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**Editorial office 'The Netherlands Journal of Medicine'**
Geeralien Derksen-Willemsen
Radboud University Nijmegen Medical Centre
Department of General Internal Medicine 541
PO Box 9101, 6500 HB Nijmegen
The Netherlands
Tel.: +31 (0)24-361 04 59, fax: +31 (0)24-354 17 34
E-mail: g.derksen@aig.umcn.nl

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EDITORIAL

# Liquorice and hypertension

**S.H. van Uum**

Department of Medicine, University of Western Ontario, Canada,
e-mail: Stan.VanUum@sjhc.london.on.ca

ABSTRACT

**Glycyrrhetinic acid, the active constituent of liquorice, inhibits renal 11β-hydroxysteroid dehydrogenase. This allows cortisol to stimulate mineralocorticoid receptors, which can result in hypertension and hypokalaemia. Treatment options are based on pathophysiological understanding.**

Liquorice, the root of the *Glycyrrhiza glabra*, has been used throughout the millennia for its taste and for medical purposes. Natural liquorice root was found in the 3000-year-old Tomb of King Tut. Soldiers of Alexander the Great's army chewed the root as a thirst quencher. Early Greek physicians as Hippocrates used natural liquorice to heal wounds and sore throats, and liquorice is an extremely important herb in Chinese medicine. In the Middle Ages it was used for treatment of hypotension. In 1946, the Dutch physician F.E. Revers demonstrated that liquorice was the active ingredient in a domestic medicine used in the Netherlands, and reported good results in the treatment of stomach ulcers. He also observed, however, that many patients developed hypokalaemia and an increase in blood pressure. Following this Borst *et al.* demonstrated that liquorice and cortisone had a synergistic effect in Addison's disease.[1] Later investigations showed that aldosterone secretion was suppressed in liquorice-induced hypertension, thus the expression 'pseudohyperaldosteronism' was used. The hypertension responds to spironolactone, a blocker of mineralocorticoid receptors (MRs), but no steroids stimulating the MRs could be identified.

The mechanism by which both liquorice and the apparent mineralocorticoid excess (AME) syndrome cause hypertension was not understood until the discovery of the 11β-hydroxysteroid dehydrogenase isozymes (11β-HSDs).[2] These isozymes catalyse the interconversion of cortisol and cortisone. 11β-HSD type 1 is most abundantly expressed in liver and adipose tissue, where it mainly functions as a reductase, converting inactive cortisone to active cortisol.[3,4]

The second isozyme, 11β-HSD2, is highly expressed in mineralocorticoid target tissues such as renal cortex[5,6] and salivary glands.[7] This isozyme has mainly dehydrogenase activity and is already active at very low cortisol concentrations. 11β-HSD2 plays a key role in regulating mineralocorticoid activity of cortisol. *In vivo* MRs are protected from exposure to cortisol by activity of 11β-HSD2. This isozyme rapidly metabolises the active mineralocorticoid cortisol to its inactive metabolite cortisone, thus preventing stimulation of MRs by cortisol. Aldosterone is not metabolised by 11β-HSD2 and can therefore bind to the MRs. Liquorice contains glycyrrhizin, in the intestine this is converted to glycyrrhetinic acid (GA) which is absorbed. GA inhibits activity of 11β-HSD2, this allows cortisol to bind to the MRs resulting in a hypermineralocorticoid state. Two case reports in this issue of the Netherlands Journal of Medicine demonstrate that liquorice-induced effects can present in very different ways. The report by Van den Bosch *et al.* reminds us that chronic liquorice intake can result in very serious symptoms, including rhabdomyolysis and paralyis.[8] The case report by Janse *et al.* demonstrates that liquorice-induced hypertension can occur at any age, and that a high level of suspicion is required to elucidate liquorice abuse.[9] This is also illustrated by the story of a 42-year-old female patient in Ontario, Canada. She developed hypokalaemia and mild hypertension without apparent cause. Only after several weeks it became clear that her family in the Netherlands had sent her boxes of liquorice as a Christmas present. As she enjoyed the taste she finished the boxes within a few weeks, resulting in the clinical situation described. It should be noted that GA can also be ingested from a variety of other products, including laxatives,[10] liquorice tea,[11] and Chinese medicines.[12]

When liquorice is suspected to be the cause of hypokalaemia and/or hypertension, the diagnosis can be confirmed by measuring plasma levels of GA,[13] or by demonstrating an increased ratio of cortisol over cortisone in plasma, saliva or urine.[14] In clinical practice, discontinuation of liquorice intake will often be sufficient.

How much liquorice is required to develop symptoms? This will depend on the amount of GA in the liquorice, as there is a clear dose-response relation between GA

and cortisol-cortisone ratio.[15] On average, 1 g of liquorice contains about 2 mg GA, but the amount of GA varies considerably, from 0.026 to 98 mg per gram liquorice.[16] The effect on blood pressure is also dose related: in a study in healthy volunteers, the increase in systolic blood pressure was 3 mmHg following 75 mg GA, and 14 mmHg following 540 mg GA a day.[17]

The sensitivity for the effect of liquorice varies between individuals. A daily dose of 100 g liquorice, containing 150 mg GA, resulted in an increase in systolic blood pressure of 15 mmHg in subjects with primary hypertension, while the increase was only 3.5 mmHg in normotensive subjects.[18] However, there was no difference in the urinary cortisol-cortisone ratio between the groups, suggesting that there was no difference in the inhibition of renal 11β-HSD2 activity by liquorice. It is not clear, therefore, whether patients with hypertension are more sensitive to the effect of liquorice on 11β-HSD2 activity, or that the difference is located at or post mineralocorticoid receptor level. Alternatively the effects could be located outside the kidneys. Some studies have suggested that there is a relation between 11β-HSD2 activity and salt sensitivity,[19] but other studies could not confirm this.[20] Case reports have shown that liquorice intake as low as 50 g a day has occasionally resulted in clinical effects.[21] One wonders if these patients had mutations in the 11β-HSD2 gene resulting in congenitally reduced activity of 11β-HSD2, rendering them more susceptible to the inhibitory effects of liquorice.

The treatment of patients with liquorice-induced hypertension and hypokalaemia is based on understanding the pathophysiology. The first step is recognition of liquorice as a cause, and discontinuation of its intake. Next steps may be administration of potassium and blockade of the MRs. In severe hypokalaemia, administration of dexamethasone could be considered, as this will suppress the endogenous production of cortisol, thus decreasing stimulation of MRs by cortisol.

