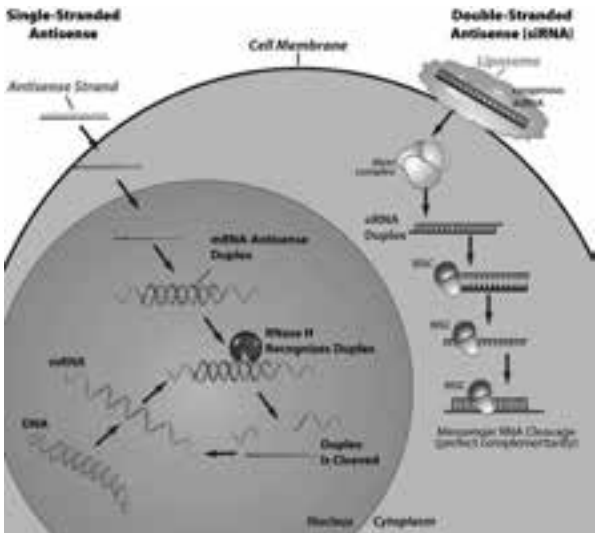
CURRENT TREATMENT OPTIONS IN LIPID DISORDERS

A decrease of LDL-C by 1 mmol/l (38.67 mg/dl) in patients with increased CVD risk reduces CVD risk by 22%.[1] In past decades the effect of statins on LDL-C and subsequent CVD risk has been extensively investigated. Statins lower LDL-C by 30-60% and CVD risk by 25-30%.[2] Unfortunately statin therapy is accompanied by side effects in a minority of subjects; 10% of treated patients develop muscle pain, which leads to discontinuation of treatment in a third of those patients.[3] In addition, a considerable number of

patients do not attain the LDL-C treatment goal despite optimal statin therapy.[4] Additional lipid-lowering drugs such as ezetimibe and fibrates only result in modest additional reductions of LDL-C. Moreover, CVD risk reduction by these drugs remains to be demonstrated.

Another lipid disorder which has gained attention is the presence of decreased high-density lipoprotein cholesterol (HDL-C).[5] HDL particles play an important role in the 'reverse cholesterol transport'. By this mechanism cholesterol is taken up from peripheral cells and transported to the liver where it is excreted via the faeces. There is a strong, inverse correlation between HDL-C and CVD which is independent of LDL-C concentration.[6] These epidemiological findings have directed the attention to HDL-increasing therapies as a promising target in CVD prevention.[5] Whether HDL increase reduces CVD risk, however, remains to be demonstrated. An example of an HDL-increasing drug is niacin which enhances HDL-C by 26% with an additional decrease in both LDL-C and triglycerides of 20%.[7] Its effect on cardiovascular disease was examined in the AIM HIGH study that was terminated prematurely, prompted by inefficacy and an unexpected high number of ischaemic strokes among treated patients.[8] More recently, the effect of niacin on cardiovascular events was addressed in the HPS2-Thrive study (ClinicalTrials.gov: NCT00461630). This study was halted by the US Food and Drug Administration (FDA) due to futility.[9] In the same year, the research on dalcetrapib, a selective HDL-C increasing drug that works by inhibiting CETP activity, was also terminated since there was no significant decrease in CVD risk.[10] Although other studies on CETP-increasing drugs, such as the CETP inhibitor anacetrapib, are still ongoing, it is safe to state that the bar for novel HDL-C raising agents in CVD prevention has been raised significantly.

Figure 1. *Steps of RNase H (A) and siRNA (B) antisense mechanisms*



The single-stranded DNA oligonucleotide passes through the cell membrane and enters into the cytoplasm of the cell. Although the RNase H enzymes are present in the cytoplasm and the nucleus of the cell, data suggest that RNase H oligonucleotides work predominantly in the nucleus. The oligonucleotide enters into the nucleus from the cytoplasm. Once in the cell nucleus, the oligonucleotide binds or hybridises to the target mRNA, resulting in the formation of a sense–antisense duplex. The formation of the duplex initiates the recruitment of the RNase H enzyme, an endogenous nuclease. RNase H degrades the target mRNA, which results in inhibition of target mRNA expression. The oligonucleotide moves on and then hybridises to another target mRNA.
The RNAi oligonucleotide (siRNA) passes through the cell membrane and enters into the cytoplasm of the cell. A helicase separates the sense and antisense strands of the oligonucleotide. RISC, an endogenous conglomerate of functional components, associates with the antisense oligonucleotide. The antisense strand of the oligonucleotide hybridises to the target mRNA, resulting in the formation of a sense–antisense duplex. The nuclease component of RISC is anendogenous nuclease that degrades the target mRNA. This results in the inhibition of target mRNA expression.

RISC: RNA-induced silencing complex; RNAi: RNA interference; siRNA: Small interfering RNA. From: Crooke RM. Antisense oligonucleotides as therapeutics for hyperlipidaemias. Expert opinion on biological therapy. 2005 Jul;5(7):907–17.

ANTISENSE OLIGONUCLEOTIDES

ASOs are short single strands of DNA comprising 12-20 nucleotides. This provides a highly specific complementary binding to mRNA. The binding prevents translation of mRNA, thereby preventing translation and the production of the respective proteins.[11] ASOs can exert their function in two ways (*figure 1*). A general advantage of ASOs in the treatment of lipid disorders is that they mainly accumulate in the liver where the majority of the proteins involved in lipid metabolism is also produced. Moreover, ASOs are not metabolised by cytochrome P450 as most other drugs, which reduces the risk of drug-drug interactions.[12] The binding of ASOs to mRNA of one specific protein is another valuable characteristic, which can be expected to decrease the chances of inducing unspecific side effects.

A disadvantage of ASOs is their rapid degradation by nucleases. To circumvent this, various chemical modifications have been designed to stabilise ASOs and promote their cellular uptake.[13] Because ASOs selectively inhibit the production of selected proteins, specific targets can be identified based on knowledge of the lipid metabolism. Below is a review of targets investigated thus far and ASOs developed against these targets.

POTENTIAL TARGETS AND AVAILABLE ASO'S

ApoB-100

ApoB-100 is an essential protein in all pro-atherogenic lipoproteins (e.g. VLDL, LDL and Lp(a)).[14] Patients

expressing increased apoB-100 are characterised by increased CVD risk. In contrast, genetically decreased apoB-100 offers protection against CVD.[15]

Mipomersen: Mipomersen (ISIS 301012) is one of the first ASOs specifically targeted against apoB-100.[16] It is administered subcutaneously followed by almost complete absorption. Due to minimal degradation by endonucleases it exhibits a relatively long half-life of 23-46 days. Following absorption, highest concentrations are measured in liver and kidneys. LDL-C reductions of 44% were achieved with 200 mg administered once a week subcutaneously, whereas on top of statins mipomersen decreased LDL-C by 36%.[17,18] In phase III studies identical dosages during 26 weeks abated both LDL-C and apoB by 36% in patients with severe hypercholesterolaemia or increased CVD risk.[19] Most of the side effects reported are related to injection site reactions. These are experienced by up to 90% of patients, comprising erythema and itching. Of the patients 50% also report flu-like symptoms.[20] Another significant side effect of mipomersen relates to its mechanism of action, namely the decreased excretion of VLDL. In 10% of patients a steatotic response of the liver was observed, which was shown to be reversible after discontinuation of treatment.[21] Increased liver transaminases were associated with hepatic steatosis.[18] Noteworthy, there were no signs of impaired liver synthesis function (PTT, bilirubin).[22]

ISIS 147764: A second ASO against apoB-100 is ISIS 147764. Thus far its effects have only been investigated in mice. In LDL-receptor knockout mice 12 weeks of treatment resulted in LDL-C reduction of 60-90% with a concomitantly decreased formation of atherosclerotic lesions.[23]

PCSK9

PCSK9 promotes the degradation of LDL receptors.[24] Gain-of-function mutations in the gene encoding this protein result in low abundance of hepatic LDL receptors and consequently increased levels of LDL-C in serum and eventually premature CVD. Conversely, loss-of-function mutations are accompanied by decreased levels of LDL-C and decreased risk of CVD.[25] These observations led to development of antibodies targeted against PCSK9, which have shown very positive effects on LDL-C in humans.[26] ASOs against PCSK9 are expected to offer potent LDL-C lowering effects, comparable with those reported following the treatment with PCSK9 antibodies. In a mouse model these ASOs produced a strong reduction in PCSK9 mRNA (92%) with a concomitant doubling of LDL receptors. This resulted in an LDL-C decrease of 38%.[27]

Apo(a)

Lipoprotein (a) (Lp(a)) is an important risk factor for myocardial infarction and other forms of coronary diseases.[28] Lp(a) is formed by the fusion of apolipoprotein (a) and apoB-100 containing lipoproteins (e.g. LDL). To date, there are no treatments to lower Lp(a) besides niacin.[29] Therefore it remains unclear whether Lp(a) reduction will lead to decreased CVD risk. Apolipoprotein (a) is assimilated in the liver and an ASO was developed against the responsible gene.[16] In mice this ASO diminished Lp(a) by 27% and total cholesterol by 22%. Interestingly, mipomersen, the apoB antisense, also lowers Lp(a).[30]

Apolipoprotein C-3

The apolipoprotein C-3 (apoC-3) is present on triglyceride-rich lipoproteins, such as VLDL. ApoC-3 impairs clearance of these particles by inhibition of the enzyme lipoprotein lipase (LPL) which has a triglyceridase effect. Patients with a genetic defect in the gene encoding apoC-3 are characterised by decreased levels of VLDL and triglycerides.[31] Furthermore these patients exhibit low coronary calcium scores, a surrogate marker for atherosclerosis. ASOs directed against apoC-3 theoretically possess therapeutic effects in patients with increased levels of triglycerides. In mice studies treatment with such ASO resulted in 90% lower apoC-3 mRNA and subsequently 80% and 95% decreased levels of triglycerides in serum and liver respectively.[11] Currently the ASO 'ISIS APOCIII Rx', targeted to apoC-3, is being examined in a phase II study.

Diglyceride acyltransferase (DGAT2)

DGAT is the enzyme catalysing the last step in the synthesis of triglycerides. It is expressed in the liver, small intestine and adipose tissue. Two subtypes of DGAT (DGAT1 and DGAT2) have been identified. DGAT2 has been shown to play a role in cholesterol metabolism as shown in several mice studies.[32] Due to its central role in triglyceride metabolism, inhibition of DGAT2 is suggested to result in decreased levels of triglycerides. To verify this hypothesis the effect of the ASO ISIS-217376 targeted against DGAT2 was investigated in mice.[32] It abates mRNA of DGAT2 in liver and adipose tissue by 75% while leaving expression of DGAT1 unaltered. Hepatic synthesis of triglycerides decreased significantly and this led to diminished secretion of triglycerides. A positive effect on lipid profile and hepatic steatosis was also observed. Antisense therapy against DGAT2 appears to have a selective effect on cholesterol metabolism which makes DGAT2 an interesting target in patients with lipid disorders.

miRNA 33a/b

ATP binding cassette A1 (ABCA1) is a transmembrane protein which exerts essential functions in cholesterol transport from peripheral cells to HDL particles.

Deficiency of this protein results in Tangier disease which is characterised by, among other things, extremely low levels of HDL-C and increased CVD risk.[33,34] Production of ABCA1 is inhibited by microRNAs 33a and 33b (miRNA 33a/b). Of note, the latter does not only inhibit ABCA1 but also other genes involved in lipid metabolism.[35] Rayner and colleagues showed an increased expression of ABCA1 in monkeys after treatment with an ASO directed against miRNA33a/b.[35] This translated into diminished plasma levels of VLDL and triglycerides (50%) and increased levels of HDL-C (50%). MiRNA 33a/b is therefore a promising target in the treatment to promote cholesterol efflux.

CONCLUSION

ASOs provide a potentially novel therapeutic paradigm allowing highly specific inhibition of selected proteins. They are considered a promising moiety to treat a wide variety of lipid disorders due to the fact that ASOs accumulate in the liver, which is also the major orchestrator of lipid metabolism. Since ASOs retain their efficacy on lipid levels, also on top of statin therapy, they hold a promise as add-ons in patients not reaching their target LDL-C despite maximum lipid-lowering therapy or those experiencing serious side effects on currently available lipid-lowering drugs. Mipomersen, the most advanced ASO decreasing apoB production in the liver, is developed for use in patients with homozygous and/or severe heterozygous FH. In view of the less favourable side effect profile, comprising injection site reactions and liver steatosis, further efforts should be aimed at reducing these side effects. In view of the high similarity of the production process of the various ASOs, it is to be expected that the development of novel ASOs for clinical use can be performed in an expedited manner.

Disclosures

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REVIEW

Blood loss after cardiopulmonary bypass, standard *vs* titrated protamine: A meta-analysis

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ABSTRACT

Background: The aim of this meta-analysis was to determine whether standard or titrated dosing of protamine is more effective in facilitating haemostasis after cardiac surgery with cardiopulmonary bypass (CPB).
Methods: We searched MEDLINE, and Biomedical Central using the terms "cardiopulmonary bypass and heparin and protamine". Studies were included in the meta-analysis if they were randomised controlled trials (RCTs), controlled clinical studies, or cohort studies with designs comparing the postoperative volume of bleeding between the study group (titrated dose) and the control group (standard dose) for protamine reversal of surgical anticoagulation in CPB procedures. The primary outcome of interest was postoperative blood loss.
Results: There were 219 studies identified in the initial search; four of these were included in the meta-analysis. All studies were RCTs, involving a total of 507 patients. Postoperative blood loss was lower in the study group (range: 625-839 ml) compared with the control group (range: 765-995 ml) in all four studies. Transfusion of packed red blood cells was also lower in the study group compared with the control group in all four studies. There was no evidence of significant heterogeneity in postoperative blood loss among the four studies ($Q=4.224$, $I^2=28.98\%$, $p=0.238$); hence, a fixed-effects model of analysis was used. The overall/combined standardised difference in means of postoperative blood loss volume significantly favoured study treatment over control treatment (-0.562 ± 0.322, $p<0.001$).
Conclusion: These findings suggest that titrated protamine dosing is more effective than standard protamine dosing for reducing postoperative bleeding after CPB.

KEYWORDS

Cardiopulmonary bypass, heparin, protamine, standard, titrated

INTRODUCTION

Management of haemostasis is a key facet of any surgical procedure. To this end, balancing the risk of thromboembolism with that of excessive bleeding is paramount. A number of anticoagulants are commonly used to reduce the risk of thromboembolism. These include heparin, vitamin K antagonists, such as warfarin, and antiplatelets, such as aspirin.[1] After surgery, the risk of bleeding complications may outweigh that of thromboembolism; hence, reversal of haemostasis is often warranted in patients taking anticoagulants. For patients on vitamin K antagonists, vitamin K or vitamin K-dependent coagulation factors may be given to ameliorate the anticoagulant effect of the antagonist.[1] For patients on antiplatelet drugs, amelioration of the anticoagulant effects may be achieved by the administration of platelet concentrate or de-amino d-arginine vasopressin (a vasopressin analogue).[1] In contrast, protamine sulphate is often used to counteract the effect of heparin.

Heparin is routinely administered prior to and during cardiopulmonary bypass (CPB) surgery to reduce the risk of thromboembolism. After surgery, however, the anticoagulant activity of heparin requires neutralisation to promote haemostasis and reduce the risk of bleeding. To this end, protamine is typically used to reverse the anticoagulant activity of heparin after CPD. Protamine exerts this effect by binding heparin (1:1 ratio) to form an inactive complex.[2,3] Approximately 1 mg of protamine has the capacity to rapidly neutralise 100 units of heparin.[3]

Although protamine is a very effective and generally safe means of reversing the anticoagulant activity of heparin, associated adverse events are not uncommon, occurring in slightly over 2% of patients after CPB.[4,5] These adverse events include various haemodynamic changes, pulmonary oedema, and anaphylactic reactions.[4-6] An increased risk of in-hospital mortality has also been reported in patients who received protamine after CPB.[6,7] Optimising the dose

of protamine is thought to be crucial for minimising the occurrence of adverse events.[2] Clearly, a balance must be struck between underdosing of protamine, which can result in inadequate haemostasis after CPB, and overdosing, which can lead to the aforementioned adverse events.

Generally, there are two options for dosing of protamine after CPB: standard or titrated. Standard dosing typically comprises giving a fixed dose of protamine per unit of heparin given, whereas titrated dosing involves assessing plasma heparin concentrations and giving an appropriately titrated dose of protamine to neutralise the measured heparin concentration.[2] In theory, titrated dosing should be optimal in terms of facilitating haemostasis and minimising the risk of adverse events; however, this has not been a consistent finding in the studies conducted to date.[8-14] In the absence of any large-scale, double-blind, randomised controlled trials examining whether standard *vs* titrated protamine dosing is more effective in facilitating haemostasis after CPB, we felt compelled to perform a meta-analysis of the available literature to seek a more definitive answer. Here we present the findings from our analysis.

MATERIALS AND METHODS

Literature Search Strategy

MEDLINE and Biomedical Central databases were searched. The search involved use of the following terms: ("cardiopulmonary bypass"[MeSH Terms] OR ("cardiopulmonary"[All Fields] AND "bypass"[All Fields]) OR "cardiopulmonary bypass"[All Fields]) AND ("heparin"[MeSH Terms] OR "heparin"[All Fields]) AND ("protamine"[MeSH Terms] OR "protamine"[All Fields] OR "protamine"[All Fields]). The following search limits were applied where possible: [Clinical Trial], [Randomized Controlled Trial], and [English]. All searches included literature published / available online from inception to November 2012.

Reference lists of pertinent articles were hand-searched to indentify further potentially relevant studies.

Selection criteria

Studies were eligible for inclusion in the meta-analysis if they were randomised controlled trials, controlled clinical studies, or cohort studies with designs comparing postoperative bleeding volume between the study group (titrated dose) and the control group (standard dose) for protamine reversal of surgical anticoagulation in CPB procedures in adult patients.

Data extraction and quality assessment

Two independent reviewers extracted the data from eligible studies. A third reviewer resolved any disagreements. The following information / data were extracted from studies that met the inclusion criteria: the name of the first author, year of publication, type of study, number of participants in each treatment group, participants' age and gender, name(s) of the drug(s) given, name(s) of comparator drug(s), and blood loss.

The primary outcome of interest was postoperative blood loss.

Data analysis

Means with standard deviations were calculated for blood loss, and were compared between participants who were treated with standard or fixed-dose protamine (control treatment) versus titrated-dose protamine (study treatment). A χ^2-based test of homogeneity was performed using Cochran's Q statistic, and the inconsistency index (I^2) statistic was determined. An I^2 >50% indicated the existence of heterogeneity between studies and a random-effects model was used. Otherwise, fixed-effects models were used. Combined summary statistics of the standardised difference in the mean for each individual study are shown. A two-sided p value <0.05 was considered to indicate statistical significance. A funnel plot and the fail-safe N (which indicates whether the observed significance is spurious or not) were used to assess possible publication bias. All analyses were performed using Comprehensive Meta-Analysis statistical software, version 2.0 (Biostat, Englewood, NJ).

RESULTS

Literature search

A total of 219 studies were identified by searching the specified databases (*figure 1*). Of these, four[8-11] met the eligibility criteria and were included in the meta-analysis.

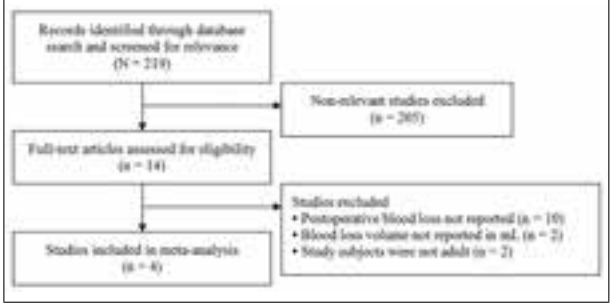
Study characteristics

The characteristics of the four studies are summarised in *table 1*.

All were randomised controlled trials, involving a total of 507 patients, ranging from 20 to 247. Patients were generally similar in mean age between studies, and within studies between treatment groups (study *vs* control). The proportion of male participants varied considerably between studies, ranging from 54[11] to 90%.[8] The proportion of males was generally similar between treatment groups within each individual study.

The most common reason for surgery was coronary artery bypass grafting.[8,10,11] The reason for CPB was not specified in the study reported by Despotis *et al.*[9] The initial heparin dose was generally 300 U/kg; however, most studies required patients to attain a specified activated clotting time (>400[8] or >480 seconds).[9-11] Study group

Figure 1. *Flow diagram of study selection*



protamine dosing was determined using the Hepcon Heparin Management System (HMS) in three studies[9-11] and using the Hemochron system in the study reported by Keeler *et al*.[8] The means of determining protamine dosing in the control group were different between all studies, but was generally based on the heparin dose.