In conclusion, the story of liquorice and its effect on blood pressure and potassium remains fascinating. It has been a very useful tool in the discovery of the importance of intracellular shuttling of cortisol and cortisone. Liquorice-induced effects can be encountered in patients of all ages and all over the world. In clinical practice, a high level of suspicion remains warranted in patients with unexplained hypokalaemia and/or hypertension.

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REVIEW

# Anti-IgE and other new immunomodulation-based therapies for allergic asthma

**R.E. Jonkers, J.S. van der Zee**

Department of Pulmonology, Academic Medical Centre, Amsterdam, the Netherlands,
tel.: +31 (0)20-566 43 56, fax: +31 (0)20-691 75 84, e-mail: r.e.jonkers@amc.uva.nl

## ABSTRACT

**Understanding of the cellular and molecular mechanisms in asthma has lead to the recognition of a number of potential therapeutic targets, a few of which have been evaluated in clinical studies. Parenteral administrations of both anti-IL-5 and IL-12 inhibit eosinophil recruitment to the airways, but display a lack of clinical efficacy. Interrupting the IL-4 pathway thus far has also shown disappointing results in clinical studies. Omalizumab is the first anti-IgE monoclonal antibody developed for the treatment of moderate to severe asthmatics to receive FDA approval. In a number of clinical trials treatment with omalizumab was associated with moderate improvements in a number of relevant endpoints, including the rate of occurrence of disease exacerbations. Newer DNA-based therapeutic strategies including DNA vaccination and the antisense oligonucleotides show promise but thus far have only been tested in animal models.**

## KEYWORDS

Anti-IgE, asthma, immunomodulation

Allergic asthma is now considered to be a complex syndrome rather than a single disease entity. Its major clinical characteristics include a variable degree of airway obstruction and bronchial hyper-responsiveness (BHR): the capacity to react with an (increase in) airway obstruction in response to a variety of nonspecific stimuli, such as cold air and exercise. In addition, the majority of patients have elevated serum levels of IgE, as well as specific IgE antibodies to common environmental allergens, such as house dust mite and animal dander.

In clinical studies substantial inflammation has been found in bronchial biopsy specimens from patients with asthma, even in those with mild disease. Current therapy therefore emphasises suppression of this airway inflammation by the regular use of inhaled corticosteroids (ICS), which provides reasonable efficacy for patients with mild asthma. In this group, as well as in patients with more severe disease, there is an obvious need to make existing therapies readily available and to educate patients as to how and why the medication must be taken on a regular basis. However, even when given appropriately, existing therapies do not completely solve the clinical problems of subjects with moderate and severe asthma. These patients still experience significant residual symptoms and are sometimes subject to frequent exacerbations of their disease associated with consumption of healthcare resources and poor quality of life, despite the use of higher doses of inhaled corticosteroids as well as adjunctive therapy, such as long-acting $\beta_2$-agonists and antileukotrienes. The prevalence of asthma in Western Europe has doubled in the last decade, leading to an estimated prevalence in the adult population of 10 to 15%.[1] It is estimated that about 5% of these patients have poorly controlled asthma despite the use of maximally recommended doses of inhaled therapy[2] and an additional number of patients can only be managed well at the cost of the use of high doses of ICS. The latter is relevant in view of concerns about systemic side effects such as cataract, osteoporosis and skin atrophy as a consequence of the long-term use of higher doses of inhaled corticosteroids. So there are a substantial number of patients who may benefit from novel therapies designed to target specific mechanisms underlying airway inflammation in asthma.

The chronic inflammatory reaction in the airway walls of asthmatics is dominated by eosinophilic and neutrophilic granulocytes and T helper lymphocytes as well as mast cells. Dendritic cells appear to be the key cells for antigen presentation in asthmatic airways. Following antigen stimulation, naive T helper precursor T cells acquire a restricted capacity for cytokine production. Those producing predominantly interleukin (IL)-4, IL-13 and IL-5 have been termed $T_h2$ cells and those producing predominantly interferon-γ $T_h1$ cells (*figure 1*). Microbes are probably the chief stimuli of 'protective' $T_h1$ immunity by stimulating macrophages to produce IL-12, which in turn stimulates $T_h1$ cells to produce interferon-γ. IL-4 is required for the development of $T_h2$ cells and together with IL-13 regulates IgE production. IL-5 is the major $T_h2$ cytokine involved in the accumulation of eosinophils in allergic inflammation (*figure 2*). The inflammatory process is accompanied by, and probably the driving force behind, structural changes in the airway wall, generally referred to as airway remodelling.

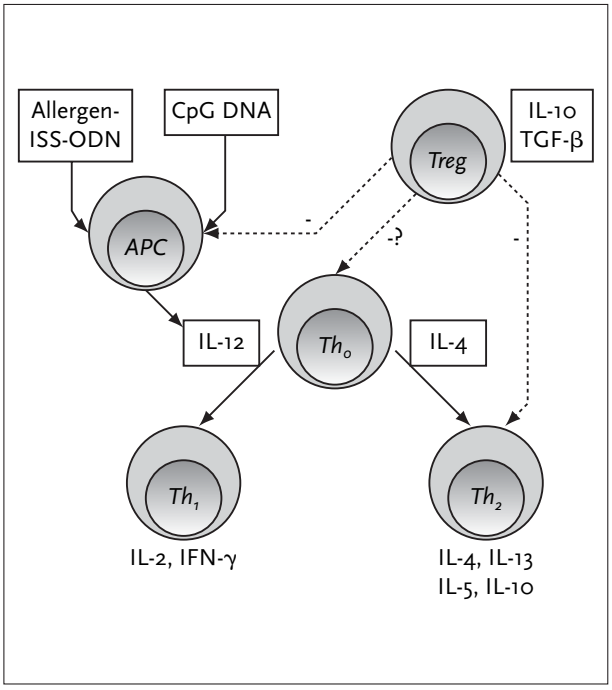


**Figure 1**

*Factors involved in the balance between lymphocytes with a predominantly Th1- or Th2-like profile of cytokine excretion*

**Within this concept immunostimulatory sequence oligodeoxynucleotide (ISS-ODN)-based therapies and CPG-DNA provide a signal which leads to the increased production of IL-12 by antigen presenting cells (APC), which in its turn shifts the Th1/Th2 balance in a Th1-like direction. Under the influence of IL-4 naive Th0 cells differentiate in a Th2-like direction. Interleukin 2 and interferon-γ are important effector cytokines of Th1 lymphocytes; IL-4, IL-13, IL-5 and IL-10 of Th2 lymphocytes. Treg = regulatory T cells.**

These structural changes are the result of the interaction of inflammatory mediators and resident cells as well as of plain tissue injury. Structural cells in the airways and the matrix respond to the inflammation in an apparently coordinated fashion, which can be viewed as an attempt to repair the damage in an effort to keep the airway intact. The net result is an increase in airway wall thickness, to which all the tissue elements can contribute, which leads to a reduction in airway luminal diameter. Remodelling and inflammation result in, or at least contribute to, airway hyper-responsiveness which together with the reduced diameter of the airways cause the (periodic) breathlessness and wheezing that are so characteristic of clinical asthma. There is at least doubt as to whether ICS are able to prevent or reverse the process of airway wall remodelling.