Postoperative blood loss was lower in the study group (range: 625-839 ml) compared with the control group (range: 765-995 ml) in all four studies (*table 1*). Transfusion of packed red blood cells (PRBCs) was also lower in the study group (range: 0.2-1.8 U; 558-659 ml) compared with the control group (range: 0.3-2.7 U; 633-1559 ml) in all four studies (*table 1*). Only two studies[9,11] reported on the transfusion of fresh frozen plasma (FFP) after surgery, with Despotis *et al*. reporting that patients in the study group received more units of FFP than patients in the control group (2.7 *vs* 1.4 U),[9] and Koster *et al*. reporting the opposite (0.2 *vs* 0.3 U) (*table 1*).[11]

Only two studies reported on complications after surgery. Ohata *et al*. reported that no patients experienced neurological accidents, myocardial infarction, or any other complications related to CPB.[10] However, in the control group one patient experienced transient pulmonary hypertension and one patient sudden systemic hypotension. There were no other complications in the study group. Koster *et al*. reported that three patients in both groups required re-exploration because of postoperative haemorrhage.[11]

Postoperative blood loss and other outcomes

Two[10,11] of the four studies included in the meta-analysis had standardised difference in means of postoperative blood loss volume that significantly favoured study treatment over control treatment (*figure 2*). There was no evidence of significant heterogeneity in postoperative blood loss among the four studies (Q=4.224, I^2=28.98%, p=0.238); hence, a fixed-effects model of analysis was used for meta-analysis and generated an overall / combined standardised difference in means of postoperative blood loss volume of -0.325 with a 95% confidence interval (CI)

Table 1. *Characteristics of studies included in the meta-analysis*

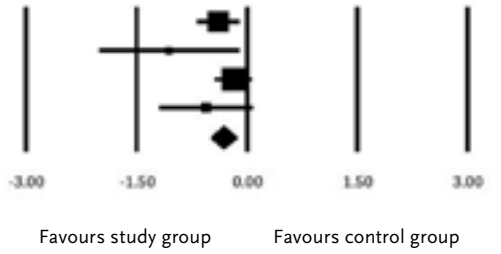
Study	Participants (study vs control)	Age, years (study vs control)	% Males (study vs control)	Surgery type	Heparin dosing	Study group protamine dosing	Control group protamine dosing	Postoperative blood loss, ml (study vs control)	Transfusion required (study vs control)
Koster et al., 2002	100 vs 100	66±17 vs 64±13	54 vs 55	CABG, valve replacement / recon-struction	300 U/kg (+ additional to achieve ACT >480 sec)	Titration by Hepcon HMS	1:1 according to initial heparin dose needed to achieve target ACT	625±312 vs 765±397	PRBCs 0.2±0.1 U vs 0.3±0.2 U FFP 0.2±0.1 U vs 0.3±0.1 U
Ohata et al., 1999	12 vs 8	59.3±2.7 vs 62.4±1.6	NA	CABG	300 U/kg (+ additional to achieve ACT >480 sec)	Titration by Hepcon HMS	1.67 mg/mg total heparin	821±131 vs 960±132	PRBCs 659±224 mL vs 1559±323 mL
Despotis et al., 1995	124 vs 123	64±11 vs 65±11	69 vs 70	NA	Control group: 250 U/kg (+ additional to achieve ACT >480 sec) Study group: dosing based dose-response assay	Titration by Hepcon HMS	0.8 mg/mg total heparin	839±377 vs 924±520	PRBCs 1.8±1.9 U vs 2.7±4.7 U FFP 2.7±4.7 U vs 1.4±2.5 U
Keeler et al., 1991	20 vs 20	58.4±7.18 vs 54.0±8.14	75 vs 90	CABG	300 U/kg (+ additional to achieve ACT >400 sec)	Titration by Hemochron system	6 mg/kg	769±286 vs 995±492	PRBCs 558±422 mL vs 633±477 mL

ACT = activated clotting time; CABG = coronary artery bypass graft; FFP = fresh frozen plasma; HMS = Heparin Management System; NA = not available; PRBCs = packed red blood cells; U = units.

Figure 2. *Forest plot showing the standardised difference in means (SD in means) of postoperative blood loss volume for the four studies included in the meta-analysis. Patients received a titrated (study group) or standard (control group) dose of protamine for the reversal of heparin after cardiopulmonary bypass. A fixed-effects model was used according to the heterogeneity test (Q=4.224, I^2=28.98%, p=0.238). The overall effect of an SD in means of -0.325 (95% confidence interval [CI]: -0.499, -0.150) indicates the results favoured the study group over the control group (p<0.001)*

Study name	SD in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	P value
Koster et al, 2002	-0.392	0.143	0.020	-0.672	-0.112	-2.746	0.006
Ohata et al, 1999	-1.056	0.486	0.236	-2.009	-0.104	-2.173	0.030
Despotis et al, 1995	-0.187	0.126	0.016	-0.434	0.059	-1.488	0.137
Keeler et al, 1991	-0.562	0.322	0.104	-1.194	0.070	-1.742	0.082
Overall	-0.325	0.089	0.008	-0.499	-0.150	-3.646	0.000

Heterogeneity: Q statistic=4.224, df=3 (P value=0.238) I2=28.975 %
Test for overall effect: Z statistic=-3.646 (P value<0.001)



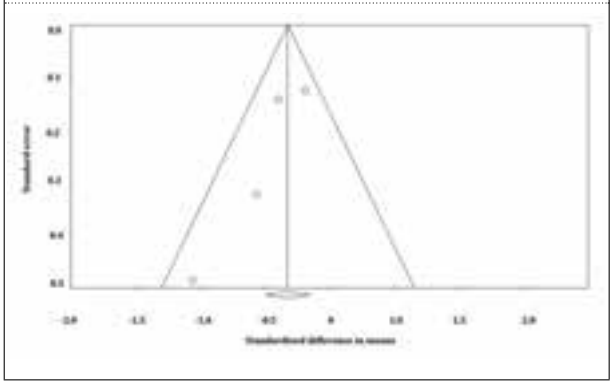
(-0.499, -0.150). The overall effect significantly favoured study treatment over control treatment (p<0.001).

Only two studies reported on postoperative complications / reoperation.[10,11] Koster *et al.* reported that three patients in the study group and five patients in the control group underwent reoperation because of postoperative haemorrhage. Ohata *et al.* reported that no patients experienced neurological accidents, myocardial infarction, or any other CPB-related complications;[10] however, two patients in the control group experienced postoperative systemic hypotension or transient pulmonary hypertension.

Publication bias

The funnel plot for publication bias (standard error by standardised difference in means of postoperative blood loss volume) demonstrated moderate asymmetry (*figure 3*), indicating the existence of moderate publication bias. For postoperative blood loss volume, the combined effect size yielded a Z value of -4.075 with a corresponding p value <0.001. The significant results indicate the fail-safe N value were relevant.

Figure 3. *Funnel plot of the standard error by standardised difference in means of postoperative blood loss volume for the four studies included in the meta-analysis. Patients received a titrated (study group) or standard (control group) dose of protamine for the reversal of heparin after cardiopulmonary bypass. The combined effect size yielded a Z value of -4.075 with a P value 0.0001*



DISCUSSION

We carried out a meta-analysis to determine whether standard or titrated dosing of protamine is more effective in facilitating haemostasis after CPB. Four RCTs met our eligibility criteria and were included in the meta-analysis. The findings from our meta-analaysis suggest that titrated protamine dosing is more effective than standard protamine dosing for reducing postoperative bleeding after CPB.

The major finding of our meta-analysis was that patients who had tirated protamine dosing experienced less postoperative blood loss after CPB than patients who had standard protamine dosing. Whether or not the decreased postoperative blood loss with titrated protamine dosing is of clinical significance remains to be determined. Indeed, there was little information on clinical outcomes in the four studies included in our meta-analysis. Although we did not perform any meta-analyses on other variables, all four studies included also reported that postoperative transfusion of PRBCs was lower with titrated *vs* standard protamine dosing. This is not surprising given the decreased blood loss. Three of the studies included in our meta-analysis

used the Hepcon HMS for titrating protamine doses,[9-11] while one study used the Hemochron system.[8] Other studies not eligible for inclusion in our meta-analyses also used the Hepcon HMS[15,16] and Hemochron system[17] for effectively titrating protamine doses. A number of studies, however, have questioned the effectiveness of protamine dose titration using these systems.[13,14] Specifically, Hardy *et al.* have suggested that the Hepcon HMS does not accurately assess heparin concentrations when compared with laboratory evaluation,[14] whereas Shore-Lesserson *et al.* found that protamine dose titration using the Hemochron system did not reduce postoperative blood loss compared with standard protamine dosing.[13] Hardy *et al.* however, did not examine clinical outcomes, specifically bleeding volume, which are clearly more important indicators of the system's utility than laboratory-equivalent accuracy in the evaluation of heparin concentrations. The disparate findings reported by Shore-Lesserson *et al.* are difficult to explain, but may reflect the somewhat small number of patients (n=28 in the protamine titration group) in the study and thus a possible lack of statistical power. We suggest that additional large-scale RCTs are needed to further explore the effectiveness of the Hemochron system for protamine dose titration.

Concerns have been raised about the safety of protamine for reversing the anticoagulant activity of heparin.[4-7] We did not focus on examining the safety of protamine dosing in this meta-analysis; hence, we cannot provide any definitive comments on this issue. However, neither of the two studies reporting complications after surgery presented evidence suggesting that safety was a significant concern.[10,12] Indeed, theoretically, one of the key advantages of protamine dose titration should be reducing the likelihood of overdosing and the associated adverse events / complications, which can occur with standard dosing.[18]

Our meta-analysis has a number of limitations. First, our analysis included only a small number of studies, and as a consequence, a relatively small number of patients for a meta-analysis. Further confirmatory, large-scale RCTs are needed. Secondly, there were some differences between studies that may have confounded the analyses, specifically differences in the type of surgery and the means of determining protamine dosing. This is reflected in our finding that there was evidence of moderate publication bias among the studies. Nevertheless, all of the studies included were RCTs (albeit non-double-blinded), and thus free of the inherent biases associated with non-randomised, retrospective, and observational studies.

In conclusion, the findings from this meta-analysis suggest that titrated protamine dosing is more effective than standard protamine dosing for alleviating postoperative bleeding after CPB. As such, titrated protamine dosing may help improve patient outcomes and reduce the need for supportive therapy due to postoperative bleeding.

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ORIGINAL ARTICLE

Bortezomib-induced polyneuropathy

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ABSTRACT

Background: Peripheral neuropathy is a frequent side effect of bortezomib chemotherapy. Relatively little is known about the clinical characteristics of this neuropathy, especially with respect to pain. Our aim was to describe the clinical characteristics and course of bortezomib-induced polyneuropathy.

Methods: This is a retrospective cohort study of 39 patients diagnosed with bortezomib-induced polyneuropathy.

Results: Pain is the most prominent symptom and 14 of 39 patients suffered from severe pain. More than 50% of our patients used analgesics due to moderate or severe pain. We found no correlation between severity of symptoms of bortezomib-induced polyneuropathy and cumulative dose or dose intensity of bortezomib. Nerve conduction studies did not correlate well with symptom severity. Dose reduction or discontinuation of treatment reduced severity in most cases.

Conclusion: Painful polyneuropathy is a frequent, dose-limiting side effect of bortezomib with a relatively good prognosis. Careful neurological monitoring of symptoms and timely dose adjustment is important.

KEYWORDS

Bortezomib, multiple myeloma, neuropathy, pain

INTRODUCTION

Bortezomib (Velcade®) is a proteasome inhibitor and registered for the treatment of multiple myeloma, a relatively frequent haematological malignancy.[1] Ongoing trials are evaluating the efficacy in other haematological malignancies and solid tumours.[2] Proteasomes are protein complexes involved in protein degradation, including pro-apoptotic proteins which induce programmed cell death in (cancer) cells. Through complex molecular cascades proteasome inhibition leads to apoptosis of cancer cells.[3]

In phase I studies polyneuropathy was already identified as a side effect of bortezomib.[4] In subsequent phase II and III investigations, the reported incidence of bortezomib-induced polyneuropathy ranged between 30% and 64%.[1,5-9] The exact causative pathway of this polyneuropathy is not yet fully understood, but seems most likely to be multifactorial, to which genetic factors of both the patient and tumour contribute.[10]

Bortezomib-induced polyneuropathy has a typical glove-stocking distribution and, similar to polyneuropathies induced by thalidomide and vincristine, besides sensory symptoms also autonomous and mild motor symptoms can develop. However, unlike other chemotherapy-induced polyneuropathies, bortezomib-induced polyneuropathy can be remarkably painful. Patients can experience different modalities of neuropathic pain (for example, a burning sensation or very painful paraesthesia), which can greatly effect quality of life.

Little is known about the course of these symptoms (specifically pain) over time and their optimal treatment.

In this retrospective study we describe a group of patients with polyneuropathy due to bortezomib, who were followed over time.

MATERIALS AND METHODS

All patients referred to the outpatient clinic Neuro-Oncology/Neurology of the ErasmusMC between 2004 and 2008 with a polyneuropathy induced or aggravated by the use of bortezomib were included.

For these patients the following information was collected from their medical records:

1. Presence of a pre-existent polyneuropathy due to, for example, diabetes mellitus or previous use of neurotoxic chemotherapeutics, especially vincristine and thalidomide.
2. Bortezomib dosage regimen and the number of doses administered, combined with reasons for any dose reduction or premature cessation of the treatment. With these data we calculated the cumulative dose and dose intensity of bortezomib per patient.
3. Symptoms and signs of polyneuropathy and any treatment for neuropathic pain. For estimating the severity of polyneuropathy we used a score list which was specifically developed for assessing chemotherapy-induced polyneuropathy.[11,12] In this score symptoms experienced by the patient are combined with abnormalities in the neurological exam (see appendix). Maximum pain intensity was recorded using a numerical rating score (NRS).[13] An NRS pain intensity of 0 means no pain, an NRS pain intensity of 10 maximum pain. In addition, the severity of motor neuropathy, sensory neuropathy and neuropathic pain was scored separately using the Common Toxicity Criteria (CTC).[14] Finally, the effect of pain medication was investigated; a reduction of at least 30% in the NRS pain intensity, which is a generally accepted outcome measure in pain studies, was considered a favourable response.[15]
4. Nerve conduction studies (NCS). The NCS consisted of sensory nerve conduction of the median, ulnar and sural nerve and motor nerve conduction of the ulnar and peroneal nerve. Using age-adjusted local reference values a distinction between normal and abnormal nerves was made.[16] Depending on the abnormalities found, we considered patients to have a normal NCS, a sensory polyneuropathy or a mixed (sensory and motor) polyneuropathy.

Mean and standard error of the mean of normally distributed continuous variables and median and range of not normally distributed continuous and categorical variables were calculated.

RESULTS

Forty-three patients presented to the Neuro-Oncology/Neurology outpatient clinic before or during use of bortezomib for the treatment of multiple myeloma. Four patients were excluded from further analysis: one patient had a severe polyneuropathy after use of thalidomide that did not worsen during use of bortezomib and for three patients insufficient data were available regarding the timing and dosage of bortezomib and other potentially neurotoxic chemotherapeutics.

Table 1. *Demographic data and medical history*

	Mean ± SEM
Age	57.6 ± 1.29
	Number of patients
Gender (n=39)	
Male	29
Female	10
Medical history (n=39)	
Diabetes mellitus	2
Previous chemotherapy (n=39)	
None	17
Vincristine	8
Thalidomide	6
Vincristine and thalidomide	8

SEM = standard error of the mean.

In *table 1* the demographic data and medical history are described. In accordance with the prevalence of multiple myeloma our study population contained more men than women. A minority of the patients had diabetes mellitus, a considerable group of patients had been treated previously with vincristine and/or thalidomide.

In 23 patients pain was the presenting symptom. In some this pain was more specifically described as painful paraesthesias (n=6), burning (n=4) and cold (n=4). Other presenting symptoms were paraesthesias (n=11), numbness (n=3) and cold sensation (n=1). In our cohort no patients presented with motor symptoms.

Median NRS pain intensity of our 39 patients at the time of maximum symptoms was 6. Twenty-seven patients had NRS pain intensity of 5 or higher (i.e. moderate pain) and in 14 patients this was even 8 or higher (i.e. severe pain).

Table 2 shows the average cumulative dose and dose intensity, the median time to development of bortezomib-induced polyneuropathy, the median time to maximum (pain) symptoms of polyneuropathy, median sum of

Table 2. *Clinical data at time of maximum symptoms*

	Mean ± SEM
Cumulative dose (n=39)	18.37 ± 1.44 mg
Dose intensity (n=39)	0.189 ± 0.0073 mg/day
	Median (range)
Number of days to first symptoms of polyneuropathy (n=39)	67 (4-288)
Number of days to maximum symptoms of polyneuropathy (n=39)	130 (28-349)
NRS pain intensity (n=39)	6 (0-10)
Score list (n=38)	6 (1-11)
CTC	
Motor neuropathy (n=32)	0 (0-3)
Sensory neuropathy (n=35)	1 (0-4)
Neuropathic pain (n=34)	1 (0-3)

SEM = standard error of the mean; NRS = numerical rating score; CTC = common toxicity criteria

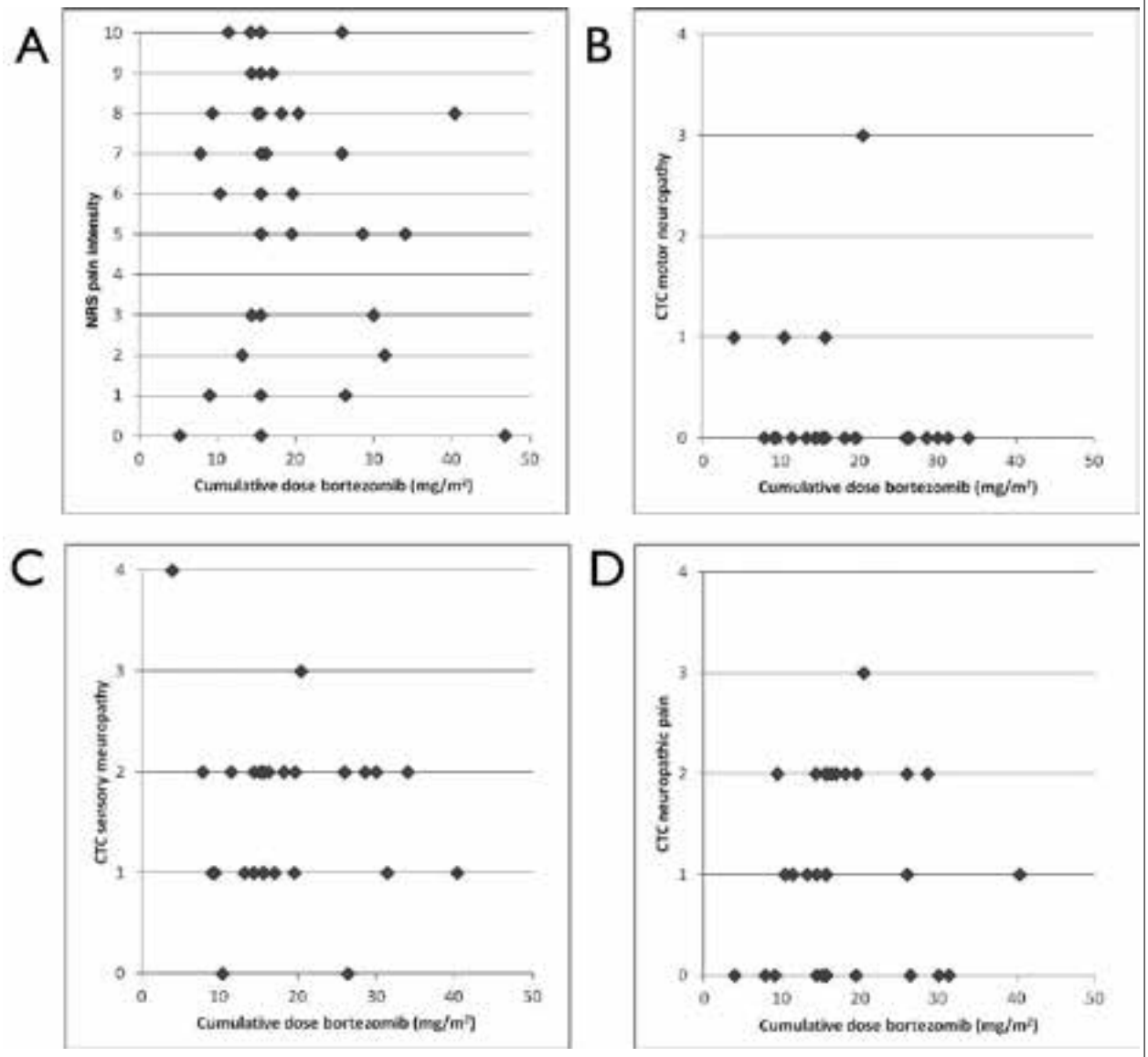
the score list, medians for the separate CTC groups and median NRS pain intensity. In this patient population there is no linear correlation between cumulative dose and NRS pain intensity or the individual CTC subgroups (*figure 1A-D*); nor between dose intensity and NRS pain intensity/CTC.