The dominant mechanism through which IgE determines the expression of atopy is through its binding to high-affinity receptors (FcεRI) expressed on the surface of tissue mast cells and basophils. During an immediate hypersensitivity reaction cross linkage of IgE with allergen results in the release of an array of preformed and newly generated mediators of inflammation, which are responsible for the early asthmatic response (EAR) (*figure 2*). During late-phase allergic reactions (LAR) eosinophils and neutrophils accumulate, followed by CD4+ T cells and basophils.

The view that microbial stimuli may skew the Th1/Th2 balance forms the basis of the so-called hygiene hypothesis. According to present insights, the development of asthma is the result of a complex interaction of a genetic predisposition and environmental factors. Asthma is strongly associated with atopy and both have shown a remarkable increase in prevalence during the last 30 to 40 years. According to the hygiene hypothesis, this rise is related to the adoption of a Western lifestyle in which the human immune system is deprived of microbial encounters. This would then lead to a lack of stimulation in a Th1-like direction, which results in a shift of the Th1/Th2 balance in a Th2 dominated direction. A subpopulation of suppressive T cells, termed regulatory T cells, recently (re)gained attention since these appear to play a key role in the maintenance of immunological balance. In animal models and *in vitro*, regulatory T cells prevent the development of autoimmunity by inducing tolerance to (Th1) self antigens, but also appear to be able to suppress allergen-induced activation of Th2 cells.[3] It seems that part of the suppressive activity is mediated via direct cell-cell interactions and part via cytokines such as IL-10 and TGF-β, but much is still to be elucidated about the precise mode(s) of action of these regulatory T cells.

The understanding of the cellular and molecular mechanisms of the allergen-induced and Th2 lymphocyte driven inflammatory process in asthmatic airways has lead to the recognition of a number of potential therapeutic targets, a few of which have been evaluated in clinical studies.

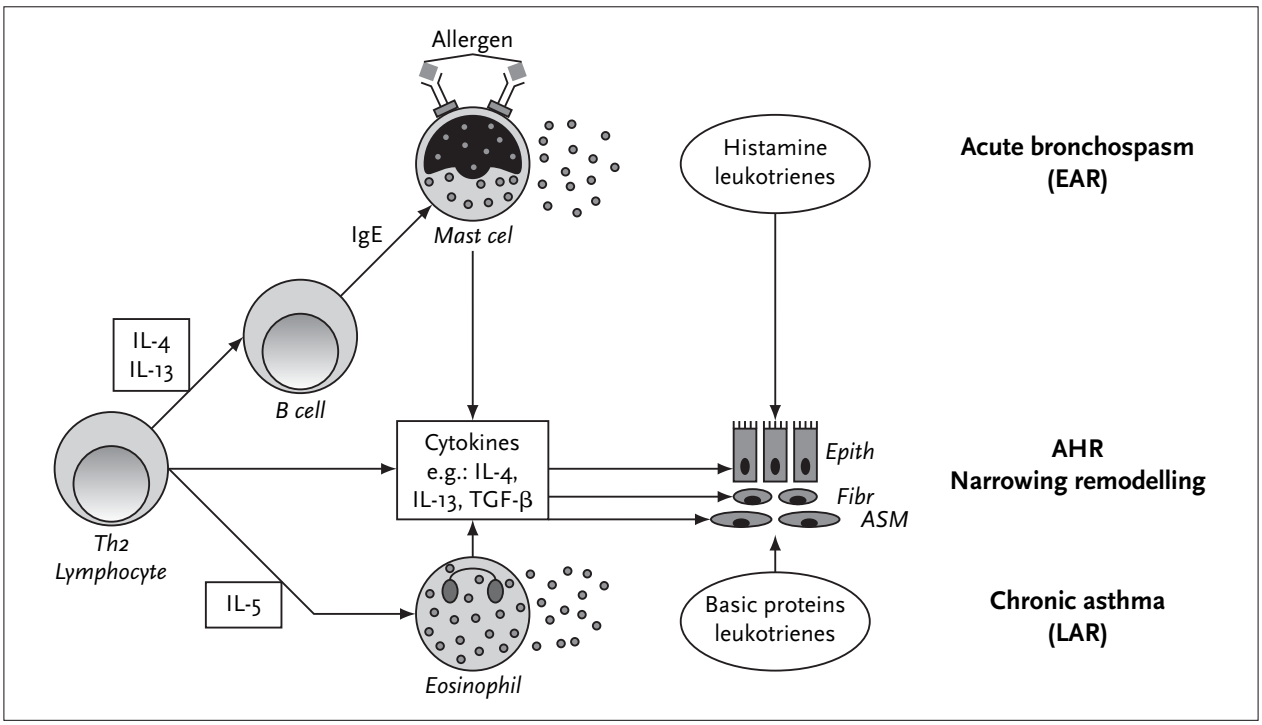


**Figure 2**
*Mechanisms involved in acute and chronic inflammatory reactions in asthmatic airways*

**Th2 lymphocytes drive the inflammatory response by producing IL-4 which stimulates the IgE production by B lymphocytes and IL-5 which is the major cytokine promoting the maturation, recruitment and activation of eosinophils. Free IgE binds to mast cells. After cross-linking of mast cell bound IgE by allergen a number of mediators are released that are able to precipitate an acute asthmatic airway reaction (early asthmatic reaction = EAR). Eosinophilic granulocytes have a direct effect on airway narrowing by the release of basic proteins and lipid mediators (late asthmatic reaction = LAR). Inflammatory cells elaborate cytokines, such as IL-4, IL-13 and TGF-β, that have direct effects on epithelial cells (epith), fibroblasts (fibr) and airway smooth muscle cells (ASM), which in turn lead to the release of growth factors and fibrogenic factors involved in the development or aggravation of airway hyper-responsiveness, airway narrowing and airway wall remodelling.**