In 23 patients the dosage regimen of bortezomib was modified because of symptoms of polyneuropathy. In nine patients (27.3%) the dose was reduced and in 17 patients (51.5%) bortezomib treatment eventually had to be discontinued. In ten patients dosage regimen was unchanged and in six patients the exact dosage regimen of bortezomib could not be retrieved from the clinical records. Noteworthy, in 21 patients pain severity initially increased after cessation of bortezomib.

Of the 27 patients with NRS pain intensity of 5 or higher, 23 used one or more analgesic drugs: 22 used antiepileptic drugs (pregabalin or gabapentin), 7 antidepressants (amitriptyline) and 11 opioids (tramadol, morphine or oxycodone). In 16 patients a follow-up NRS pain intensity was known. Eventually in all of these cases at least a 30% reduction in pain was reached after a median of 64 days (range 18-430).

Because of differences in the bortezomib dosage regimen between previously untreated and treated patients, we were unable to perform an analysis of the effects of previous

Figure 1. *Correlation between cumulative dose of bortezomib and A) NRS pain intensity, B) CTC motor neuropathy, C) CTC sensory neuropathy, and D) CTC neuropathic pain*



NRS = numerical rating score, CTC = common toxicity criteria.

Table 3. *Nerve conduction studies*

	Normal	Abnormal	Missing
Median nerve (sensory)	14	7	5
Ulnar nerve (sensory)	9	15	2
Sural nerve (sensory)	6	14	6
Ulnar nerve (motor)	8	10	8
Peroneal nerve (motor)	16	8	2

treatment with neurotoxic chemotherapy on NRS pain intensity and CTC scores.

In 26 patients NCS at the time of bortezomib-induced polyneuropathy were available (*table 3*). In most patients a predominantly sensory (n=9) or mixed axonal polyneuropathy (n=12) was found. There was no clear correlation between EMG abnormalities and severity of the pain. Most striking were three patients with severe pain (NRS pain intensity score 8 or 9) and completely normal findings on the EMG. Additionally, in ten patients NCS had also been performed before initiation bortezomib treatment. Two of them were not previously treated with neurotoxic chemotherapy and had normal baseline NCS that deteriorated during treatment with bortezomib to a sensory and mixed polyneuropathy with a pain score of 6 and 7 respectively. Six out of the eight previously treated patients had abnormal NCS. Two patients who were previously treated with thalidomide had normal baseline NCS; in one of them it remained normal, despite developing a pain score of 9 during treatment with bortezomib.

DISCUSSION

Bortezomib is a proven effective treatment for multiple myeloma, whose frequently occurring painful polyneuropathy is an important dose-limiting side effect. This polyneuropathy typically occurs rather early during treatment. In our group, the first symptoms of polyneuropathy developed after a median time of 67 days, which is similar to a previously reported duration of 42 days to 2.5 months.[7,8,10]

More than two-thirds of the patients in our cohort had a pain score of 5 or more, i.e. moderate to severe pain.[17,18] Although most authors mention pain as a prominent symptom, there is only one other study which actually quantified pain by means of NRS.[19] In this prospective study on multiple myeloma patients who developed bortezomib-induced polyneuropathy, the percentage of patients with pain (less than 25%) was lower than in our study. In another study the percentage of patients who experienced pain during treatment with bortezomib varied between 50% for previously untreated patients and 81% in previously treated patients.[8]

We did not find a clear linear correlation between the cumulative dose or dose intensity and severity of polyneuropathy, indicating that some patients developed a severe polyneuropathy after a relatively low dose of bortezomib and vice versa. A phase II study with patients who received previous chemotherapy,[20] and two recent phase III trials, with both previously treated and chemotherapy-naive patients with multiple myeloma,[7,9] all showed that the risk of developing bortezomib-induced polyneuropathy did not increase above a cumulative dose of 30-45 mg/m^2, confirming the absence of linear dose-toxicity.

Although often bortezomib-induced neuropathic pain is severe and prolonged, most patients improve over time. In the majority of patients in our cohort bortezomib dosage was adjusted and various medications against neuropathic pain were prescribed to reduce pain. These data confirm the general view that bortezomib-induced polyneuropathy has a favourable outcome regarding symptoms,[1,9,20] provided that the dose is adjusted in time to prevent progression of symptoms. Currently a validated algorithm based on CTC criteria for the severity of the neuropathy is used.[7] Recently an even stricter guideline was proposed, which recommends dose adjustment when symptoms regarding neuropathic pain first occur and are still minimal.[21] The rationale is that once severe symptoms have developed resolution takes more time and therefore the occurrence of severe symptoms must be prevented.

Different dosage regimens appear to influence the incidence of bortezomib-induced polyneuropathy. Recent investigations regarding the effectiveness of bortezomib in treating multiple myeloma showed a lower incidence of bortezomib-induced polyneuropathy with subcutaneous administration and less intensive dosage regimen (for example, once instead of twice weekly).[22-24]

Symptomatic treatment is still empirical in the absence of specific studies on the effectiveness of neuropathic analgesics in bortezomib-induced polyneuropathy. Studies investigating possible neuroprotective medication for the prevention of bortezomib-induced polyneuropathy have not been conclusive.[25]

A pre-existent polyneuropathy, for example caused by diabetes mellitus, excessive alcohol use or multiple myeloma, has been shown to be a risk factor for developing bortezomib-induced polyneuropathy.[6,9] Many multiple myeloma patients receive different potentially neurotoxic agents (vincristine, thalidomide). In our cohort we could not assess a predisposing effect of this previous treatment on the development of bortezomib-induced polyneuropathy, because of differences in dosage regimen between previously treated and untreated patients. Some studies found no clear link between previous neurotoxic chemotherapy and the emergence of bortezomib-induced

polyneuropathy.[6,7,20,26] In contrast, others described that patients who had previously used neurotoxic chemotherapy could be at an increased risk to develop neuropathic pain during treatment with bortezomib.[8,19,27]

A direct comparison between these neurotoxic drugs is difficult because of clinical and pathophysiological differences. Thalidomide-induced polyneuropathy is a predominantly sensory, dose- and duration-dependent polyneuropathy with both clinical and neurophysiological evidence for axonal involvement.[28] The data on vincristine-induced polyneuropathy, a sensorimotor, duration-dependent neuropathy, are also consistent with direct axonal toxicity. Pathophysiologically microtubules are involved, though the exact mechanism is still not fully clarified.[29]

Contrary to the axonal neuropathies caused by thalidomide and vincristine, bortezomib-induced polyneuropathy is a small diameter neuronopathy. In an animal model bortezomib mainly caused toxicity to the dorsal root ganglia, Schwann cells and myelin and axonal damage to a lesser extent.[30] Genetic studies also identified a different set of genes for bortezomib-induced polyneuropathy compared with vincristine-induced polyneuropathy suggesting different molecular mechanisms.[10]

No correlation was found in our cohort between the severity of pain and severity of abnormalities in NCS. Some patients with much pain had normal NCS. This implies that conventional NCS have limited diagnostic value. This is probably explained by the fact that pain is an expression of small fibre damage and NCS are not an adequate tool for evaluation these fibres. One other study described small fibre neuropathy due to bortezomib using quantitative sensory testing, which may be more sensitive for the detection of small fibre dysfunction than NCS.[31]

In conclusion, bortezomib-induced polyneuropathy is a common, painful and dose-limiting side effect, however with a favourable outcome if bortezomib treatment is adjusted timely. It is therefore advisable to establish an accurate clinical neurological diagnosis and to follow-up symptoms of polyneuropathy, specifically paraesthesias and (neuropathic) pain. This should be done during and preferably also before initiation of treatment with bortezomib. Since bortezomib-induced polyneuropathy preferentially affects small diameter nerve fibres, conventional NCS are of limited additional value. The history of the patient and the severity of complaints are the decisive factors in the management of this sometimes severe iatrogenic complication.

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Appendix. *Score list to assess the severity of chemotherapy-induced polyneuropathy*[11,12]

	Absence/presence
Paresthesias	0/1
Numbness	0/1
Loss of dexterity	0/1
Unsteadiness of gait	0/1
	Normal/abnormal
Position sense hallux	0/1
Vibration sense hallux	0/1
Pin-prick sensation hallux	0/1
Romberg's sign	0/1
Romberg's sign with heel-to-toe stand	0/1
Knee tendon reflexes	0/1
Ankle tendon reflexes	0/1
Total	0-11

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CASE REPORT

Central retinal vein occlusion as an uncommon complication of sarcoidosis

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ABSTRACT

A 23-year-old black women with acute blurred vision of the right eye was referred for ophthalmological examination. Fundus examination and fluorescence angiography showed a non-ischaemic central retinal vein occlusion (papillophlebitis). The diagnosis of sarcoidosis, suggested by the presence of bilateral hilar and mediastinal lymphadenopathy, was confirmed by transbronchial biopsy of the lymphadenopathy demonstrating noncaseating granulomas. The eye problems were successfully treated with systemic corticosteroids. Central retinal vein occlusion is a rare complication of sarcoidosis.

KEYWORDS

Sarcoidosis, central retinal vein occlusion, complication

INTRODUCTION

Sarcoidosis is a multisystem disorder of unknown aetiology that is characterised by tissue infiltration of noncaseating granulomas. Tissue biopsy is needed for a definitive diagnosis. Although the lungs are the most common site of inflammation, sarcoidosis can also involve other organs such as the skin, nervous system, heart, spleen and eyes.[1] Ocular involvement occurs in 25 to 50% of patients with sarcoidosis.[2-5] The most common ocular manifestations are uveitis and conjunctival nodules.[5] Central retinal vein occlusion, however, is an uncommon complication and only reported in a few case reports.[6-11] We describe a patient whose presenting clinical manifestation of sarcoidosis was a unilateral central retinal vein occlusion.

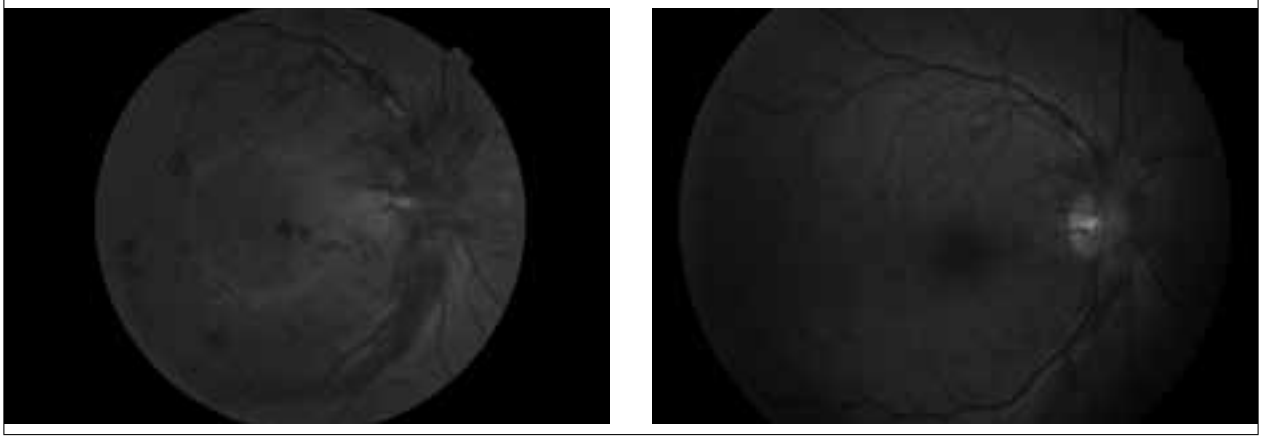
CASE REPORT

A 23-year-old black woman was referred to our hospital because of acute blurred vision in the right eye. Her medical history was unremarkable and she was not on any medication. There was no history of eye trauma. She had been consulting her general practitioner for four months because of dyspnoea and coughing; treatment with different antibiotics and inhalation therapy with corticosteroids and sympathicomimetics had no effect. Furthermore she complained of peaks of fever, fatigue, arthralgias of her hips, knees and ankles and she had lost 10 kg of weight.

Physical examination showed a moderately dyspnoeic young woman with a saturation of 97%, temperature of 36.0°C, blood pressure 110/65 mmHg, and pulse 90 beats/min. No pathological lymph nodes were found. Heart and breath sounds were normal. There was no hepatosplenomegaly, and no skin changes or signs of arthritis. Neurological examination was normal. Ocular examination showed a visual acuity of 20/30 in the right eye and 20/20 in the left eye and normal intraocular pressures. Slit lamp examination showed fine keratic precipitates and cells in the anterior chamber of both eyes. There were no clear signs of vitritis. Fundus examination of the right eye showed multiple flame-shaped haemorrhages surrounding the optic disc, tortuous retinal veins and dot-blot haemorrhages in all quadrants of the retina (*figure 1A*). The left eye showed some small retinal haemorrhages.

Laboratory tests showed an increased erythrocyte sedimentation rate, a mild anaemia and leucocytosis and an increased level of angiotensin-converting enzyme (ACE). Blood cultures remained negative. Chest X-ray demonstrated bilateral mediastinal and hilar lymphadenopathy. A high resolution computed tomography

Figure 1. *A) Fundus examination of the right eye: multiple flame-shaped haemorrhages surrounding the optic disc, tortuous retinal veins and dot-blot haemorrhages in all quadrants of the retina; B) Fundus examination of the right eye ten days after starting systemic prednisolone: the fundus lesions have almost entirely disappeared*



(HRCT) scan of the chest showed extensive bilateral symmetrical hilar and mediastinal lymphadenopathy and multiple nodular lesions in both lungs. Lung function testing was performed and showed static and dynamic values within the normal range and a normal diffusion. Transbronchial biopsy of the mediastinal lymph nodes was performed without complications and the specimens showed noncaseating granulomas confirming the diagnosis of systemic sarcoidosis. Because of the eye involvement high-dose systemic and local prednisolone was instituted and the eye lesions diminished within days (*figure 1B*).

DISCUSSION

Sarcoidosis is a multisystem, granulomatous disease of unknown aetiology which is characterised by noncaseating epitheloid granulomas in affected organs. The disease can affect every organ but the most common organs are the lungs.[1] Involvement of the eye is reported in up to 50% of the patients and is the presenting symptom in 5%.[2-5] Ocular findings that support the diagnosis of sarcoidosis are the following: mutton-fat keratic precipitates, iris nodules, trabecular meshwork nodules, peripheral anterior synechia, vitreous opacities (snowball/string or pearls-like appearance), multiple chorioretinal peripheral lesions, retinal perivasculitis (periphlebitis), and/or candlewax retinochoroidal exudates and/or laser photocoagulation spot-like retinochoroidal atrophy, optic disc nodule(s)/granuloma(s) and/or solitary choroidal nodule and bilaterality.[12,13] Ocular involvement in sarcoidosis encompasses a wide range of clinical manifestations. The most frequently seen are uveitis and conjunctival nodules. In addition, secondary glaucoma, cataract formation and blindness are late complications in untreated patients.[4,5,14,15].

A central retinal venous occlusion (CRVO) is an important cause of visual loss among older adults throughout the world and is mostly associated with arteriosclerotic ischaemic changes of the veins. However, the occurrence of a non-ischaemic CRVO in a young patient is rare. Moreover, when retinal venous occlusion occurs, it usually involves the smaller peripheral vessels, and has been attributed to retinal phlebitis, choroidal granuloma as well as impaired ocular circulation.[7,8,11,16,17] Only a few case reports have described a central venous occlusion as a symptom of sarcoidosis.[6-11,15] The exact pathogenesis of CRVO in sarcoidosis is unknown. Possibly, it is a clinical manifestation of microangiopathy that is also found in other organs affected with sarcoidosis.[18] It can also be caused by infiltration of the retinal vessels by sarcoid granulomata manifesting in a perivascular mantling on the vessels with lymphocytes and epithelioid cells and perivascular proliferative changes compressing the vessels leading to luminal occlusion.[19]

Treatment of ocular sarcoidosis is indicated even when symptoms are slight, because of the risk of severe loss of vision. Corticosteroids are the mainstay of treatment. Mild cases of uveitis can usually be treated adequately with topical corticosteroids alone. Systemic corticosteroids are indicated in uveitis not responding to topical corticosteroids or in the presence of bilateral posterior involvement, especially with macular oedema and occlusive vasculitis. In 5 to 20% of the patients who are corticosteroid resistant or require an unacceptable dose to maintain remission, various cytotoxic steroid-sparing drugs are used. Methotrexate is the most commonly used steroid-sparing agent in the treatment of sarcoidosis, but

azathioprine, mycophenolate mofetil and leflunomide have also been shown to be useful.[2,20-23] Anti-TNF biological therapies such as infliximab and adalimumab may be helpful for those patients experiencing persistent disease or an intolerance to cytotoxic immunosuppressive therapy. However, these agents themselves are associated with uveitis as well.[24]

The main cause of visual loss is cystoid macular oedema.[25] Poor visual prognosis is associated with advanced age of the patient, black race, female sex, chronic systemic disease, and also with posterior segment involvement, peripheral punched out lesions, and the presence of cystoids macular oedema and glaucoma. However, the visual prognosis of sarcoidosis is usually good.[25]

CONCLUSION

Our patient presented with a central retinal venous occlusion of the right eye as the presenting symptom of sarcoidosis. This is an uncommon complication of sarcoidosis.

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PHOTO QUIZ

Pancreatitis and a sudden loss of sight

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A 32-year-old female presented to the emergency department with a one-day history of malaise, nausea, vomiting and colic pain in the right upper abdominal quadrant. Clinical examination showed a suffering non-jaundiced female patient with a body temperature of 37.7°C, a normal blood pressure and intense abdominal pain in the right upper quadrant. Laboratory tests revealed a gamma-glutamyltranspeptidase of 84 U/l (0-35 U/l), alkaline phosphatase 73 U/l (0-120 U/l), aspartate aminotransferase 59 U/l (0-45 U/l), alanine aminotransferase 43 U/l (0-45 U/l), bilirubin 14 µmol/l (0-17 µmol/l), p-amylase 41 U/l (0-220 U/l), and C-reactive protein 7 mg/l (0-5 mg/l). An ultrasound of the liver showed dilated intrahepatic biliairy ducts, and sludge in the distal part of the common bile duct. There were no signs of cholecystitis. The next day the patient's liver enzymes increased and an endoscopic retrograde choledochography (ERC) was performed. After papillotomy some small pigmented stones were removed. During the next day the patient developed severe abdominal pain and an elevation in the serum amylase concentration of 8098 U/l (normal 0-220 U/l) was measured, which indicated a post-ERC pancreatitis. She was treated with supportive care including pain control, intravenous hydration and enteral tube feeding. On the fifth day of admission the patient reported a sudden loss of her visual acuity and she noticed (para)central scotomas in her visual field.

The consulted ophthalmologist performed a fluorescein angiography (*figure 1*). Angiography five months later showed complete disappearance of the prior lesions (*figure 2*).