## INTERLEUKIN 12

IL-12 is considered to be a key cytokine involved in regulating the balance between Th1 and Th2 cells (*figure 1*). Furthermore, IL-12 inhibits airway hyper-responsiveness and airway eosinophilia after antigen challenge in several animal models for allergen sensitisation.[4-7]
In patients with mild asthma weekly infusions of human recombinant IL-12 in escalating doses over a four-week period caused a progressive fall in peripheral blood eosinophil numbers, a reduction in the rise in circulating eosinophils after allergen challenge and a concomitant reduction in eosinophils in induced sputum.[8] However, this was not accompanied by a suppression of the late asthmatic airway reaction nor an increase in bronchial hyper-reactivity after allergen challenge. Furthermore, due to the IL-12 infusions most of the patients suffered from malaise and one had an episode of cardiac arrhythmia. These side effects in combination with lack of clinical efficacy suggest that IL-12 is not a suitable therapy for patients with established asthma. In a mouse model administration of an IL-12-allergen fusion protein remarkably resulted in the development of a specific Th1 response to the allergen, with increased production of allergen-specific IgG2, instead of a Th2 response with IgE formation.[9] This indicates the possibility of (local) administration of IL-12 as an adjuvant in conjunction with specific allergens to provide a more effective form of immunotherapy, which may even be preventive or curative when given early in the course of atopic disease, in early childhood for instance.

## ANTI-IL-5

Eosinophilic infiltration is a characteristic feature of asthma, and based upon its biological properties this cell has assumed a position as the principal inflammatory cell in asthma. The eosinophil is a rich source of inflammatory proteins, such as major basic protein, which can damage

airway epithelium and increase bronchial hyper-reactivity. IL-5 came forward as a key target in the Th2 cascade in mouse models for asthma using allergen sensitisation and challenge that were extrapolated to the delayed allergen response in humans. In a monkey model with ascaris-induced asthma a monoclonal antibody to IL-5 almost completely abolished eosinophilia and airway hyper-reactivity.[10]

An initial study evaluated the effect of a high-affinity humanised monoclonal anti-IL-5 antibody on the airway responses to allergen challenge in patients with mild asthma.[11] Although the usual increase in blood and sputum eosinophils to allergen challenge was nearly abolished by anti-IL-5 treatment, it did not affect either the EAR or the expected post-allergen-challenge increase in BHR.

In a follow-up study, anti-IL-5 treatment nearly ablated circulating and bronchial lavage fluid eosinophils, but in contrast in mucosal biopsies the reduction of eosinophils was only about 50%, and the deposition of major basic protein was unaffected.[12] The different susceptibility for anti-IL-5 treatment of eosinophils in different biological compartments may explain why airway functional outcomes appear to be resistant to this therapy.

The biology of eosinophil recruitment and activation in the airways appears to be more complex than traditionally viewed, which may explain the absence of significant clinical efficacy of anti-IL-5, also in patients with more severe asthma.[13] There is evidence to support *in situ* eosinophilopoiesis in asthmatic airways.[14,15] However, the number of the eosinophil progenitor cells is hardly if at all decreased by anti-IL-5 therapy.[14] Furthermore, the role of IL-5 may be limited to the recruitment of mature eosinophils from the bone marrow, since airway eosinophils express reduced numbers of surface IL-5 receptors relative to their circulating counterparts; the decrease in IL-5 receptors is associated with a loss in capacity to degranulate to IL-5.[16,17] Eosinophil recruitment to the airways and the local biological activity almost certainly requires a coordinated effort of IL-5 and other cytokines and chemokines such as eotaxin.[18]

As stated earlier, asthma is probably a complex syndrome rather than a single disease. Therefore, anti-IL-5 therapy may only benefit those patients in whom eosinophilic inflammation contributes significantly to the signs and symptoms of disease. It may be important to design studies that allow identification of such patients. It is noteworthy that variability in the response to medication is characteristic in asthma. In a study in mild to moderate asthmatics, one third of patients did not respond to therapy with the leukotriene receptor antagonist montelukast.[19] Furthermore, it may be worthwhile to look for treatment regimens that lead to more profound reductions of numbers of eosinophils and their biological activity in the airways. Since corticosteroids reduce eosinophil numbers in the airways by apoptosis, such reduction may be achieved by the administration of high doses of systemic corticosteroids followed by anti-IL-5 as a kind of maintenance treatment to prevent repopulation of the airways with eosinophils. Support for such an approach can be found in a recent study showing significant reductions in airway eosinophils by high-dose systemic corticosteroids in severe asthmatics.[20]

## INTERRUPTING THE IL-4 PATHWAY

In view of its role in $T_h2$ cell development, neutralisation of IL-4 is likely to be most effective at the time of initial allergen encounter. However the contribution of IL-4 in the effector phase of immunity and the theoretical role in promoting ongoing $T_h2$ cell commitment during established inflammation provide a rational basis for interrupting IL-4 signalling in asthma. Treatment with soluble recombinant IL-4 receptor (to compete with the endogenous ligand) showed encouraging results in an initial placebo-controlled trial in atopic asthma. A single nebulised dose of soluble IL-4 receptor prevented the fall in lung function induced by the withdrawal of inhaled corticosteroids in patients with moderately severe asthma[21] and sustained asthma control was obtained by weekly nebulisations over a 12-week period.[22] However, the results of an as yet unpublished large-scale trial indicate that the agent has no clinical efficacy in asthma. This negative result for IL-4 blockade does not preclude that other approaches aiming at the inhibition of the IL-4 pathway, such as interrupting downstream IL-4 receptor signalling by targeting transcription factors such as Stat6 and GATA-3,[23] may be more successful.

## ANTI-IGE

Preclinical studies have convincingly shown that anti-IgE antibodies are able to abrogate IgE-mediated responses in allergic disease. Omalizumab is a humanised recombinant monoclonal anti-IgE antibody that selectively binds to the Cε3 domain of free IgE at the FcεI receptor binding site. It is very important that these antibodies bind to the FcεI receptor binding part of IgE, to prevent cross-linking of mast cell bound IgE and subsequent anaphylactic reactions. Omalizumab appears to be safe and well-tolerated during long-term administration to adults, adolescents and children with asthma. In patients with allergic asthma, omalizumab results in a rapid decline in free serum IgE levels in a dose-dependent manner.[24] In addition, FcεI receptor expression on basophils[25] and dendritic cells[26] markedly decreases. In clinical trials of a six-month duration, treatment with subcutaneously administered omalizumab

resulted in reductions in serum IgE levels of 90% or more.[27-29] Short-term treatment of asthmatic patients with omalizumab inhibited the EAR and LAR after allergen challenge, as well as the accompanying increases in airway eosinophilia and BHR.[24] These observations suggest anti-inflammatory effects of maintenance therapy with omalizumab.