Figure 1. *Five white spots inferotemporal of the optic disc in the left eye. In fluorography only little leakage, appropriate for cotton wool spots. The macula itself showed no alteration or leakage of fluorescence in fluorography*

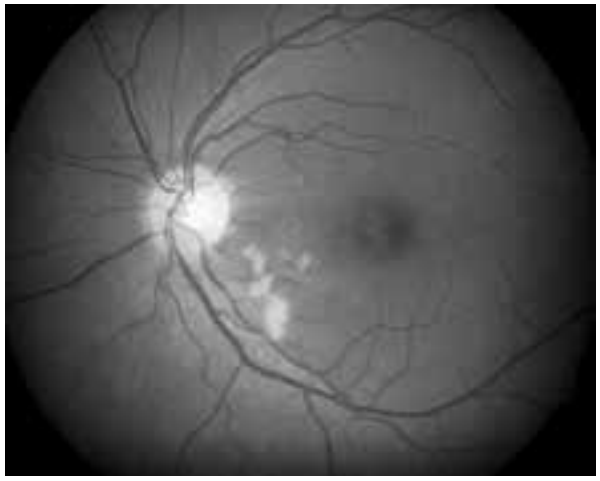
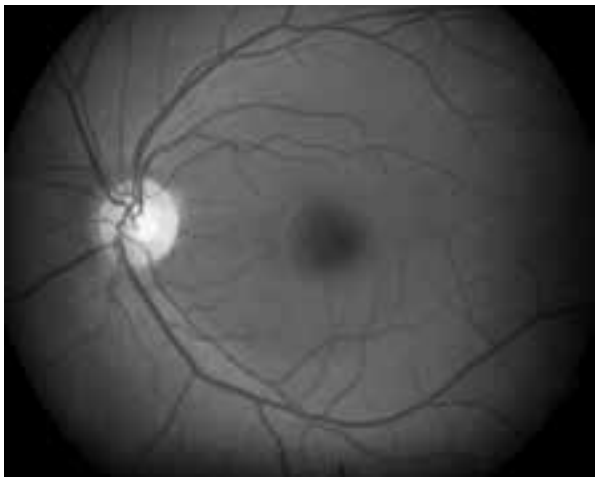


Figure 2. *Complete disappearance of cotton wool spots after five months*



WHAT IS YOUR DIAGNOSIS?

See page 141 for the answer to this photo quiz.

Netherlands
The Journal of Medicine

PHOTO QUIZ

Bluish-grey pigmentation of the facial skin and lower legs

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CASE REPORT

A 77-year-old woman was referred to our outpatient clinic because of bluish-gray pigmentation of the facial skin and lower legs (*figures 1A* and *1B*).

Her medical history revealed hypertension, hypercholesterolaemia and chronic obstructive pulmonary disease Global Initiative for Chronic Obstructive Lung Disease (Gold) classification 3. Home medication included metoprolol, barnidipine, irbesartan, omeprazole and gemfibrozil. Her COPD medication included acetylcysteine, inhaled steroids, short-acting beta-agonists and anticholinergics. Due to recurrent disease exacerbation complicated by bronchiectasis, she had additionally been taking prophylactic minocycline 100 mg/day for the past two years. On physical examination the patient had a temperature of 36.7°C and diffuse ronchi in both lungs. Skin examination revealed blue-grey hyperpigmentation in the zygomatic region, nose and lower legs. She had no lymphadenopathy. Laboratory findings showed a subclinical hypothyroidism. Complete blood counts, inflammation parameters, liver and kidney function tests were not remarkable.

WHAT IS YOUR DIAGNOSIS?

See page 142 for the answer to this photo quiz.

Figure 1. *A) Bluish-grey pigmentation of the facial skin and B) irregular patchy blue-grey pigmentation in the pretibial regions*

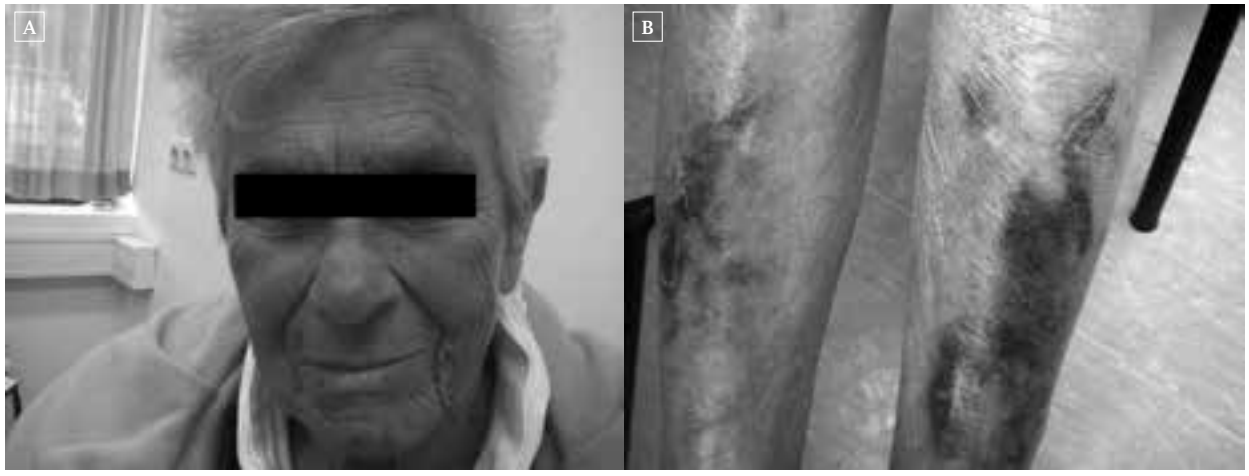

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PHOTO QUIZ

What's happening under the diaphragm?

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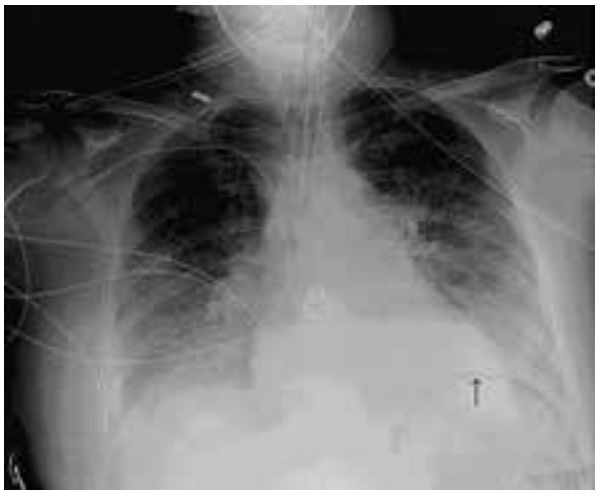
CASE REPORT

A 73-year-old woman with no relevant medical history besides progressive dyspnoea on exertion for four months was admitted to the intensive care unit with cardiogenic shock due to severe mitral valve stenosis. Cardiac failure was provoked by new-onset high-frequency atrial fibrillation. She developed multiorgan failure including ischaemic hepatitis, disseminated intravascular coagulation and acute kidney injury. The patient received mechanical ventilation, support of the circulation and continuous venovenous haemofiltration. Mitral valve replacement would only be considered if the multiorgan failure phase were to improve substantially.

After slight clinical improvement in the first ten days, she deteriorated due to hypovolaemic shock caused by a haemothorax, a complication of pleural fluid drainage under heparin therapy. Coagulation was corrected and the bleeding stopped. However, five days later the patient became hypotensive for which vasopressor therapy was started. On physical examination the abdomen was tender. *Figure 1* shows the chest X-ray taken on that day.

Figure 1. *Chest X-ray showing air configuration under left hemi-diaphragm (arrow)*



WHAT IS YOUR DIAGNOSIS?

See page 143 for the answer to this photo quiz.

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A male with a painful left knee

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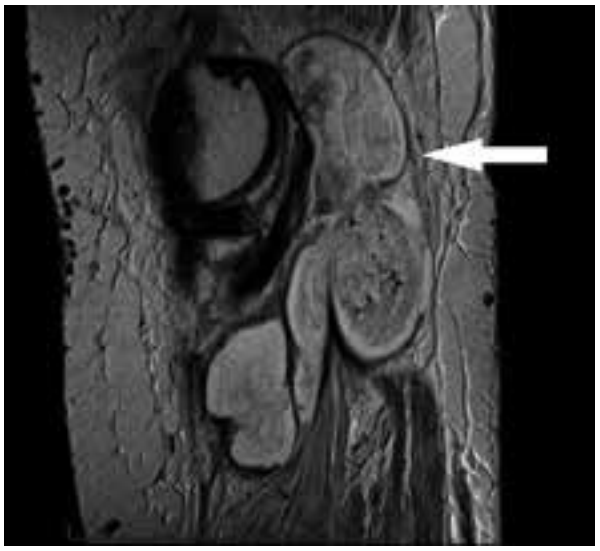
CASE REPORT

A 40-year-old male was admitted to the emergency department with fever and progressive pain of the left leg. His medical history included kidney transplantation for loss of kidney function due to hereditary glomerulonephritis, obesity, thromboembolic events and erysipelas. The patient had been on long-term immune suppression (ciclosporine and prednisone). Examination of the left leg showed no signs of acute infection, besides a minimal difference in temperature between the left and right knee. Extended lower extremity ultrasound was performed to exclude deep vein thrombosis, showing no signs of intravascular coagulation. However, a hydrops of the left knee was seen. Magnetic resonance imaging of the left knee showed the following picture (*figure 1.*)

Figure 1. *A sagittal T2-weighted magnetic resonance imaging showing a large collection in the medial femorotibial compartment of the left knee*



WHAT IS YOUR DIAGNOSIS?

See page 144 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 137)

PANCREATITIS AND A SUDDEN LOSS OF SIGHT

DIAGNOSIS

The acute pancreatitis in this patient was complicated by Purtscher's retinopathy, an unusual complication demonstrating a multisystem disorder. It is characterised by sudden unilateral or bilateral loss of acuity or visual field or both. In Purtscher's retinopathy the typical Purtscher flecken are the most specific feature, although cotton wool spots and retinal haemorrhage are more often present.

The incidence of this ocular complication in pancreatitis is unknown. Systematic ophthalmoscopy and continuous staging for multiple-organ failure in pancreatitis patients showed that Purtscher's retinopathy cannot been seen as a prognostic factor for pancreatitis, nor is the severity of pancreatitis a predictive value for the risk of development of Purtscher's retinopathy.[1]

The exact pathophysiology of the retinal whitening has not been completely elucidated but is mostly seen in pancreatitis of alcoholic aetiology. Fibrin clots, fat embolism, complement-granulocyte or platelet-fibrin aggregates, and coagulative necrosis of the retinal vessels may cause the retinopathy associated with pancreatitis.[2,3]

Therapeutic consequences for early recognition are unknown. In literature only Hackert *et al.*[4] underline the importance of treatment with high-dose low-weight heparin, crystaloid infusion, steroid application and/or somatostatin analogues, but no standardised therapeutic procedures exist.

Remnant visual symptoms at the time of ophthalmoscopic control are absent in half of cases published in English literature. Although visual acuity of our patient after five months suggested the existence of very small paracentral scotomas, angiography showed complete disappearance of the cotton wool spots.

Recognition and diagnosis of Purtscher's retinopathy in patients with pancreatitis may extend our knowledge and understanding of this ocular complication for better future patient care.

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ANSWER TO PHOTO QUIZ (PAGE 138)

BLUISH-GREY PIGMENTATION OF THE FACIAL SKIN AND LOWER LEGS

DIAGNOSIS

The bluish-grey pigmentation was caused by the tetracycline derivate minocycline. Pigment disorders are well-recognised adverse effects of tetracyclines, while tetracyclines and doxycycline cause mainly teeth and oral cavity pigmentation. Skin hyperpigmentation is the most common adverse reaction to long-term use of minocycline and it is reported to occur in about 3-15% of patients taking cumulative doses greater than 100 g.[1] Older age and diagnosis of rheumatoid arthritis or rosacea are more often associated with this adverse reaction. However, this can be explained by higher cumulative minocycline doses in these groups of patients.[2]

The exact mechanism of minocycline-induced pigmentation is unknown. Minocycline and its iron-chelated derivates have been identified in pigmented skin and in insoluble complexes within the granular pigment deposits in dermal macrophages of affected areas.[3] There are three types of minocycline-induced pigmentation.[2] Type I is characterised by dark-blue pigmentation in areas of inflammation and scarring such as those frequently described within resolving acne lesions. Type II pigmentation presents as blue-black patches in the lower legs, ankles and arms. Type III is a symmetric and generalised pigmentation on sun-exposed area and can appear all over the body. In our case pigmentation was thought to be of type II. Histopathologically, type I and II lesions demonstrate iron-, melanin- and minocycline-containing granules in the dermic macrophages. Type III lesions contain no iron and are characterised by increased melanin in basal keratynocytes.[3]

Type I pigmentation is independent of duration of exposure and dosage while type II and III appear after a long period of drug exposure. The diffuse pigmentation of the sun-exposed areas may remain permanently while type I and II pigmentation may vanish upon cessation of minocycline, though it can take months to years to fade up. Although minocycline-induced pigmentation does no harm, the drug should be discontinued if this adverse event occurs. In this case, this patient should have been switched to an alternative to minocycline much earlier. In general, patients receiving minocycline for long periods of time should be informed about the possible occurrence of hyperpigmentation. In fact, good patient education and early recognition of this side effect can prevent further exposure to the drug and consequent development of a florid stage of this dermatosis.

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ANSWER TO PHOTO QUIZ (PAGE 139)

WHAT'S HAPPENING UNDER THE DIAPHRAGM?

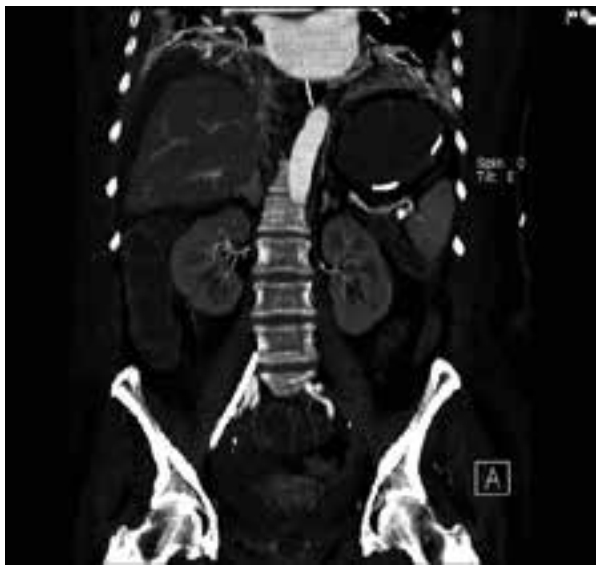
DIAGNOSIS

The chest X-ray suggests free air under the left diaphragm. However, the abdominal CT scan (*figure 2*) showed pneumatosis of the oesophagus and stomach, and non-enhancement after intravenous contrast, indicating ischaemia of the wall. There was no abdominal free air. The superior mesenteric and celiac artery were patent. The CT scan was suggestive for ischaemia of the stomach and distal part of oesophagus, in this case most likely caused by the hypovolaemic shock. Oesophagogastroscopy confirmed the diagnosis of severe diffuse ischaemia of the distal oesophagus and stomach. Due to her cardiovascular status surgical intervention was not possible. Because of the poor prognosis, treatment was withdrawn and the patient died. Gastric pneumatosis is rare and the least common location for intramural gas. There are a wide range of causes, from life-threatening to self-limiting, with mortality ranging from 21-68%.[1,2] Besides ischaemia (occlusive or non-occlusive), trauma, vomiting, nasogastric tube placement gastroparesis, malignancy, gastric ulcer, phytobezoar, infection, excessive soda drink ingestion are all causes of gastric pneumatosis. Treatment of gastric pneumatosis is subordinated to the cause, but in benign causes often conservative.[1-4]

This case report illustrates the known low sensitivity and specificity of plain upright chest X-rays for diagnosing free intra-abdominal air.[5] The CT scan following the conventional imaging illustrates this by showing pneumatosis of the stomach. It also showed patency of the superior mesenteric and celiac artery, suggesting the cause was a low-flow state. The hypovolaemic shock due to the thoracic haemorrhage in combination with the severe mitral valve stenosis caused the low-flow state which led to gastric and oesophageal ischaemia, four days later.

Diagnosis: gastric pneumatosis due to ischaemia caused by low-flow state, masquerading as free abdominal air.

Figure 2. *Coronal CT image showing gastric pneumatosis (arrow)*



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ANSWER TO PHOTO QUIZ (PAGE 140)

A MALE WITH A PAINFUL LEFT KNEE

DIAGNOSIS

This sagittal T2-weighted image shows a distended gastrocnemius–semimembranosus bursa in the medial femorotibial compartment of the left knee. This popliteal cyst consisted of fluid collections with an inhomogeneous aspect, making it more suspect for infection. Therefore a needle aspiration was performed revealing a *Klebseilla pneumoniae*.

Popliteal cysts were first described in the 19th century by Morrant Baker as a cystic mass in the popliteal fossa (also known as Baker's cyst). Most popliteal cysts are asymptomatic and are detected incidentally by an imaging study performed for another reason. Several risk factors for the development of popliteal cysts have been reported including trauma and coexisting joint disease such as rheumatoid arthritis. Popliteal cysts may enlarge as a consequence of rupture or dissection giving symptoms resembling deep vein thrombosis.

In rare cases popliteal cysts can become infected. *Staphylococcus aureus* infections are most commonly found. *Mycobacterium tuberculosis* infections of popliteal cysts are also reported. Popliteal cysts are an ideal seeding ground for circulating bacteria and can therefore get infected in patients suffering from bacteraemia.[1]

In our patient a *Klebsiella pneumoniae* was cultured from aspiration fluid. To our knowledge *K. pneumoniae* infections of popliteal cysts were not previously described. *K. pneumoniae* infections are generally found in patients with a reduced immune status. *K. pneumoniae* infections are most commonly found in the respiratory tract and urinary tract although cases of liver and renal cyst infections have been reported. Our patient had been on systemic immune suppression for a longer period of time to prevent rejection after kidney transplantation, making him more susceptible for *K. pneumoniae* infection.

In most cases treatment with antibiotics alone is not sufficient. Nevertheless, operative treatment to reduce symptoms is also a topic of discussion, especially in cases of ongoing cyst infections. Therefore, the patient was treated primarily with antibiotics (cotrimoxazole) for a long period of time. Treatment led to regression of the swelling and reduction of the clinical symptoms. Follow-up will determine if surgical treatment is required.

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SPECIAL ARTICLE

A clinical approach to pharmacogenetics

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ABSTRACT

Taking into account the high frequency of adverse drug reactions (ADRs) in the clinic and taking into account the growing knowledge of the genetic mechanisms underlying some of these ADRs, we believe that every clinician should know at least the basic principles of pharmacogenetics. However, our experience is that many clinicians are unaware of the potential contribution of pharmacogenetic testing and have not implemented this new modality in their daily practice.

We present a case of Stevens-Johnson syndrome in a patient treated with carbamazepine. Following the pathways of clinical reasoning, we describe the possibilities of pharmacogenetic testing in the clinic (HLA-B*1502 and HLA-A*3101 in our patient). We describe the pharmacological and pharmacogenetic aspects relevant for the clinician's daily practice (the existence of ADR subtypes, cytochrome P450, drug-drug interactions, genetic variations, CYP450 and HLA genotyping). Based on the Dutch top 100 of most prescribed drugs, we provide data on CYP450 and HLA genotypes relevant to those 100 most commonly used drugs. We discuss the availability and costs of pharmacogenetic testing, show a calculation of the 'number needed to genotype' and, based on these data, we propose a decision model for pharmacogenetic testing by clinicians.

KEYWORDS

Adverse drug reaction, clinical medicine, decision modelling, pharmacogenetics

We provide clinically relevant pharmacological and pharmacogenetic information for the most commonly used drugs and we propose a decision model for pharmacogenetic testing by clinicians.

Key messages

Medication-related emergencies are a frequent cause of unplanned hospital admissions and adverse drug reactions are common in the clinic.

Every clinician should know at least the basic principles of pharmacogenetics.

INTRODUCTION

Medication-related emergencies account for 5.6% of unplanned hospital admissions in the Netherlands,[1] with comparable frequencies found in other countries;[2-7] see reference 8 for a review. Almost 50% of these cases are potentially preventable. Drugs most often associated with potentially preventable medication-related hospital admissions are those that affect blood coagulation, such as antiplatelet drugs (8.7%), oral anticoagulants (6.3%), NSAIDs (5.1%), a combination of these (10.5%), antidiabetic drugs (12.3%) and drugs that act on the central nervous system (5.1%).[1]

These numbers implicate that all clinicians, from internal medicine residents through emergency physicians to cardiologists, are facing medication-related clinical problems and adverse drug reactions (ADRs) on a regular, if not daily, base.

Some patients are more prone to adverse drug reactions than others. Apart from the impact that comorbidity, comedication, nutrients or herbal supplements can have on the occurrence of ADRs, recent developments have revealed underlying genetic mechanisms for these inter-individual differences. Pharmacogenetics is the study of how the actions of and reactions to drugs vary with the patient's genes. Taking into account the high frequency of ADRs in the clinic (in-hospital adverse drug events occur in up to one fourth of the patients [9]) and taking into account the growing knowledge of the genetic mechanisms underlying

some of these ADRs, we believe that every clinician should know at least the basic principles of pharmacogenetics. However, our experience is that many clinicians are unaware of the potential contribution of pharmacogenetic testing and have not implemented this new modality in their daily practice. With this article, we aim to provide comprehensive basic pharmacogenetic information for the most frequently used drugs in the Netherlands (as a country representative of the Western world): drugs that every clinician meets in everyday practice.

Firstly, we present a case of Stevens-Johnson syndrome (SJS) in a patient treated with carbamazepine. Based on the Dutch drug top 100, we discuss the pharmacological and pharmacogenetic aspects relevant for these frequently prescribed drugs in the Netherlands. After discussing the non-genetic issues, we provide data on CYP450 and HLA genotyping relevant to those 100 drugs, we give information about the availability and costs of pharmacogenetic testing and, based on these data, we propose a decision model for pharmacogenetic testing by clinicians.

Figure 1. *Picture of our patient with SJS due to carbamazepine treatment. A) shows the normal situation, B) shows the patient during presentation at the emergency room*

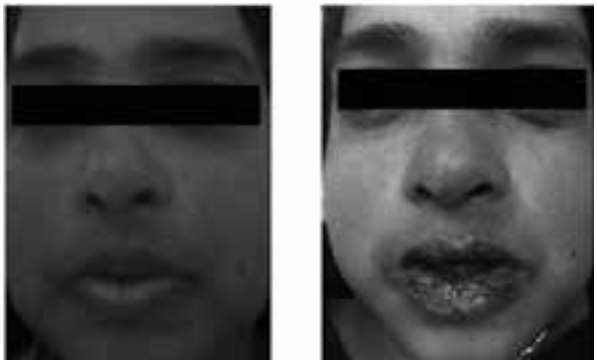
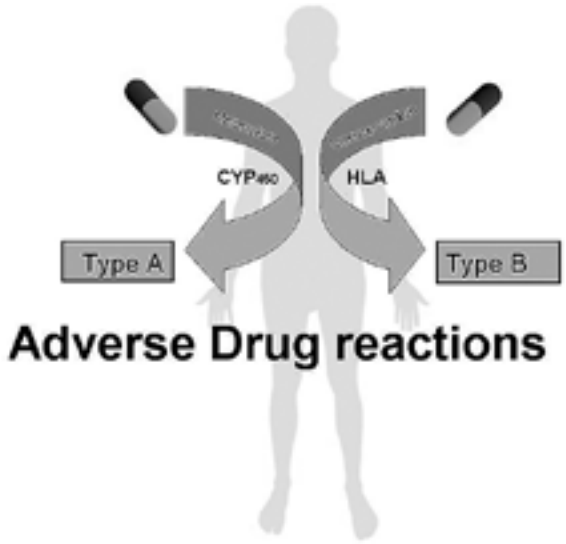


Figure 2. *Simplified model to provide basic understanding of the genetic causes underlying different types of ADR*



METHODS

From the database of the Dutch Health Care Insurance Board (College Voor Zorgverzekeringen, www.gipdatabank.nl), we downloaded the list of 100 most frequently used drugs in the Netherlands. In PubMed, we selected articles about the most clinically relevant cytochrome P450 enzymes (search strategy: "cytochrome P450 AND (1A2 OR 2B6 OR 2C8 OR 2C9 OR 2C19 OR 2D6 OR 2E1 OR 3A4) AND clinical (significance OR relevance OR implication)", limited to review articles).

We collected pharmacogenetic data of these 'top 100 drugs' on PubMed and from the cytochrome P450 Drug Interaction Table, which is a frequently updated table supplied by the Division of Clinical Pharmacology of the Indiana University School of Medicine, covering most of the clinically relevant CYP450 information from peer-reviewed biomedical journals cited by PubMed.[10] Finally, we summarise the information relevant for the drugs encountered daily in the clinic, in two 'white coat pocket size' figures *(figures 3 and 4)*.

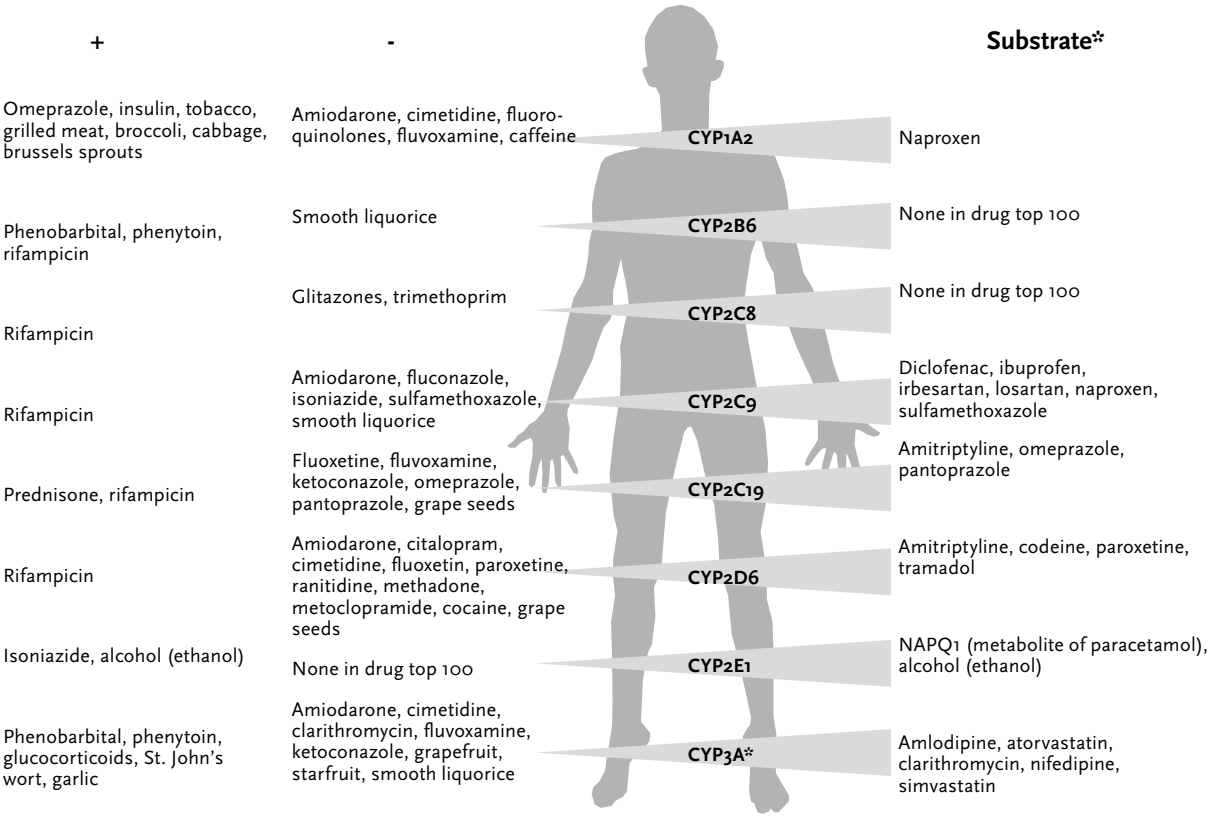
CLINICAL CASE

Our patient, a 47-year-old woman of Asian descent, presented to our emergency department with painful ulcers in her mouth. The ulcers had first appeared three days before. They were progressive and hindered her from eating and drinking. The patient's medical history was unremarkable, apart from epilepsy, diagnosed several months before presentation, for which she had been on carbamazepine 300 mg twice daily for one month. She was not on any other medication.

Physical examination revealed a blood pressure of 140/60 mmHg, a pulse of 100 beats/min and a body temperature of 38.7°C. The patient had confluent erosions on the lips and ulceration over the buccal mucosa *(figure 1)* as well as conjunctival inflammation of the left eye. She had a widespread erythematous bullous rash with target-shaped lesions over the upper trunk and the extremities. There was auricular erythema and swelling as well as tenderness of the plantar and palmar surfaces. There were no genital ulcers.

On admission, the C-reactive protein level was 61 mg/l (normal <10 mg/l) and the serum gamma-glutamyl

Figure 3. *CYP450 inhibitor, inducer and substrate data for drugs present in the Dutch drug top 100 and frequently used food components*



* P-450 3A4, 3A5, and 3A7 have overlapping catalytic specificities, although some selectivity exists

transpeptidase, aspartate transaminase and alanine transaminase were elevated: 478 U/l (normal <40 U/l), 85 U/l (normal <34 U/l) and 267 U/l (normal <44 U/l), respectively. The creatine kinase and lactate dehydrogenase were normal.

Based on the history and clinical findings, Stevens-Johnson's syndrome related to carbamazepine use was suspected. The carbamazepine was ceased and corticosteroids were started at a high dose (1 mg/kg), after which the patient's condition improved rapidly. She was released from hospital six days after presentation.

PHARMACOLOGICAL AND PHARMACOGENETIC ASPECTS INVOLVED IN MANAGEMENT OF PATIENTS WITH ADR'S

In the next paragraphs, we will discuss the basic pharmacological and pharmacogenetic principles each clinician needs to be aware of for the clinical management of patients with adverse drug reactions. We discuss the existence of different types of ADRs, cytochrome P450, drug-drug interactions, genetic variations involved in ADRs, CYP450 and HLA genotyping and the number needed to genotype.

Types of ADR

An adverse drug reaction is defined as 'harm associated with the use of given medications at a normal dosage during normal use'. Drug reactions can be divided into several subtypes, the major two being types A and B. Type A reactions are expected exaggerations of the drug's known effect. These are usually dose dependent and predictable and they account for the majority of ADRs. An example could be the occurrence of bleeding in a patient on anticoagulation therapy. Clinical factors contributing to the occurrence of type A reactions include: impaired metabolism or excretion and increased drug sensitivity. Type B reactions are idiosyncratic and usually unrelated to the drug's known pharmacology. Normally they are not related to the dose, are unpredictable, uncommon, and usually more serious than type A. Most of the type B reactions are mediated by the immune system and thus also termed drug-hypersensitivity reactions.[11] Our case history is an example of a type B reaction.

There is increasing knowledge of the molecular basis underlying ADRs. Studies investigating type A ADRs have revealed associations with genetic variations in cytochrome P450 enzymes, among others. For type B drug hypersensitivity reactions, immunological studies have shown clear associations with HLA-class I alleles.[11] Although there is no 100% correlation between ADR subtypes (A or B) and the possible underlying genetic factors, one could make a general distinction as shown in *figure 2*.

Cytochrome P450

Cytochrome P450 (CYP450) enzymes are important for the oxidative metabolism of drugs. There are more than 50 CYP450 enzymes, but only six (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5) metabolise 90% of drugs (for a comprehensive overview, see reference 12). The majority of these enzymes are expressed in the liver.

Some drugs are inactivated by CYP450 enzymes, whereas other drugs, such as losartan, tamoxifen, codeine and tramadol, need to be activated by CYP450 enzymes.[13] These prodrugs may cause an increased effect or adverse effects when their corresponding CYP450 enzyme activity is increased. Conversely, therapeutic failure is likely due to little or no production of the active drug when CYP450 enzyme activity is decreased.

Drugs that alter CYP450 enzyme activity are referred to as either inhibitors or inducers. The extent to which an inhibitor affects the metabolism of a drug depends upon the dose and the ability of the inhibitor to bind to the enzyme. For instance, sertraline is considered a mild inhibitor of CYP2D6 at a dose of 50 mg, but if the dose is increased, it becomes a potent inhibitor.[14]

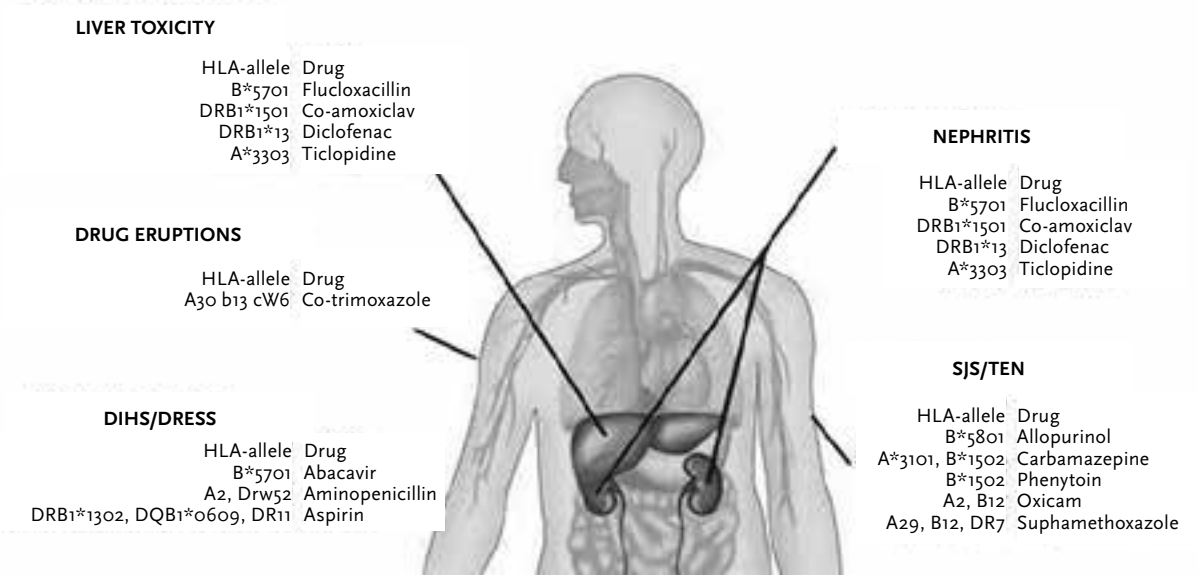
When patients are extremely sensitive or resistant to drug effects at normal doses, the clinician should exclude drug-drug interactions before searching for genetic variations in CYP450 metabolism as the underlying cause.

Drug-drug interactions

When a CYP450 enzyme inhibitor or inducer is added to drugs metabolised by one or more CYP450 enzymes, this can result in an increased or decreased effect of the drug in question. The following drugs are particularly known to cause clinically significant CYP450 drug interactions (*figure 3*): amiodarone, antiepileptic drugs such as carbamazepine, antidepressants as paroxetine, antitubercular drugs as rifampicin, macrolide antibiotics such as clarithromycin and protease inhibitors as ritonavir (the last-mentioned is not shown in *figure 3*: because the figure only shows drugs from the Dutch drug top 100).

A drug can either be both metabolised by and inhibit the same CYP450 enzyme (e.g., erythromycin,[15]) or it can be metabolised by one enzyme and inhibit another enzyme (e.g., terbinafine). Drugs may be combined on purpose, in order to take advantage of CYP450 inhibition. For example ritonavir, a protease inhibitor and potent CYP3A4 inhibitor, is added to lopinavir to increase serum levels in patients with an HIV infection.[16]

Figure 4. *HLA genotypes associated with ADR in reaction to drugs from the Dutch drug top 100, as well as specific drugs discussed in this article (underlined)*



SJS = Stevens-Johnson syndrome; TEN = toxic epidermal necrolysis; DIHS/DRESS = drug-induced hypersensitivity syndrome/drug rash with eosinophilia and systemic symptoms (for a description of the differences between mild drug eruptions and the severe cutaneous adverse reactions SJS/TEN and DIHS/DRESS, see references 30 and 31).

Genetic variations

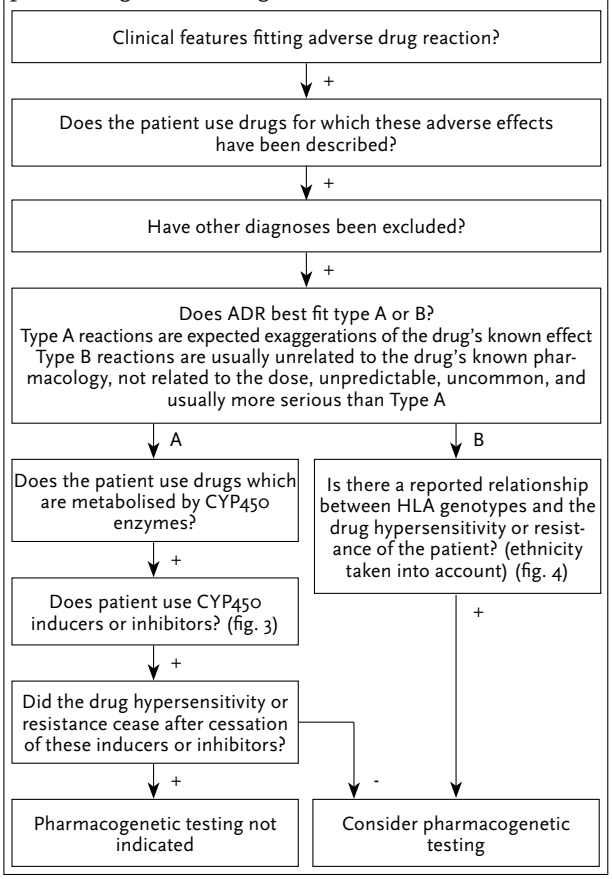
After excluding drug-drug interactions as the explanation for hypersensitivity or resistance to medication, the clinician should consider genetic variations as the underlying cause.

Variations in drug response among patients can be explained by polymorphisms in genes encoding CYP450 enzymes (for a recent review, see reference 17) as well as genetic variations in other drug-metabolising enzymes, drug transporters and drug receptors.[18] For this article, we focus on CYP450 and HLA genotyping because those are the genes most relevant for the drugs in the Dutch drug top 100.

Each CYP450 enzyme is encoded by a specific gene. Every person inherits one allele from the father and one from the mother. The wild-type allele is per definition the allele most commonly found in the general population, although in practice 'wild type' is interpreted as the most common allele encoding *active* enzyme. CYP3A5*3, for example, has an allele frequency of 90% in the Caucasian population,[19] but since it is a splice variant with a premature stop codon, which encodes a truncated *nonfunctional* protein, this allele is not referred to as 'wild type'.

A normal metaboliser has two copies of the wild-type allele. When a variant allele replaces one or both wild-type alleles, this may result in altered enzyme activity. Poor metabolisers are persons with two variant alleles, whereas slightly reduced enzyme activity is found in heterozygous individuals (those with one wild-type and one variant allele). Some persons have excess enzyme activity because they possess multiple copies of wild-type alleles. These individuals are called ultrarapid metabolisers.

Figure 5. *Proposed clinical decision model for the use of pharmacogenetic testing*



CYP450 genotyping

Several tests are available for genotyping CYP450 enzymes. The Amplichip® CYP450 test is an FDA-approved DNA microarray that can detect a large number of polymorphisms of CYP2D6, analysing 33 variant alleles in combination with two CYP2C19 variant alleles.[20] Costs of this analysis are €400 to €600. Costs of CYP450 genotyping in general vary between laboratories, and range from €50 to €700 per gene, mostly depending on the number of variant alleles being analysed and the analysing system used. Alternatively, CYP450 can be genotyped using Taqman®, LightCycler, PCR-RFLP, Luminex or DNA chip techniques such as Infiniti. At the moment, the results are usually available within one to two weeks after ordering, which depends more on the frequency of the analysis being carried out than on the testing time, which is one to four hours. Results are usually provided with a genotype to phenotype interpretation ('poor metaboliser', 'ultra-rapid metaboliser' etc.), based on the general observed activity of the enzyme studied on probe drugs. In the Netherlands, at least six hospitals offer pharmacogenetic testing for clinical use, mostly connected to psychiatric hospitals. The Erasmus MC offers pharmacogenetic testing for 19 genes involved in drug metabolism or drug effect on a regular (weekly) basis. In the US, the Mayo clinic performs CYP genotyping on a regular basis.

An international consensus or guideline for the use of pharmacogenetic testing has not yet been broadly established. Currently, pharmacogenetic testing is performed in individual cases, and mostly retrospectively, for example in patients who experience adverse effects. The indication depends on the ethnicity of the patient, since the frequency of the various genotypes differs between populations (see *figure 6,* reference 21 and paragraph 'number needed to genotype').