In a number of well-designed clinical studies, the efficacy of omalizumab as add-on with ICS has been evaluated in patients with moderate to severe allergic asthma.[27-30] In these studies patients typically received omalizumab subcutaneously every two or four weeks. The results showed improvements in symptom scores, reduced use of rescue $\beta_2$-agonists and improvements in lung function. Furthermore, significant reductions in exacerbation rates were observed in adults and adolescents (*table 1*). Treatment with omalizumab allowed reductions in the doses of inhaled and oral corticosteroids, while effects obtained during the corticosteroid stable phase generally could be maintained during the corticosteroid reduction phase (*table 1*). Although the aforementioned effects were all clinically significant, it might be argued that they are relatively modest in view of the nearby elimination of systemic IgE that can be achieved by treatment with omalizumab. This is consistent with observations in animal models suggesting that it might be possible to dissociate T cell-induced asthmatic airway reactions from IgE-dependent ones.[31] In addition, late-phase allergic airway reactions have been induced in patients with atopic asthma in the absence of an immediate hypersensitivity reaction and mast cell activation.[32,33] The observation of such IgE-independent and major histocompatibility complex (MHC)-restricted late-phase reactions indicates that the activation of T lymphocytes alone may be sufficient to initiate airway reactions in sensitised individuals, which may underlie the limited clinical efficacy of anti-IgE. Alternatively, the amounts of specific IgE still present on pulmonary mast cells related to the residual presence of total IgE in the circulation may be sufficient to precipitate mast cell activation and degranulation.

## IMMUNOSTIMULATORY SEQUENCE OLIGODEOXYNUCLEOTIDE (ISS-ODN)-BASED STRATEGIES

The therapeutic goal of allergen immunotherapy is the induction of protective immunity to an allergen to which a clinical hypersensitivity pre-exists, by inducing a change in the allergen-specific adaptive immune response. Desensitisation can be achieved by traditional protein-based immunotherapy. However, allergen immunotherapy requires multiple injections, takes at least several months to achieve a therapeutic effect, is generally less effective than pharmaceutical therapeutics for the treatment of allergic airway symptoms, and has associated risks, such as potentially life-threatening anaphylaxis. Although it is has been shown that effective therapy is associated with an increase in the level of specific IgG and a shift from Th2 to Th2 responses,[34,35] precise information on what immunological mechanisms lead to a reduction in allergic effector functions is lacking. Promising results have been achieved in patients suffering from allergic rhinitis or allergies to bee and wasp venom[36,37] but there is a need for more effective strategies to prevent and reverse the Th2-biased immune deviation that drives the pathogenesis of allergic airway conditions. In this respect regulatory T cells are an emerging target for immunomodulation. In a DNA vaccine (*figure 3*) the gene for the antigen of interest is cloned into a bacterial plasmid that is engineered to augment the expression of the inserted gene in mammalian cells. After being injected, the plasmid enters the host cell, where it stays in the nucleus but is not integrated into the host's DNA. Using the host cell protein-synthesising 'machinery', the plasmid DNA directs the synthesis of the antigen it encodes. This approach involving the synthesis of antigen within host cells has potential advantages over immunisation with exogenous (recombinant) proteins. A protein produced by transfected cells is more likely to be folded in its native configurations, which favours the production of effectively neutralising antibodies. Furthermore, in the course of

**Table 1**

*Observed effects of treatment with omalizumab in randomised controlled trials in adults and children*

| STUDY | AGE GROUP | ASTHMA SEVERITY | REDUCTION IN ICS DOSE *VS* PLACEBO | REDUCTION IN ORAL CS DOSE *VS* PLACEBO | REDUCTION IN EXACERBATIONS *VS* PLACEBO* |
|---|---|---|---|---|---|
| Milgrom[28] | 12-18? | Moderate-severe | 33% | | NS, 85% |
| Milgrom[27] | 11-50 | Moderate-severe | | 60% | |
| Soler[29] | 12-75 | Moderate-severe | 33% | | 58%, 52% |
| Busse[30] | 12-75 | Severe | 25% | | 48%, 41% |

**Reduction during stable corticosteroid phase and corticosteroid reduction phase, respectively. (I)CS = (inhaled) corticosteroids.*

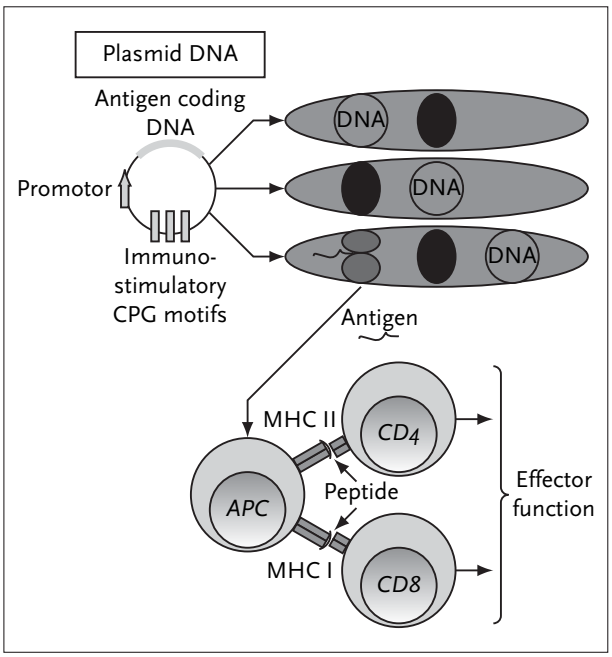


**Figure 3**
*The concept of DNA vaccination*

**After intramuscular injection the DNA plasmid is actively or passively taken up by host cells. Antigens produced by transfected myocytes can be taken up and processed by antigen presenting cells (APCs). Alternatively APCs can be transfected directly. APCs can process and present peptides complexed with MHC molecules to the immune system after migration to lymphoid tissues.**

evaluating the immunogenicity of various gene vaccination vectors, it was discovered that effective vaccination was dependent upon the presence of immunostimulatory DNA sequences - unmethylated cytidine phosphate guanosine (CpG) motifs - within the plasmid, which provide adjuvant Th1 immunity for the responses that develop towards the gene product.[38] These unmethylated CpG motifs, which are more highly present in microbial DNA, are recognised as foreign by the innate immune system via Toll-like receptor 9.
Several ISS-ODN-based therapeutic strategies were evaluated and proved to be effective for the prevention and reversal of Th2-mediated models of allergic disease in animals (for review see Horner & Raz 2002).[39] Vaccination with allergen mixed with ISS-ODN proved more effective than vaccination with allergen alone in the induction of Th1-biased and the reversal of Th2-biased immune responses. In their turn, allergens physically conjugated to ISS-ODN have shown improved immunogenicity and reduced allergenicity compared with allergen-ISS-ODN mixtures. In animal models, ISS-ODN conjugate vaccine induced antigen-specific Th1-biased immune responses that were maintained for at least one year.[40,41]
Preliminary clinical data in patients with ragweed-sensitive allergic rhinitis show that allergen-ISS-ODN conjugate vaccination is feasible and well tolerated.[42]