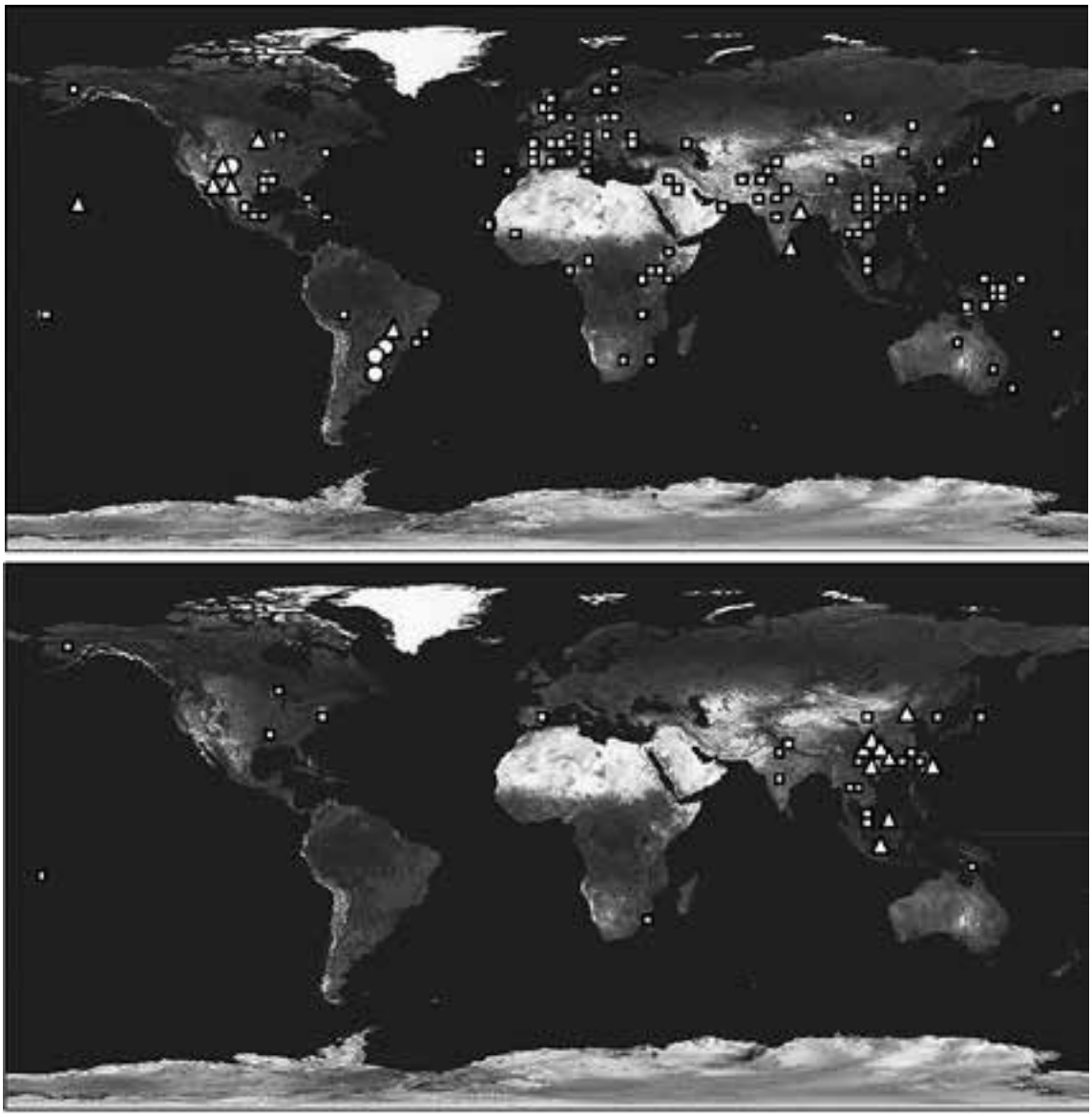
In 2011, Swen *et al*. published therapeutic (dose) recommendations for a large number of genotype/phenotype-drug combinations, including CYP2D6, CYP2C19 and CYP2C9.[22] From their list, we selected the drugs present in the Dutch drug top 100 (*table 1*). This table lists the most commonly used drugs for which therapeutic dose recommendations have been published.[22] The actual dose recommendations can be found on the website of the Royal Dutch Association for the Advancement of Pharmacy ('KNMP kennisbank') and on www.pharmgkb.com. The FDA has added pharmacogenetic information to the labels

of a number of drugs (for a list of these drugs, see http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm).

HLA

Apart from studies focusing on metabolic factors, predominantly involved in type A ADRs, recent immunological studies have revealed clear associations of certain type B drug hypersensitivity reactions with HLA-class I alleles (*figure 4*).[11] For example, in about 5% of treated patients, the antiviral drug abacavir causes a severe hypersensitivity reaction affecting multiple organs. The majority of these patients with drug hypersensitivity carries the HLA-B*5701 allele. This association is strongest in Caucasians[23] and the particular allele is present in 94.4% of patients who develop this drug hypersensitivity, but in only 1.7% of controls.[24] Partly based on these data, in 2008 an amendment was made to the product information of abacavir. Based on this change, before starting treatment with abacavir each HIV-infected patient should be screened for presence of the HLA-B*5701 allele, irrespective of race. Abacavir should not be used in patients who test positive for the HLA-B*5701 allele, unless there is no other therapeutic alternative available for these patients based on treatment history and resistance tests. To date, HLA-B*5701 testing

Figure 6. *Worldwide allele frequencies of two HLA alleles associated with carbamazepine hypersensitivity in Europeans and Han-Chinese, respectively A) HLA-A*3101, B) HLA-B*1502*[32]



Squares: 0-10%, triangles: 10-25%, circles: > 25%.

is the only pharmacogenetic HLA test that is performed on a regular basis in the Netherlands.

Another HLA allele which is clearly associated with severe type B ADRs is HLA-B*1502. This allele is strongly associated with the occurrence of Stevens-Johnson syndrome in Han-Chinese on carbamazepine treatment.[25] However, although the association with HLA alleles is very strong, many patients with HLA-B*1502 are exposed to carbamazepine without developing hypersensitivity.[23,24]

Another allele associated with type B ADRs in patients treated with carbamazepine, is HLA-A*3101. In subjects of Northern European ancestry, the HLA-A*3101 allele is associated with 'carbamazepine-induced hypersensitivity' being either the hypersensitivity syndrome (including rash, fever, eosinophilia, hepatitis and nephritis), maculopapular exanthema or SJS/TEN. The HLA-A*3101 allele has a prevalence of 2 to 5% in Northern European populations. The presence of the allele increases the risk of carbamazepine-induced hypersensitivity reactions from 5.0 to 26.0%, whereas its absence reduces the risk from 5.0 to 3.8%.[26]

Table 1. *Drugs from Dutch drug top 100 with therapeutic (dose) recommendations based on CYP450 genotype (adapted from reference 22)*

Amitriptyline	AD	2C19, 2D6
Aripiprazole	AP	2D6, 3A
Clomipramine	AD	2C19, 2D6
Clopidogrel	PAI	2C19
Clozapine	AP	1A2
Codeine	Opium alkaloid	2D6
Doxepin	AD	2D6
Duloxetine	AD	1A2, 2D6
Felodipine	Calcium antagonist	3A
Flecainide	AA	2D6
Haloperidol	AP	1A2, 2D6, 3A4
Imipramine	AD	1A2, 2D6
Lansoprazole	PPI	2C19
Olanzapine	AP	1A2
Omeprazole	PPI	2C19
Ondansetron	Aem	2D6
Pantoprazole	PPI	2C19
Paroxetine	AD	2D6
Phenytoin	Aep	2B6, 2C9, 2C19, 3A
Propafenone	AA	2D6
Rabeprazole	PPI	2C19
Risperidone	AP	2D6
Tolbutamide	SUd	2C9
Tramadol	Opioid	2D6
Venlafaxine	AD	2D6

See the website of the Royal Dutch Association for the Advancement of Pharmacy ('KNMP kennisbank') for the actual dose recommendations for these drugs. AD = antidepressant; AP = antipsychotic drug; PAI = platelet aggregation inhibitor; AA= antiarrhythmic drug, PPI = proton pomp inhibitor; Aep=antiepileptic drug; Aem = antiemetic drug, SUd = sulphonylurea derivative.

Number needed to genotype

To assess the clinical value of pharmacogenetic testing, one should consider the 'number needed to genotype' (NNG), which is an equivalent to the well-known 'number needed to treat'. The NNG is the average number of patients who need to be genotyped to prevent one additional ADR.

Ferrell *et al.*[27] recently calculated this NNG for carbamazepine in the Han-Chinese population. Assuming a 0.25% incidence of carbamazepine SJS/TEN in newly prescribed carbamazepine patients in Taiwan, one in every 400 newly treated patients would develop SJS/TEN. Since the sensitivity of genotyping of the HLA-B*1502 allele is 98.3%, this test would detect 98.3% of this one (out of 400) patients, being one in every 407 (400/0.983) patients. This means the number needed to screen is 407 people, with subsequent carbamazepine avoidance, to prevent one case of SJS/TEN.

For other ethnic groups, the situation is different: in Caucasians, at least two studies have failed to show a correlation between HLA-B*1502 status and carbamazepine SJS/TEN.[28,29] Based on the lack of evidence and relative infrequency of HLA-B*1502 in those with non-Asian ancestry, genotypic screening for HLA-B*1502 in non-Asian patients is of little value.

Based on the figures reported by McCormack,[26] who studied the association between carbamazepine-induced hypersensitivity and the HLA-A*3101 allele in Europeans, the NNG for the HLA-A*3101 allele would be 83.[26] This would mean that genotyping and carbamazepine avoidance of 83 individuals would prevent one case of carbamazepine-induced hypersensitivity. However, this calculation is based on carbamazepine-induced hypersensitivity in general (including milder forms), whereas the calculation by Ferrell strictly considers severe SJS/TEN. To date, HLA-A*3101 screening before starting carbamazepine treatment is not common in the Netherlands.[22]

CLINICAL DECISION MODEL

In order to assess whether pharmacogenetic testing is useful for a given patient presenting with an adverse drug reaction, we propose the decision model for clinicians, as shown in *figure 5*. Following our own clinical decision model, we performed HLA genetic testing in our patient, and found she was HLA-B*1502 positive. HLA-A*3101 was also tested, and was negative.

In summary, in this article we have provided basic pharmacological and pharmacogenetic information for the 100 most commonly prescribed drugs in the Netherlands, which are relevant in the daily clinical practice of every clinician. Based on the clinical case of Stevens-Johnson syndrome (SJS) in a patient treated with carbamazepine, we have described the basic principles of pharmacogenetic

testing, relevant for clinicians. We have provided clinically important CYP450 and HLA data for the drugs present in the Dutch drug top 100, we have given information about the availability and costs of pharmacogenetic testing and, based on these data, we have proposed a decision model for pharmacogenetic testing in order to apply pharmacogenetics to daily clinical practice. Finally, we have summarised this information in two 'white coat pocket size' figures.

Conflict of interests: None declared.

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SPECIAL REPORT

Dutch guideline for the management of electrolyte disorders – 2012 revision

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ABSTRACT

Electrolyte disorders are common and often challenging in terms of differential diagnosis and appropriate treatment. To facilitate this, the first Dutch guideline was developed in 2005, which focused on hypernatraemia, hyponatraemia, hyperkalaemia, and hypokalaemia. This guideline was recently revised. Here, we summarise the key points of the revised guideline, including the major complications of each electrolyte disorder, differential diagnosis and recommended treatment. In addition to summarising the guideline, the aim of this review is also to provide a practical guide for the clinician and to harmonise the management of these disorders based on available evidence and physiological principles.

KEYWORDS

Algorithm, hyperkalaemia, hypokalaemia, hypernatraemia, hyponatraemia

INTRODUCTION

The first Dutch guideline on electrolyte disorders was published in 2005 under the auspices of the Dutch Society of Internal Medicine.[1] This guideline was recently revised not only to include new evidence but also to make the guideline more clinically applicable. For example, recommended tests, diagnostic algorithms, treatment options including dosing, and recommended formulae have now been incorporated. In addition, for each electrolyte disorder we have specified which key questions should be asked to assess whether the patient is in immediate danger and what the most likely cause of the electrolyte disorder is. In addition to these practical elements, the emphasis on physiological principles has remained.[1] The recommendations for the acute treatment of electrolyte disorders have been synchronised with another commonly used national reference work ('acute boekje' – guidelines for the diagnosis and management of disorders in internal medicine).[2] This article is not only a summary of the 2012 revision of the Dutch guideline on electrolyte disorders,[3] it also aims to be a practical summary of the diagnosis and treatment of hypernatraemia, hyponatraemia, hyperkalaemia, and hypokalaemia. The reader is referred to other reviews for more detailed background information.[4-9] The guideline can be accessed at www.internisten.nl/gzi2 and http://internisten-apps.nl/elektrolytstoornissen/index.php/Hoofdpagina.

METHODS

The 2012 guideline on electrolyte disorders is a revision of the first guideline that was published in 2005.[1,3] The guideline aims to facilitate and harmonise the management of electrolyte disorders. The guideline is primarily intended for use by internal medicine trainees and specialists, but also for other specialists who are confronted with electrolyte disorders such as cardiologists, lung physicians, gastroenterologists, intensivists, and anaesthesiologists. The guideline was developed by a working group consisting of five experts in the field and one methodological expert (M.K.T.). None of the guideline working group members had a conflict of interest during the guideline development process. At the start of the guideline development, the working group agreed that

Table 1. *Signs, symptoms, and clinical dangers*

	Hypernatraemia	Hyponatraemia	Hyperkalaemia	Hypokalaemia
Signs and symptoms	Thirst Fever Sensorium changes Seizures Focal neurological deficits Hyperventilation	Nausea Vomiting Headache Diplopia Falls Seizures Coma	Muscle cramps Muscle weakness ECG changes	Muscle cramps Muscle weakness Paresthesia ECG changes
Complications	Brain cell shrinkage Intracranial haemorrhage Dural sinus thrombosis Osmotic demyelination Cerebral oedema*	Cerebral oedema Osmotic demyelination*	Arrhythmia Paralysis	Arrhythmia Paralysis Ileus Respiratory failure due to respiratory muscle weakness Rhabdomyolysis Glucose intolerance Urinary concentrating defect Hypokalaemic nephropathy

*** During treatment.**

electrolyte disorders constitute an area of medicine that lacks studies with a high level of evidence. Instead of restricting the guideline to available evidence, the working group decided to develop a didactic and practical guideline supported by evidence if available. In addition, 17 clinical questions were elaborated in evidence reviews based on a systematic review of the literature. After preparing a draft guideline, all members of the Dutch Society of Internal Medicine were invited to comment. All comments were reviewed by the working group and incorporated in the final guideline, which was endorsed at the 2012 annual meeting of the Dutch Society of Internal Medicine.

HYPERNATRAEMIA

Hypernatraemia is defined as a serum sodium concentration >145 mmol/l and can be further classified as acute or chronic and symptomatic or asymptomatic. Patients are more likely to be symptomatic when hypernatraemia develops acutely (usually <48 hours, *table 1*). However, when patients present to the hospital with hypernatraemia, the time in which it developed is usually unknown. Therefore, for the assessment whether hypernatraemia is acute or chronic, one often needs to rely on symptoms. The same holds true for hyponatraemia (see further). Acute hypernatraemia causes brain cell shrinkage due to a shift of water from the intracellular to the extracellular fluid compartment.[5] In severe cases and especially in neonates, this can cause intracerebral haemorrhage because vessels are stretched when brain volume decreases rapidly.[10] When hypernatraemia is documented to be acute or when severe symptoms are present, immediate treatment is indicated and should precede diagnostic evaluation (*table 2*). Conversely, when hypernatraemia is chronic or when few symptoms are present, the underlying cause should be identified and serum sodium should be corrected gradually. Indeed, when serum sodium is corrected too rapidly during chronic hypernatraemia, there is a risk of cerebral oedema (*table 2*).[5] The most common causes of hypernatraemia are shown in *table 3*. In general, the causes of hypernatraemia can be divided into those primarily caused by a negative water balance (due to a water or solute diuresis) and those primarily caused by a positive sodium balance, although combinations also exist.[11] The recommended diagnostic tests in a patient with hypernatraemia are shown in *table 4*. Hypernatraemia can usually be differentiated by using three parameters, including urine osmolality, urine sodium concentration, and urine output (*table 5*).[5] The differentiation between central and nephrogenic diabetes insipidus requires a functional test, namely the response in urine osmolality to a single dose of desmopressin.[12] A water restriction test is indicated to evaluate whether

Table 2. *Recommendations regarding the correction of hypernatraemia*

- When hypernatraemia is acute or severely symptomatic, immediate treatment with hypotonic fluids should be started, regardless of the underlying cause
- When a patient with hypernatraemia is hypotensive, isotonic fluids should be started
- When hypernatraemia is chronic, rapid correction should be avoided to prevent cerebral oedema and treatment should be directed to the underlying cause
- For all causes of hypernatraemia the correction rate is *limited* to 8 mmol/l in the first 24 hours and 18 mmol/l in the first 48 hours
- Acute hypernatraemia may be corrected faster initially (1-2 mmol/l/hour); a rise of 5 mmol/l is usually sufficient to improve symptoms

Table 3. *Most common causes*

Hypernatraemia	Hyponatraemia	Hyperkalaemia	Hypokalaemia
Osmotic diuresis (e.g. hyperglycaemia)	Pseudohyponatraemia	Pseudohyperkalaemia	Redistribution (shift)
High protein enteral feeding	Hyperglycaemia	Redistribution (shift)	Diarrhoea
Diabetes insipidus (central, nephrogenic, gestational)	Diuretics	Acute or chronic kidney disease	Laxative abuse
Breastfeeding	SIADH	Drugs inhibiting RAAS	Vomiting
Infusion of fluids hypertonic to the urine	Adrenal insufficiency	Primary adrenal insufficiency	Tube drainage
Primary aldosteronism (mild)	Cerebral salt wasting	Renal tubular acidosis (type IV)	Diuretics
	Heart failure	Pseudo-hypoaldosteronism (types I and II)	Primary or secondary aldosteronism
	Liver cirrhosis		Renal tubular acidosis (types I and II)
	Nephrotic syndrome		Bartter, Gitelman, and Liddle syndromes
	Primary polydipsia		Nonreabsorbable anions
	Low solute intake		Hypomagnesaemia
	Nonrenal sodium loss		Dialysis

RAAS = renin-angiotensin-aldosterone system; SIADH = syndrome of inappropriate antidiuretic hormone secretion.