## (R)ASONS

Antisense oligonucleotides (ASONs) are short oligonucleotides, modified to slow degradation, which code for peptides that match the non-coding strand of DNA. When introduced into a cell they inhibit protein synthesis by binding RNA in a sequence-specific manner and blocking translation. The advantages of the antisense technology include its high specificity, ability to be delivered locally to the lung (respirable antisense oligonucleotides (RASONs)) avoiding systemic side effects, and relative low production cost. The main disadvantages are the tedious work to find the appropriate antisense and evaluate its specificity and effectiveness, and the generation of anti-DNA antibodies.[43]
A human antisense against the adenosine receptor has been tested in a rabbit model for allergic inflammation.[44] Adenosine A1 receptor binding activity as well as receptor numbers were significantly (75%) and specifically reduced by administration of the antisense by direct inhalation of the nebulised material. In physiological terms the RASON reduced the BHR and the EAR in rabbits sensitised to house dust mite. Recently an antisense oligodeoxynucleotide for IL-4 was found to inhibit allergic inflammation *ex vivo* in nasal mucosal biopsies of ragweed allergic rhinitis patients. These data support the concept of treating allergic airway diseases by local administration of antisense oligonucleotides.

## CONCLUSIONS

Although it may be too early to draw firm conclusions, the rather limited beneficial effects in clinical studies interrupting the IL-4 and IL-5 pathways at least challenge the Th2 paradigm in human asthma. However, even if these observations are supported by further studies, this would not be the definite proof that these cytokines are not important in the pathogenesis of asthma. Blockade of Th2-mediated or other pathways relevant in asthma will not necessarily lead to a rapid reversal of physiologically relevant abnormalities that were in fact initially the result of it. Therefore, lack of a rapid meaningful clinical effect cannot be taken as evidence that a specific cytokine is not involved in asthma pathogenesis, but rather might be taken as an indication that therapy must be started much earlier in the course of the disease.
In the majority of patients with persistent asthma the disease can be well controlled by the currently available

inhaled anti-inflammatory (ICS) and bronchodilator agents. The new recombinant DNA-based high-molecular-weight proteins such as anti-IL-5 and anti-IgE are expensive drugs that need parenteral administration at least partly under medical supervision.

None of the above-mentioned treatment modalities are likely to lead to a cure of asthma. Additive value above existing therapies must be based on a better control of asthma, reduced need for the use if inhaled therapy or the induction of long-term disease remissions with infrequent (parenteral) administration.

Studies showing that omalizumab can be an effective and well-tolerated add-on therapy for moderate to severe patients with allergic asthma already on ICS have formed the basis of the recently obtained FDA approval. Procedures to allow omalizumab on the European market are ongoing, but it is to be foreseen that in this license the emphasis will be on more severe asthmatics.

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REVIEW

# Is chronic HIV infection associated with venous thrombotic disease? A systematic review

**S.K. Klein[1*], E.J. Slim[1], M.D. de Kruif[1], T.T. Keller[1], H. ten Cate[2], E.C.M. van Gorp[1], D.P.M. Brandjes[1]**

[1]Department of Internal Medicine, Slotervaart Hospital, Amsterdam, the Netherlands, [2]Department of Internal Medicine and Cardiovascular Research Institute, Maastricht University Hospital, the Netherlands, tel.: +31 (0)20-512 93 33, e-mail: igskk@slz.nl, *corresponding author

## ABSTRACT

**Infection with the human immunodeficiency virus (HIV) is still a major health problem world-wide. HIV infection has changed into a chronic infection with the chance of developing long-term complications. Vascular complications are frequently reported in the current literature. HIV and treatment by highly active antiretroviral therapy (HAART) are associated with many cardiovascular risk factors. An increased risk of arterial cardiovascular complications was found in a number of studies. However, data about the risk of venous thrombotic disease (VTE), including potentially fatal conditions as pulmonary embolism, were limited. In a systematic review of the literature, ten relevant epidemiological studies were identified that investigated the risk of venous thrombotic disease in HIV-infected patients. The incidence was increased two- to tenfold in comparison with a healthy population of the same age. However, these studies were mainly retrospective cohort studies that were prone to selection bias, confounding factors were not always mentioned and in all but three control populations were missing. An increased risk of venous thrombotic disease in HIV-infected patients could be explained by the presence of a hypercoagulable state, characterised by an increase in procoagulant factors, such as endothelial TF expression and thrombogenic properties of microparticles, and a decrease in anticoagulant factors, including AT III, HC II and the protein C pathway. Furthermore, the risk of VTE was associated with an increased risk of infections and autoimmune haemolytic anaemia, and was weakly associated with HAART. All together, quite some evidence pointed towards a relationship between HIV infection and venous thrombotic disease, but the association still needs to be established in properly designed epidemiological studies.**

## KEYWORDS

AIDS, coagulation, complication, fibrinolysis, HAART, HIV, thromboembolism, venous thrombosis

## INTRODUCTION

Infection with the human immunodeficiency virus (HIV) is increasingly becoming a chronic disease in the developed world. Treatment with highly active antiretroviral therapy (HAART) has successfully prolonged the life expectancy of HIV-infected patients. However, as a consequence, chronic HIV infection and HAART are now increasingly associated with long-term complications.

HAART is a combination of therapy by protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI) and non-nucleoside reverse transcriptase inhibitors (NNRTI). In particular the use of PI is complicated by disorders of lipid metabolism, insulin resistance, osteoporosis, nephrotoxicity and neurotoxicity. Furthermore, the use of NRTI is associated with lactic acidosis. HIV infection itself is associated with a number of complications, including increased general risk of infections and various malignancies. Many of these complications are potential risk factors of cardiovascular disease. In the recent literature, the possibility of a relationship between HIV infection and cardiovascular disease has been frequently discussed. A number of studies have shown an increased risk of arterial vascular diseases in HIV patients. A prospective trial in 1551 HIV-infected patients showed an increased risk of coronary artery disease for patients using PIs.[1] A cohort study comprising 4993 patients revealed an incidence of 0.59 to 3.41/1000 patient-years[2] and a very large prospective observational study enrolling 23,468 HIV-positive patients found an incidence

of 3.5/1000 patient-years for myocardial infarction.[3] Apart from research on arterial vascular diseases, a number of case reports were also published describing venous thrombotic events in HIV-infected patients. Recurrent episodes of deep venous thrombosis (DVT) and pulmonary embolism were relatively frequently reported. Further clinical observations in our own clinic raised questions about the possibility of an increased risk of venous thrombotic disorders in HIV-infected patients. Venous thrombotic disorders could be a serious, potentially fatal complication of HIV infection and clear insight is needed into the risk of venous thrombotic disorders in HIV-infected patients to judge the nature of the relationship, mechanism, risk factors and necessity of intervention.