Table 4. *Recommended diagnostic testing*

	Hypernatraemia	Hyponatraemia	Hyperkalaemia	Hypokalaemia
Always	Serum creatinine Serum urea Serum glucose Serum calcium Serum potassium Urine sodium Urine osmolality	Serum creatinine Serum glucose Serum potassium Serum osmolality Urine sodium Urine potassium Urine osmolality	Serum creatinine Thrombocytes Leukocytes Serum bicarbonate (or blood gas)	Serum creatinine Serum magnesium Urine potassium Serum bicarbonate (or blood gas) Blood pressure
Sometimes	Response in urine osmolality to desmopressin Urine urea	Serum uric acid Urine chloride FE uric acid FE urea FE sodium TSH and free T4 Cortisol or Synachten test	Urine potassium Urine creatinine Plasma renin and aldosterone TTKG	Urine potassium-to-creatinine ratio Urine chloride Plasma renin and aldosterone TTKG TSH and free T4

FE = fractional excretion; TSH = thyroid stimulating hormone; TTKG = transtubular potassium gradient.

polyuria is due to diabetes insipidus or polydipsia, but is *not* indicated in hypernatraemia, because polydipsia does not cause hypernatraemia.[13] For the various causes of diabetes insipidus, the reader is referred to two review articles.[12,14] Hypernatraemia is most commonly caused by a negative water balance, especially in patients with community-acquired hypernatraemia. Hypernatraemia should therefore be considered primarily a disorder of *water balance*. Prior to starting treatment, this water deficit can be estimated using the following formula: 0.6 * lean body weight * ([serum sodium/140] – 1). When a patient with hypernatraemia is hypotensive, we recommend to start with isotonic intravenous fluids (either crystalloids or colloids), because they restore haemodynamics more efficiently and are still hypotonic relative to the patient (*table 2*).[2] In all other settings, hypernatraemia can be treated with hypotonic fluids. These can be administered orally, via nasogastric tube or intravenously as half-isotonic saline with glucose (0.45% NaCl–2.5% glucose) or glucose 5%. Because glucose is metabolised to water, intravenous glucose infusions will generate electrolyte-free water. To calculate the anticipated decrease in serum sodium with 1 litre of infusate, we recommend the Adrogué-Madias formula (*figure 1*),[5] which has been validated in one study.[15] In addition to hypotonic fluids, patients with central and gestational diabetes insipidus can be treated with desmopressin, while patients with nephrogenic diabetes insipidus can be treated with thiazide diuretics or amiloride.[14] A recent cross-over trial demonstrated the efficacy of amiloride in curtailing polyuria in lithium-induced nephrogenic diabetes insipidus.[16] An important question to ask when evaluating patients with hypernatraemia is why thirst and subsequent water intake did not prevent hypernatraemia. A good example is patients with diabetes insipidus who under normal circumstances remain normonatraemic by increasing water intake, often even without additional medication. Possible causes why hypernatraemia develops include no access to water, immobility, inability to express thirst, or a defective thirst mechanism (hypodipsia). This also explains why certain groups are at increased risk of developing hypernatraemia, including children, the elderly, and patients admitted to the intensive care unit (ICU). In neonates who are exclusively breastfed,

Table 5. *Differentiation of hypernatraemia*

	Inadequate water intake	Diabetes insipidus	Osmotic diuresis	Extrarenal water loss	Positive sodium balance
Urine osmolality	Maximal	$U_{osm} < P_{osm}$	$U_{osm} > P_{osm}$	Maximal	Maximal
Urine sodium	<25 mmol/l	<25 mmol/l	>25 mmol/l	<25 mmol/l	>25 mmol/l
Urinary flow rate	Oliguria	Polyuria	Polyuria	Oliguria	Normal to high

Table 6. *Recommendations regarding the correction of hyponatraemia*

- When hyponatraemia is acute or severely symptomatic, immediate treatment with hypertonic saline should be started, regardless of the underlying cause
- When hyponatraemia is chronic, rapid correction should be avoided to prevent osmotic demyelination and treatment should be directed to the underlying cause
- For all causes of hypotonic hyponatraemia the correction rate is *limited* to 10 mmol/l in the first 24 hours and 18 mmol/l in the first 48 hours
- Acute hyponatraemia may be corrected faster initially (1-2 mmol/l/hour); a rise of 5 mmol/l is usually sufficient to improve symptoms and treat cerebral oedema
- The strategy to correct serum sodium rapidly to 120 mmol/l and then more slowly has no evidence base and does not prevent osmotic demyelination. It should therefore be abandoned
- Overcorrection and autocorrection should be anticipated during treatment with hypertonic or isotonic saline

Figure 1. *Adrogué-Madias formula*

$$\text{Change in serum } Na^+ = \frac{(\text{infusate } Na^+ + \text{infusate } K^+) - \text{serum } Na^+}{\text{total body water} + 1}$$

$$\text{Volume (liter)} = \frac{\text{Desired } \Delta [Na]_s}{\Delta [Na]_s \text{ (with 1 liter)}}$$

The formula in the upper panel calculates the anticipated rise in serum sodium ($\Delta[Na]_s$) after the administration of 1 litre of a selected infusion. The required information includes the sodium and potassium concentrations of the infusion (infusate Na^+ + infusate K^+), the serum sodium concentration of the patient (serum Na^+) and total body water (usually 0.6 * body weight). The formula in the lower panel calculates how much volume of the selected infusion should be given based on the desired rise in serum sodium (desired $\Delta[Na]_s$) and the calculated rise in serum sodium using the formula in the upper panel ($\Delta[Na]_s$ (with 1 litre)). For example, if the anticipated rise in serum sodium with 1 litre of 3% NaCl is 10 mmol/l, and the desired rise in serum sodium is only 5 mmol/l, then 5/10 = 0.5 litre of the infusion should be administered. The time in which this infusion is administered should be based on the recommended correction limits (tables 2 and 6).

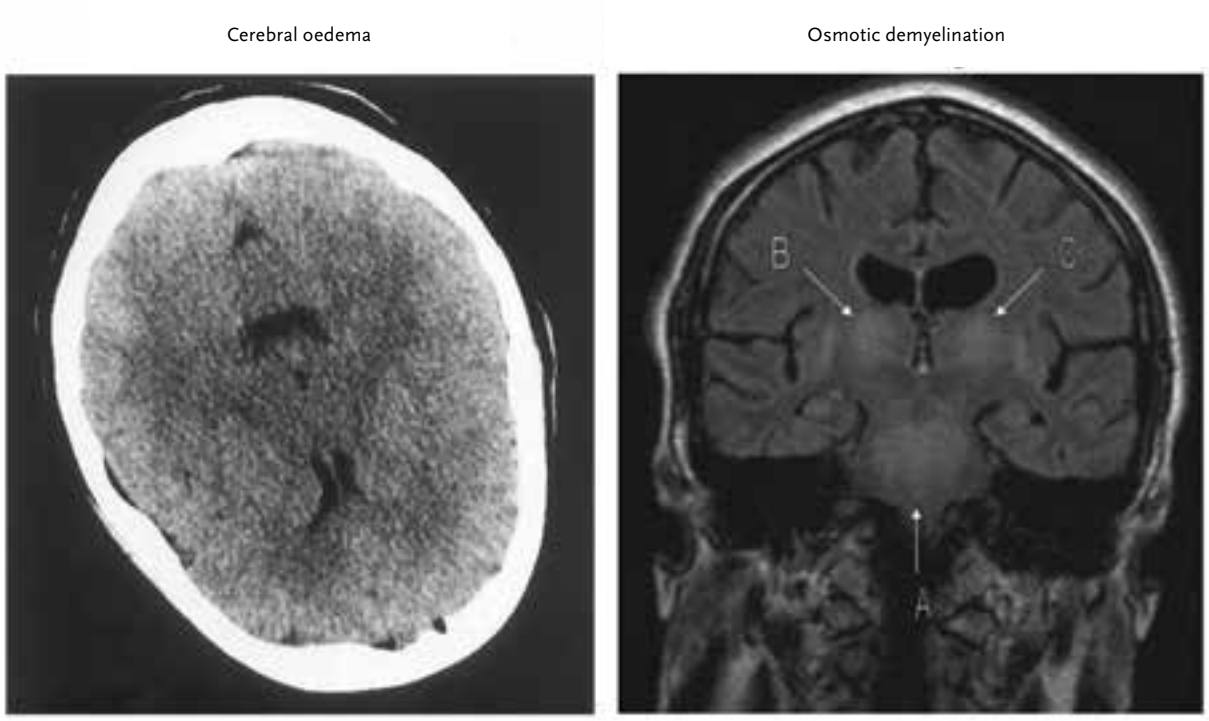
hypernatraemia may develop when there is lactation failure.[17] In the elderly a reduction in thirst sensation and urinary concentration ability as well as an increase in insensible loss predispose to hypernatraemia.[18] In the ICU, hypernatraemia is usually hospital-acquired, suggesting an iatrogenic component.[19,20] Indeed, studies have shown that a positive sodium balance often plays a role in the pathogenesis of ICU-acquired hypernatraemia.[11,19] This may be due to a shift towards using primarily isotonic intravenous fluids. If the patient for some reason has a reduced urinary concentrating ability, isotonic fluids are hypertonic to the urine and result in a positive sodium balance. In this setting treatment should rely on adding more water, but diuretics may also be useful.[21,22]

Importantly, hypernatraemia in the ICU is independently associated with mortality,[19,23,24] although it is unknown whether preventing or correcting hypernatraemia improves outcome. Although hyperglycaemia usually causes hyponatraemia (see further), it can also present with hypernatraemia if there is a large osmotic diuresis with renal water loss that is not compensated by intake.[25] Hypernatraemia also develops frequently during the treatment of hyperglycaemia because less water is attracted from the intracellular fluid compartment when serum glucose is lowered during therapy. Hypernatraemia in this context is often useful to prevent a rapid decrease in effective serum osmolality caused by decreasing serum glucose and helps to prevent the development of cerebral oedema.[26,27]

HYPONATRAEMIA

Hyponatraemia is defined as a serum sodium concentration <135 mmol/l and can be further classified as acute or chronic and symptomatic or asymptomatic. Patients are more likely to be symptomatic when hyponatraemia develops acutely (usually <48 hours, *table 1*). Acute hyponatraemia causes brain cell swelling due to a shift of water from the extracellular to the intracellular fluid compartment.[4] Cerebral oedema due to acute hyponatraemia is a medical emergency especially when there is concurrent hypoxia (*figure 2*).[28,29] Therefore, when hyponatraemia is documented to be acute or when severe symptoms are present, immediate treatment with hypertonic saline is indicated and should precede diagnostic evaluation (*table 6*). As for hypernatraemia, we recommend the use of the Adrogué-Madias formula

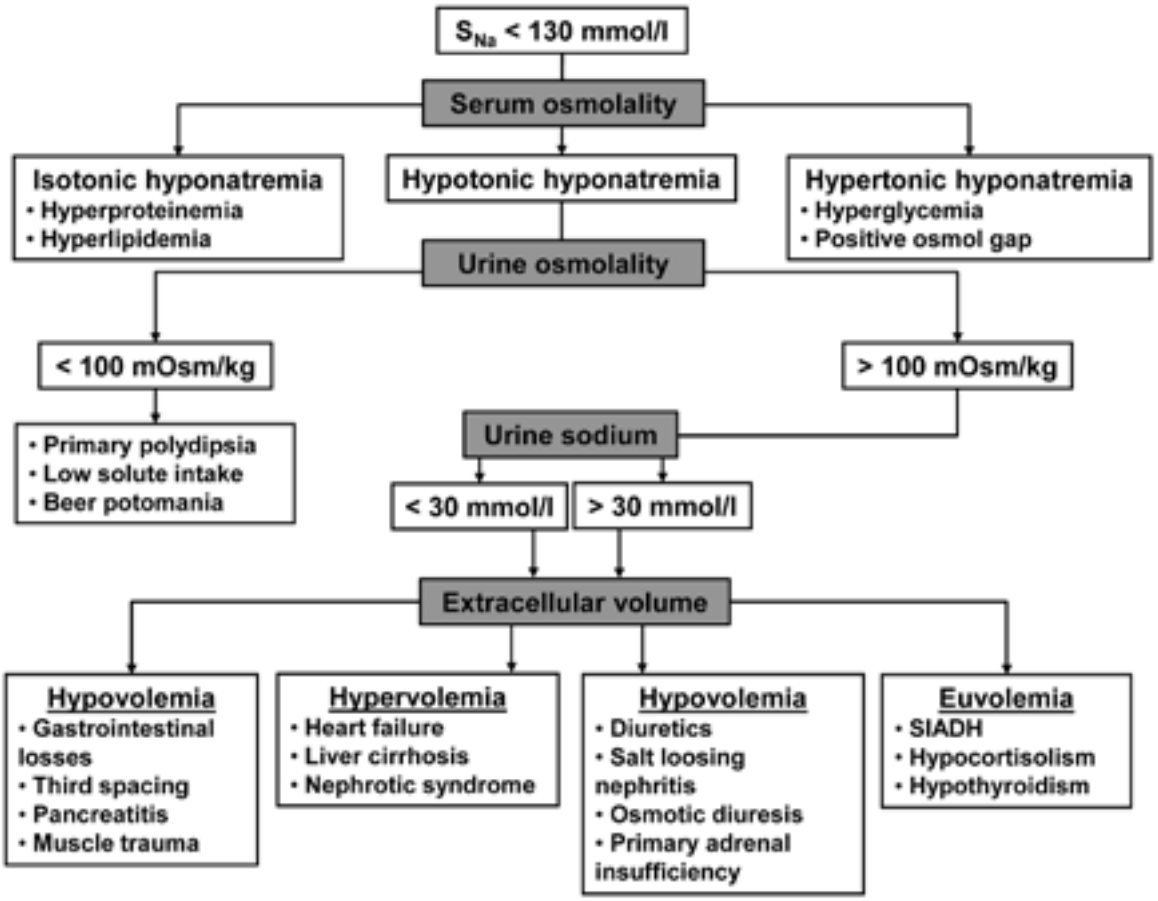
Figure 2. *Complications of hyponatraemia: cerebral oedema versus the osmotic demyelination syndrome*



Left panel: computed tomography image of a patient with acute cerebral oedema due to desmopressin-induced hyponatraemia (serum sodium 111 mmol/l). Right panel: magnetic resonance image of a patient with the osmotic demyelination syndrome due to too rapid correction of chronic hyponatraemia (14 mmol/l in 18 hours; A indicates the pons, while B and C indicate the basal ganglia). Sources: Schreiber A et al., Am J Kidney Dis 2004; 44: e3-6 and Abbott R et al., BMJ 2005; 331: 829-30. Reprinted with permission.

to estimate the anticipated rise in serum sodium during treatment with hypertonic saline (*figure 1*). Although a bolus of 100 ml of 3% NaCl has recently been advocated as a simple alternative to a continuous infusion of hypertonic saline,[30] there is as yet no evidence to support this. Conversely, when hyponatraemia is chronic or when few symptoms are present, the underlying cause should be identified and serum sodium should be corrected gradually. Indeed, when serum sodium is corrected too rapidly in chronic hyponatraemia, there is a risk of developing the so-called osmotic demyelination syndrome (*table 6, figure 2*).[31] Patients at increased risk of cerebral oedema due to hyponatraemia include postoperative patients, children, elderly women using thiazides, psychiatric patients with primary polydipsia, and patients with hypoxia.[32] Risk factors for the osmotic demyelination syndrome during overly rapid correction of chronic hyponatraemia include alcoholism, thiazide diuretics, malnutrition, hypokalaemia, and hypoxia.[32] It is important to note that some risk factors predispose to both cerebral oedema and osmotic demyelination. In addition to cerebral oedema and osmotic demyelination, which are relatively rare, hyponatraemia in general is independently associated with increased mortality.[33] It remains unknown, however, whether hyponatraemia contributes directly to mortality and whether correction of hyponatraemia reduces mortality.[34,35] The most common causes of hyponatraemia are shown in *table 3*. The differential diagnosis of hyponatraemia is often challenging.[36] The recommended diagnostic tests in a patient with hyponatraemia are shown in *table 4*. A diagnostic algorithm illustrates how serum osmolality, urine osmolality, urine sodium, and assessment of volume status help to differentiate hyponatraemia (*figure 3*). Although many algorithms are available, we recommend this specific algorithm for three reasons. First, a recent study showed that the diagnostic accuracy of junior physicians following this algorithm was higher than that of senior physicians not using the algorithm.[37] Second, this algorithm relies primarily on objective diagnostic tests and does not start with a clinical assessment of the extracellular fluid status, which has a low sensitivity and specificity in patients with hyponatraemia.[38] Third, this algorithm starts with a differentiation between hypotonic, isotonic, and hypertonic hyponatraemia, which is an important diagnostic step. Isotonic hyponatraemia is usually caused by pseudohyponatraemia, which is a laboratory artefact due to the fact that all venous samples undergo a dilution step prior to measurement.[39] Pseudohyponatraemia should be distinguished from

Figure 3. *Diagnostic algorithm for hyponatraemia*



See text for details. S_{Na} = serum sodium; SIADH = syndrome of inappropriate antidiuretic hormone secretion.

hyperglycaemia-induced hyponatraemia, which is usually a form of hypertonic hyponatraemia and is caused because glucose (an effective osmole) attracts water from the intracellular fluid compartment.[40] The syndrome of inappropriate antidiuretic hormone secretion (SIADH) is a common cause of hyponatraemia with a specific set of essential and supplemental diagnostic criteria (*table 7*).[41] A number of causes should be excluded prior to establishing a diagnosis of SIADH, including diuretic use, adrenal insufficiency, and hypothyroidism. Especially secondary adrenal insufficiency mimics SIADH because hypocortisolism increases vasopressin secretion.[42] However, primary adrenal insufficiency can also present with isolated hyponatraemia while other characteristic signs are absent, including hyperkalaemia and orthostatic hypotension.[43,44] Although hypothyroidism can cause hyponatraemia, this appears to be rare and probably only occurs in myxoedema coma when there is also a decrease in cardiac output and glomerular filtration rate.[45] A recent study did identify a correlation between hypothyroidism and decreased serum sodium, but found this effect to be small and clinically irrelevant.[46] The causes of SIADH are myriad, but can be classified into malignancy (e.g., small cell lung carcinoma), pulmonary disease (e.g., pneumonia), neurological disease (infections, stroke, neurodegenerative diseases), and drugs (e.g., antidepressants, antiepileptics, and antipsychotic drugs).[41,47,48] A number of miscellaneous causes is also important and include transient causes such as the postoperative state, nausea, pain, and exercise.[41] The nephrogenic syndrome of inappropriate antidiuresis is a relatively novel cause of SIADH and is caused by an activating mutation in the gene encoding the vasopressin type 2 receptor.[49] A complete list of all possible causes of SIADH can be found in a recent review article.[41] Cerebral salt wasting is a rare and incompletely understood cause of hyponatraemia that is sometimes difficult to differentiate from SIADH.[50] It has been described most clearly after subarachnoid haemorrhage and does not always lead to hyponatraemia.[51] Cerebral salt wasting can cause a contracted extracellular fluid volume due to profound natriuresis. Therefore, polyuria, a very high urine sodium concentration, a high serum urea, orthostatic

Table 7. *Criteria for the syndrome of inappropriate antidiuretic hormone secretion*

Essential features	Supplemental features
• Decreased effective serum osmolality (<275 mOsm/kg)	• Serum uric acid <0.24 mmol/l
• Urine osmolality >100 mOsm/kg during hypotonicity	• Serum urea <3.6 mmol/l
• Clinical euvolaemia	• Fractional sodium excretion >1%
• Urine sodium > 30 mmol/l with normal dietary sodium intake	• Fractional urea excretion >55%
• Normal thyroid and adrenal function	• Failure to correct hyponatraemia after 0.9% saline infusion
• No recent use of diuretic agents	• Correction of hyponatraemia by fluid restriction
	• Abnormal results on test of water load*
	• Elevated plasma vasopressin levels despite the presence of hypotonicity and clinical euvolaemia

*** < 80% excretion of 20 ml water/kg during 4 hours and/or inability to dilute urine to osmolality < 100 mOsm/kg.**

hypotension, and a low central venous pressure argue in favour of cerebral salt wasting.[52] The treatment options for hyponatraemia, including indications, advantages and disadvantages, are shown in *table 8*. The vasopressin type 2 receptor antagonist tolvaptan was recently approved by the European Medicines Agency for treatment of hyponatraemia secondary to SIADH. All studies have shown that tolvaptan increases serum sodium effectively in SIADH,[53] but tolvaptan does not reduce mortality and can result in overcorrection.[54,55] In addition, tolvaptan has only been compared against placebo and not against one of the other treatment options for SIADH. Finally, it is important to factor in the cost of tolvaptan (~80 euro per tablet). At present, we therefore not recommend tolvaptan as a first-line treatment of hyponatraemia secondary to SIADH. When hyponatraemia is treated with hypertonic or isotonic saline, one should be aware of the possibility of overcorrection or autocorrection (*table 6*). Overcorrection is defined as exceeding the anticipated rise in serum sodium and usually occurs during treatment with hypertonic saline.[56] Autocorrection can also lead to overcorrection and this can occur when during treatment of hypovolaemic hyponatraemia with isotonic saline, the trigger for volume-mediated vasopressin release suddenly abates. Therefore, during treatment with hypertonic or isotonic saline, serum sodium should be monitored regularly (up to every three hours). Impeding overcorrection should be curtailed by discontinuing hypertonic or isotonic saline and starting a hypotonic infusion.[57] One study suggests that combining hypertonic saline with desmopressin may help prevent overcorrection.[58]

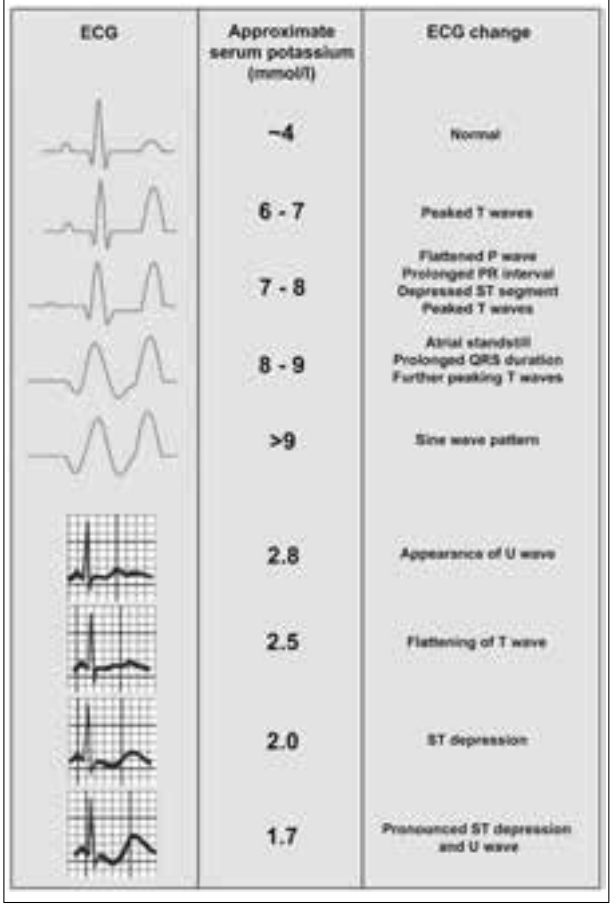
HYPERKALAEMIA

Hyperkalaemia is defined as a serum potassium concentration >5.5 mmol/l. The signs, symptoms and complications of hyperkalaemia are shown in *table 1* and *figure 4*. Hyperkalaemia and hypokalaemia can impair cardiac conduction in the heart and cause muscle weakness or paralysis. Therefore, hyperkalaemia can lead to ECG changes, arrhythmia, and even ventricular fibrillation. The most common causes of hyperkalaemia are listed in *table 3*. Pathophysiologically, these causes can be divided into pseudohyperkalaemia, redistribution of potassium from the intracellular to the extracellular compartment ('shift hyperkalaemia'), reduced glomerular filtration of potassium, and reduced tubular secretion of potassium (*figure 5*). Pseudohyperkalaemia (also called spurious

Table 8. *Therapeutic options in hyponatraemia*

Therapy	Indication(s)	Advantages	Disadvantages
Cause-directed therapy	E.g., steroids in adrenal insufficiency, discontinuation of diuretics	Cause specific	Effect may not be immediate (e.g., half-life medication)
Hypertonic saline (e.g., 3% NaCl)	Acute and/or symptomatic hyponatraemia	Treats or prevents cerebral oedema	Risk of overcorrection and sodium overload
Isotonic saline (e.g., 0.9%)	Hyponatraemia with hypovolaemia; cerebral salt wasting	Corrects hypovolaemia	Risk of autocorrection
Fluid restriction	SIADH, hyponatraemia in heart failure and liver cirrhosis, primary polydipsia	Inexpensive	Compliance
Vasopressin-receptor antagonists	SIADH	Specific and effective	Costs; risk of overcorrection; may be dangerous in hypovolaemia
Loop diuretics	SIADH, primary polydipsia, hyponatraemia in heart failure or liver cirrhosis	Increases free water clearance	Hypokalaemia; ineffective during diuretic resistance
Demeclocycline	SIADH	Inexpensive	Liver toxicity, phototoxicity, risk of overcorrection
Urea	SIADH	Inexpensive and effective	Pallatability

Figure 4. *ECG changes associated with hyperkalaemia and hypokalaemia*



hyperkalaemia) is usually caused by haemolysis related to blood withdrawal (e.g., due to forearm contraction, fist clenching or tourniquet use). When this is suspected (some laboratories report the presence of haemolysis), serum potassium should be measured again by venipuncture or arterial puncture. Less common causes of pseudohyperkalaemia include thrombocytosis and leucocytosis, because these cells can continue to secrete potassium in the blood collection tube.[59,60] Causes of shift hyperkalaemia include acidosis (for each 0.1 fall in pH, potassium increases ~0.4 mmol/l), cell death (tumour lysis, rhabdomyolysis, intravascular coagulation, trauma), drugs (succinylcholine, thalidomide, minoxidil), exercise, and the hyperkalaemic paralysis disorder (see 'hypokalaemia' for further discussion).[9] Any cause of a reduced glomerular filtration rate can cause hyperkalaemia, including acute kidney injury and chronic kidney disease. Reduced renal tubular secretion of potassium can be caused by a disruption of the aldosterone-renal axis, including reduced secretion of aldosterone, inhibition of the renin-angiotensin-aldosterone system, inhibition of the mineralocorticoid receptor, or inhibition of the epithelial sodium channel (ENaC). ENaC is electrochemically coupled to the main transporter responsible for potassium secretion, the renal outer medullary potassium channel (ROMK). Many drugs interfere with the aldosterone-renal axis and drug-induced hyperkalaemia is therefore a common clinical entity (*table 9*).[61] Other causes include primary adrenal insufficiency and genetic causes, including pseudohypoaldosteronism type 1 and type 2.[62] The recommended diagnostic tests to differentiate between the causes of hyperkalaemia are shown in *table 4*. The cause of hyperkalaemia is usually evident from the previous medical history, medication, or accompanying laboratory results. When the cause of hyperkalaemia is less evident, the measurement of plasma renin and aldosterone may be useful. The transtubular potassium gradient (TTKG) is an indirect measure to evaluate whether hyperkalaemia is due to hypoaldosteronism.[63] The treatment of hyperkalaemia is summarised in *table 10*, and relies on membrane stabilisation to prevent cardiac arrhythmia, induction of a shift of potassium into cells, and removal of potassium either naturally (binding in the gut or promoting kaliuresis) or artificially (haemodialysis).[6] The treatment options listed in *table 10* are primarily for the emergency treatment of hyperkalaemia. Chronic hyperkalaemia can usually be treated with ion exchange resins (sodium or calcium polystyrene sulphonate), a low potassium diet, diuretics, or alkali therapy. Recently, a critical review was published on ion exchange resins stating that little evidence is available on their efficacy and that they may cause colonic necrosis.[64]

Table 9. *Drugs that can cause hyperkalaemia*

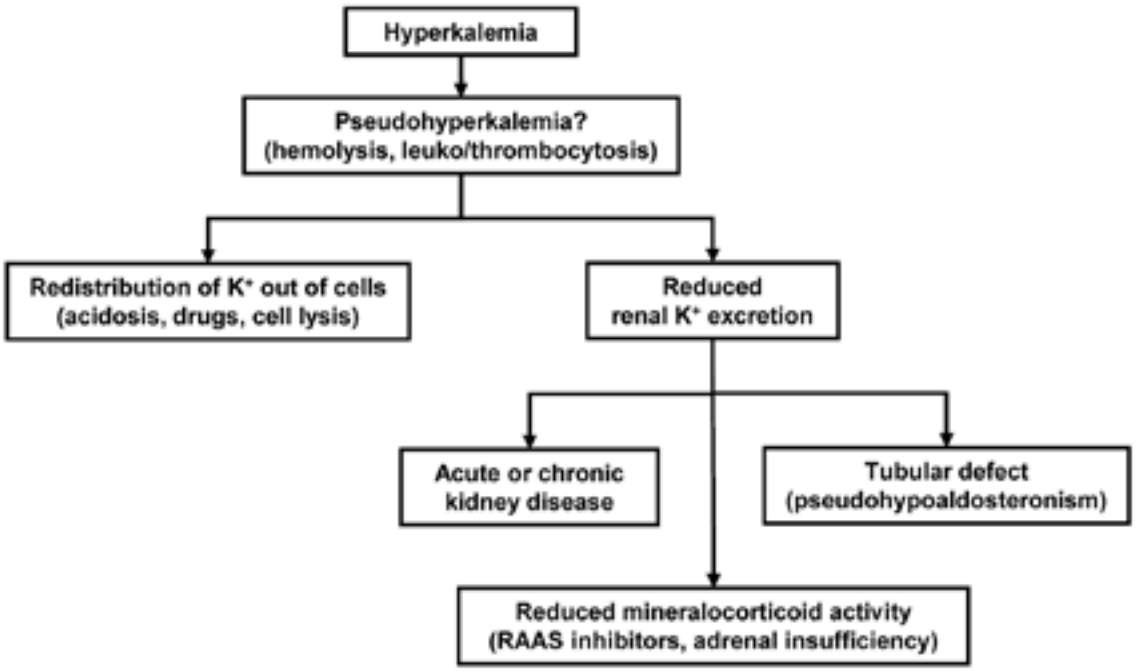
Drugs interfering with the renin-angiotensin-aldosterone system
- Beta-blockers (lower renin)
- Angiotensin-converting enzyme (ACE) inhibitors
- Angiotensin receptor blockers
- Mineralocorticoid receptor blockers (spironolactone, eplerenone, drosperinone)
- Renin inhibitors
- Nonsteroidal anti-inflammatory drugs
- Heparin
- Antifungal drugs
- Calcineurin inhibitors (cyclosporine, tacrolimus)*

Drugs that block the epithelial sodium channel
- Amiloride
- Triamterene
- Trimethoprim and co-trimoxazole
- Pentamidine

Potassium-containing compounds
- Potassium supplementation
- Potassium chloride in table salt
- Medicinal herbs
- Blood transfusions

*** Calcineurin inhibitors also have direct tubular effects that contribute to hyperkalaemia.**

Figure 5. *Diagnostic algorithm for hyperkalaemia*



See text for details. K^+ = potassium; RAAS = renin-angiotensin-aldosterone system.

HYPOKALAEMIA

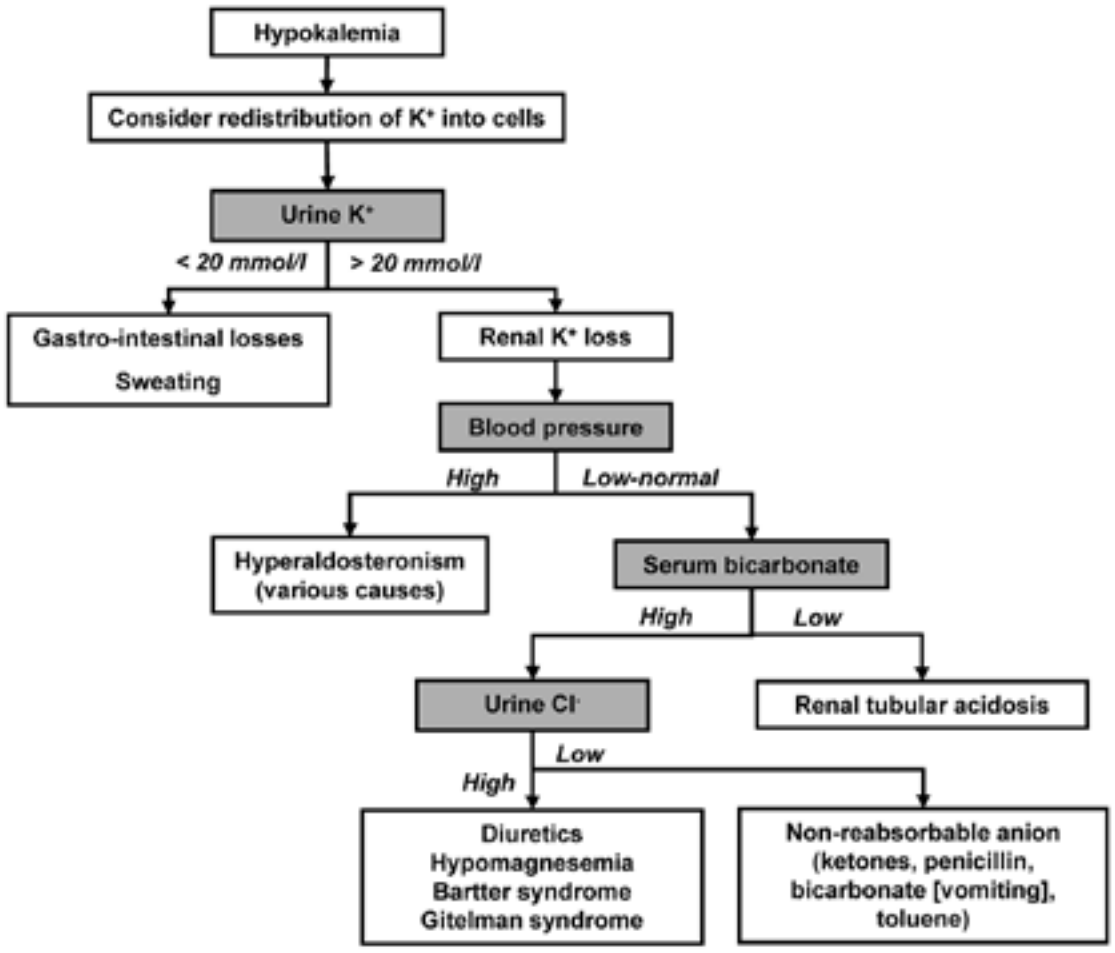
Hypokalaemia is defined as a serum potassium <3.5 mmol/l. When hypokalaemia is severe (usually serum potassium <2.5 mmol/l), it can cause ECG changes, arrhythmia, muscle weakness or paralysis (*table 1, figure 4*). The most common causes of hypokalaemia are listed in *table 3*. The causes of hypokalaemia can be divided into redistribution of potassium from the extracellular to the intracellular fluid compartment ('shift hypokalaemia'), extra-renal loss of potassium, and renal loss of potassium (most common). The recommended diagnostic tests to differentiate between these causes are shown in *table 4*. *Figure 6* shows a diagnostic algorithm which uses the urine potassium concentration, blood pressure, serum bicarbonate, and urine chloride to differentiate between the various causes of hypokalaemia. The urine potassium cut-off of 20 mmol/l is somewhat arbitrary and does not account for the concentration of the urine sample. Instead, the urine potassium to creatinine ratio may be

Table 10. *Treatment of hyperkalaemia*

Principle	Treatment	Comment
Membrane stabilisation	Calcium	• Only if serum potassium >7 mmol/l and/or ECG changes • Does not lower potassium, but reduces risk of arrhythmia
Move potassium into cells ('shift')	Insulin with glucose	• 10-20 units may be needed • Serum potassium and glucose should be monitored frequently
	Sodium bicarbonate	Especially if there is concurrent acidosis
	β2-adrenergic agonists	Because of side effects only recommended as rescue therapy
Remove excess potassium ('drift')	Restore diuresis	Intravenous fluids in hypovolaemia; urinary catheter or nephrostomy drain placement in post-renal causes
	Haemodialysis or haemofiltration	Especially in dialysis patients, patients with chronic kidney disease or in the intensive care
	Ion exchange resin (e.g., sodium or calcium polysterene sulphonate)	• Can be given orally or rectally • Takes ~4 hours before it works
	Loop diuretics	Especially if there is hypervolaemia
	Fludrocortisone	Especially if there is adrenal insufficiency

a better parameter and a cut-off of 2.5 mmol/mmol has been suggested by one study.[65] This study also showed that the urine potassium to creatinine ratio and the TTKG can be used to differentiate shift hypokalaemia from renal causes of hypokalaemia.[65] During shift hypokalaemia, the kidney will retain potassium, and therefore the urine potassium to creatinine ratio and the TTKG will be low. Causes of shift hypokalaemia include alkalosis (for each 0.1 increase in pH, the potassium falls ~0.4 mmol/l), insulin, hypothermia, increased production of erythrocytes (e.g., during folate or vitamin B12 therapy), stimulation of β2-adrenergic receptors, drugs (chloroquine, risperdal, quetiapine), and hypokalaemic paralysis.[7] Hypokalaemic and hyperkalaemic periodic paralysis represent a group of rare disorders in which patients experience periodic shifts of potassium into or out of cells triggered by food intake or exercise.[66] The cause is often genetic and various mutations in calcium and sodium channels in muscle have been identified.[67] An important cause of acquired hypokalaemic paralysis is thyrotoxicosis.[68] The combination of hypertension and hypokalaemia has a specific differential diagnosis that requires the measurement of plasma renin and aldosterone. Possible causes include renal artery stenosis and renin-producing tumour (*high renin, high aldosterone*), primary aldosteronism (Conn's disease) and glucocorticoid remediable hyperaldosteronism (*low renin, high aldosterone*), Liddle's syndrome, syndrome of apparent mineralocorticoid excess, and the use of liquorice *(low renin, low aldosterone*). Of these, renal artery stenosis, primary aldosteronism, and liquorice use are the most common. Hypokalaemia with non-anion gap metabolic acidosis also has a specific differential diagnosis that includes diarrhoea and renal tubular acidosis (RTA). These two causes can be differentiated based on the urine potassium excretion (low in

Figure 6. *Diagnostic algorithm for hypokalaemia*



See text for details. Cl^- = chloride; K^+ = potassium.

diarrhoea, high in RTA), the urine anion gap (negative in diarrhoea, positive in RTA), or the urinary electrogram which estimates renal ammonium secretion (high in diarrhoea, low in RTA). RTA can be further classified as type I (distal) and type II (proximal), which can be distinguished based on the presence of proximal tubular dysfunction (type II), fractional excretion of bicarbonate after bicarbonate infusion (type II), or a urinary acidification test (type I).[69] For a complete list of the causes of RTA, the reader is referred to two reviews.[70,71] Hypomagnesaemia can also cause hypokalaemia likely because a low intracellular magnesium concentration activates the renal potassium transporter ROMK to secrete more potassium.[72] A cause of drug-induced hypomagnesaemia that was identified relatively recently are proton pump inhibitors.[73] Although the mechanism remains elusive, several reports suggest that proton pump inhibitors can cause severe hypomagnesaemia due to gastrointestinal magnesium loss that was often accompanied by secondary hypokalaemia and hypocalcaemia.[74,75] The treatment of hypokalaemia relies largely on potassium supplementation. Potassium chloride is the preferred compound, although potassium bicarbonate, citrate or acetate can be given if there is concurrent acidosis, and potassium phosphate can be given if there is concurrent hypophosphataemia. Potassium supplementation should be given with caution when there may be shift hypokalaemia because of the risk of rebound hyperkalaemia.[76] Symptomatic hypokalaemia should be treated intravenously and, in severe cases, may require a central venous catheter and continuous ECG monitoring. Less severe cases of hypokalaemia can be treated with oral potassium supplementation either as liquid or as tablet. When hypokalaemia is due to renal potassium loss, a potassium-sparing diuretic may be added as treatment such as amiloride or spironolactone. Importantly, the treatment of hypokalaemic periodic paralysis differs from other forms of hypokalaemia.[77] First, less potassium supplementation should be given (<10 mmol/hour not exceeding a total of 60 mmol) because of the risk of rebound *hyperkalaemia* when the shift has ceased.[78] Second, the shift of potassium into cells can be reversed by giving high doses of propranolol (3 mg/kg).[79] When hypomagnesemia is present, hypokalaemia usually does not respond to potassium supplementation until magnesium supplementation is also started.

CONCLUSIONS

Although electrolyte disorders are not often the primary reason for admission to hospital, they are common and may contribute to adverse outcomes. More specifically, all the electrolyte disorders reviewed here can cause an immediate threat to the patient that may require emergency treatment. At the same time, too aggressive treatment can also cause complications and therefore frequent monitoring is necessary. Diagnostically, the presence of one or more of these electrolyte disorders may be the first sign of an important underlying disease. Although little high-level evidence is available to guide the management of electrolyte disorders, we believe the revised guideline will help to harmonise the diagnosis and treatment of these disorders. We therefore recommend the use of this guideline in daily practice. We believe future revisions of the guideline should also be based on European guidelines, which are currently being developed. We welcome any suggestions for improvement of this guideline based on experiences with its use.

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LETTER TO THE EDITOR

Doctor Google, Mister PubMed?

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To the Editor,

To support diagnostic decisions, a search of PubMed is usually considered to be the best source. Our recent experience with a single patient suggests that an alternative search strategy can be even more effective at times.

A young healthy woman who lives in a southern village noted an itch in her index finger around midnight and saw a pinpoint black mark on her skin. Soon it became swollen and within 5 hours intense pain in her finger developed associated with local discoloration, malaise and fever of 38.6°C. She was admitted with normal vital signs and white blood cell count of 11 x 10^3/µl. Despite adequate intravenous antibiotic treatment, the pain in her finger required morphine and the finger became necrotic around the middle phalanx. Fasciotomy along the lateral aspects of the digit was performed, subcutaneous low-molecular-weight heparin administered and hyperbaric oxygen therapy in a hyperbaric chamber were commenced to try and save the finger.

In the absence of a viable alternative explanation the possibility of a spider bite was raised but its association with digital gangrene remained doubtful.

A PubMed search took a few minutes and yielded nil results in any combination tried (digital/finger gangrene/necrosis AND spider/loxosceles – altogether eight combinations using filters of English, humans and abstracts available).

The impression was that spider bites have not been previously reported as a possible cause of the patient's lesion.

However, the attending physician, unaware of the negative PubMed search, conducted a similar simple Google search. The single search took a second and yielded 54,900 results (finger loxosceles). Among the first ten hits, one article provided clinical pictures virtually identical to our patient's condition.[1] Another added a vivid description of the vasoconstrictive action of the loxosceles (brown recluse spider) venom and an exhaustive list of differential diagnosis.[2] Both articles were fully available. The same information could be rapidly retrieved from the first ten hits of similar Google searches (e.g. finger necrosis spider). Thus, not even resorting to Google Scholar, physicians' Google-based search for diagnostic information may at times be more rapid and efficient than initiating a PubMed query. This appealing alternative option[3] should be kept in mind.

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INFORMATION FOR AUTHORS

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2. Kaplan NM. Clinical Hypertension. 7th ed. Baltimore: Williams & Wilkins; 1998.
3. Powell LW, Isselbacher KJ. Hemochromatosis. In: Braunwald E, Fauci AS, Kasper DL, et al., editors. Harrison's Principles of Internal Medicine. 15th edition. New York: McGraw-Hill; 2001. p. 2257-61.

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

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

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