In a systematic review, all relevant articles published from 1986 to 2004 studying the relationship between HIV infection and venous thrombotic disease are presented here. Clinical epidemiological studies are reviewed together with studies investigating the underlying pathogenic mechanisms. Special attention is paid to factors interfering with blood coagulation, which are particularly relevant for the occurrence of venous thromboembolism, whereas their significance for arterial thrombotic complications is still unknown.[4]

## METHODS

Citations were retrieved from English, French and German language based studies from PubMed and MEDLINE databases, from 1986 to 2004. Using the terms "HIV", "AIDS", "infectious disease", "thrombosis", "deep venous thrombosis", "thrombo-embolism", "pulmonary embolism", "coagulation" and "fibrinolysis", in single terms or in combinations, titles, abstracts and references were systematically scanned by two reviewers for relevant articles on the topic of HIV infection associated with venous thrombotic disorders. With the electronic search approximately 500 articles were found. Case reports, letters, comments and abstracts were excluded. Eventually a reference list of 63 articles remained.

## EPIDEMIOLOGY

In the developed world, the risk of DVT in the general population is approximately 0.10% a year.[5-7] This incidence increases sharply with age, from 0.001% a year in childhood to nearly 1% a year in the elderly[7]. Because most HIV-infected people are relatively young,[8] their background risk of DVT should be expected to be lower than the overall incidence.

Ten epidemiological studies reported on the occurrence of DVT and venous thromboembolic complications among HIV-infected patients (*table 1*). Most of these studies were retrospective cohort studies. One study was both a retrospective and prospective study and one study did not mention the study design. The population sizes in the studies ranked from 60 to 42,935. The first study by Hassell *et al.* in 1994 reported a high incidence of DVT of 18% in 60 HIV-infected people, and of 6.6% in HIV patients who were followed prospectively over a median follow-up of 12 months.[9] In subsequent studies, the risk varied from 0.19 to 7.63%.[10,11] One very large study containing 42,935 patients found an incidence of 0.26%,[12] the same incidence was found for pulmonary embolism by Howling *et al.*[13] Considering these studies, the overall risk of DVT in patients with HIV infection may be roughly estimated to be a two- to tenfold higher in comparison with a healthy population of comparable age.

An important risk factor for developing venous thrombosis in these patients could be severity of HIV infection. One study reported an incidence of venous thrombosis of 0.96% in 728 patients infected by HIV and a twofold higher incidence of 1.9% in 250 patients suffering from AIDS.[14] A second study found a significantly higher incidence of thromboembolic events of 24% in 37 patients with low CD4 counts (<200/mm$^3$) in comparison with 1.1% in 94 patients with higher CD4 counts.[11]

Another risk factor could be related to the introduction of protease inhibitors for the treatment of HIV infection in 1996. George *et al.*[10] found that the incidence of venous thrombotic events increased dramatically from 0.19% before the introduction of protease inhibitors to 1.07% afterwards. However, recently Fultz *et al.*[15] reported finding no significant increase.

## INCREASE IN PROCOAGULANT FACTORS

The increased risk of DVT in HIV-infected patients could be related to increased levels of procoagulant factors. Endothelial cells could play an important role in the activation of the coagulation cascade during HIV infection.[16-18] Activation of endothelial cells, which normally behave as anticoagulant regulators, occurred during infections with viruses including HIV,[19] cytomegalovirus,[20] herpes virus and many others.[21-23] From *in vitro* work it is known that infection initiates intracellular signalling through the NFκB pathway, which results in both stimulation of an inflammatory response and in enhanced expression of tissue factor (TF) on the cell membrane. TF induces the extrinsic pathway of coagulation by binding to factor VIIa and therefore is the major initiator of the coagulation cascade.

Another triggering factor of the coagulation cascade in HIV patients could be stimulation of microparticles.

**Table 1**
*Incidence of deep venous thrombosis and complications in HIV-infected patients: overview of studies*

| AUTHOR | YEAR | DESIGN | SIZE | POPULATION | INCIDENCE | ENDPOINTS | DIAGNOSTICS |
|---|---|---|---|---|---|---|---|
| Jenkins[57] | 1991 | R | 243 | AIDS | 3.29% | DVT, PE | Phlebography, V/Q scan |
| Hassell[9] | 1994 | R+P | 60 | HIV | 18% | DVT, PE, thrombo-phlebitis, stroke | Doppler, V/Q scan, ICD-9 code |
| Laing[13] | 1996 | - | 728 | HIV | 0.96% | DVT | Doppler, MRI, venography, V/Q scan |
| | | | 250 | AIDS | 1.60% | | |
| Howling[13] | 1999 | R | 3792 | HIV | 0.26% | PE | CT, pulmonary angiography, V/Q scan, autopsy |
| George[10] | 1999 | R | 650 | HIV* | 1.07% | DVT, PE | Plethysmography, venography, pulmonary angiography, V/Q scan |
| | | | 1050 | HIV** | 0.19% | | |
| Sullivan[12] | 2000 | R | 42935 | HIV | 0.26% | DVT | ICD-9 code |
| Saber[58] | 2001 | R | 4752 | HIV | 0.95% | DVT | Doppler, venography |
| Saif[11] | 2001 | R | 131 | HIV | 7.63% | DVT, PE | Plethysmography, venography, pulmonary angiography, V/Q scan |
| Copur[8] | 2002 | R | 362 | HIV | 2.76% | DVT, PE | Duplex, CT, V/Q scan, ICD-9 code |
| Fultz[15] | 2004 | R | 13549 | HIV** | 2% | DVT, PE, phlebitis, thrombophlebitis | ICD-9 code |
| | | | 5114 | HIV* | 1.6% | | |

**After 1996/ PI introduction, **before 1996/PI introduction. R = retrospective; P = prospective; DVT = deep venous thrombosis; PE = pulmonary embolism.*

Microparticles are relatively small cellular remnants circulating in plasma, originating from platelets and endothelial cells. In HIV patients, microparticles also originate from CD4+ lymphocytes, as a direct consequence of HIV infection and possibly as a reflection of CD4+ lymphocyte apoptosis.[24] Elevated concentrations of microparticles were found in HIV-infected individuals,[25] and, in general, high numbers of microparticles are associated with activation of the coagulation cascade.[25] The procoagulant properties of microparticles are believed to be caused by the clustering of coagulation factor complexes on the activated phospholipid surface serving as catalysts of coagulation reactions. Even in the absence of high levels of microparticles, these elements may still contribute to enhanced coagulation activity, as seen in patients with multiple organ dysfunction syndrome and sepsis.[26]

## DECREASE IN ANTICOAGULANT FACTORS

The increased risk of DVT during HIV infection could also be related to the impaired functioning of several important anticoagulant proteins. In HIV-infected patients with thrombosis, lowered levels of antithrombin (AT) were reported.[27] AT is the most important physiological inhibitor of activated coagulation factors (IIa, IXa, Xa, XIa and XIIa). An inherited heterozygous deficiency predisposes to venous thrombosis and a homozygous deficiency is not compatible with life. Acquired AT deficiency frequently occurs in the course of disseminated intravascular coagulation (DIC). Acquired deficiencies may occur by different mechanisms, including decreased synthesis by the liver, increased loss via the kidneys in the nephrotic syndrome, or inactivation by proteolytic enzymes. In DIC, a combination of impaired production, increased utilisation and clearance of AT protease complexes and accelerated cleavage may occur simultaneously. In HIV infection, the contributing role of these factors is not known.
Other anticoagulant proteins affected by HIV infection are protein C and protein S. These are vitamin K-dependent glycoproteins which are mainly synthesised in the liver. Protein C is a potent anticoagulant which is activated after the binding of thrombin to thrombomodulin on the endothelial cell surface. Activated protein C (APC) inactivates the activated clotting factors V and VIII.[28,29] Protein S has no known enzymatic activity but is an important co-factor for protein C. Most of the plasma protein S is bound to C4 binding protein and approximately 40% is free and active. Reduced concentrations of protein S are associated with an increased risk of DVT.[30] In HIV-infected patients,

a number of abnormalities in this anticoagulant system have been described. Decreased levels of protein C were detected in HIV-infected patients.[31,32] Also, reduced protein S plasma levels and diminished activity were reported.[33,34] In one study, decreased concentrations of protein S were more prevalent in subjects with CD4+ T lymphocyte counts <200/mm$^3$ compared with patients with counts >200/mm$^3$.[35] The reduced total protein S levels in HIV could be related to enhanced activation or apoptosis of circulating T cells, generating microparticles that may bind protein S. This cellular binding process could explain some of the low values of free protein S that were measured by the PEG precipitation technique.[25] Theoretically, lower levels of active protein S could also be caused by downregulation of protein S synthesis[36] or by anti-protein S antibodies.[37]

Heparin cofactor II (HC II) is another anticoagulant protein associated with HIV infection. HC II is a natural thrombin inhibitor. Although the relationship between HC II and DVT is still controversial, the congenital deficiency was reported to be associated with recurrent venous thrombosis.[38] The proportion of subjects with presumably acquired HC II deficiency was significantly greater in HIV-positive individuals than in healthy subjects.[27] A link between HC II and immunodeficiency was suggested by a significant correlation between HC II activity and both the absolute number of CD4+ T lymphocytes and the CD4/CD8 ratio. HC II deficiency was significantly more pronounced in AIDS patients compared with HIV patients.[27] Possible reasons for HC II deficiency could be decreased synthesis, enhanced proteolysis or consumption.

Antiphospholipid antibodies (APL) are proteins directed against different phosphor-containing lipids, the main constituents of cell membranes. The best known of these APL are anticardiolipin antibodies and lupus anticoagulants. Studies have shown APL to be present in 82 to 92% of patients with AIDS.[39] APL are related to an increased occurrence of both venous and arterial thrombosis.[40,41] However, in 63 HIV-infected patients, Palomo *et al.*[42] could not find a correlation between the presence of APL antibodies and development of thrombosis. The increased risk for thrombosis can at least in part be explained by inhibition of activated protein C by anticardiolipin antibodies and their co-factor $\beta_2$-glycoprotein I. Lupus anticoagulants in particular are associated with acute infections by opportunistic organisms such as *Pneumocystis carinii*. However, the presence of APL in HIV-infected patients is not associated with the stage of disease, CD4 cell count, viral load, medication, or with a hypercoagulable state.[9,43] An association described between microparticles and IgG-APL titres may be a consequence of microparticle generation,[24] but the mechanism is not known. Furthermore, increased titres of APL in HIV infection may reflect polyclonal B cell expansion.[44]

## MISCELLANEOUS FACTORS OF HAEMOSTATIS

Endothelial cell activation does not only lead to enhanced expression of procoagulant proteins, but is also related to altered functioning of various other haemostatic factors. In HIV-infected patients, significant increases of von Willebrand factor were described.[45] Von Willebrand factor is a large endothelium-derived protein that mediates platelet adhesion to damaged endothelium, which is the first step in haemostasis.

Furthermore, raised levels of both tissue type plasminogen activator (tPA) and its inhibitor, plasminogen activator inhibitor I (PAI-I), were found in HIV patients.[45-49] Activation of both proteins indicates a general activation of the fibrinolytic system, probably as a reaction to the enhanced tendency to thrombosis (and secondary fibrinolysis).

Endothelial cell activation was also reflected in the detection of increased levels of soluble thrombomodulim (sTM) in HIV-infected patients. Soluble TM is an important co-factor for protein C, and probably the raised levels should also be considered a reaction to the various haemostatic changes.

## SPECIFIC HIV-RELATED FACTORS

A number of specific HIV infection related factors could also contribute to the higher risk of DVT. Treatment with HAART was epidemiologically linked to an increased risk for DVT in one study but this association could not be confirmed in another study. Thus, it is not yet clear whether the occurrence of these events should be attributed to chance, HIV infection or HAART. The mechanism could be related to increases in PAI-1 and fibrinogen levels that were found in patients treated by HAART[50] and could be associated with increased lipid levels[51,52] and in particular with the lipodystrophy syndrome.[52-55] There are no studies published about the association between other antiretroviral therapy (nucleoside reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors alone) and venous thrombosis. In one study, no relationship was found between the use of nucleoside analogues, protease inhibitors, or non-nucleoside reverse-transcriptase inhibitors and the risk of cardiovascular or cerebrovascular events.[56]

In spite of the efficacy of HAART, HIV patients are still at increased general risk of infections. These concomitant infections are an additional risk factor for thrombosis.[11,57,58] Cytomegalovirus infections were associated with pulmonary embolism and cerebral venous thrombosis.[59]

In *Pneumocystis carinii* infection, elevated levels of APL were found in up to 94% of infected AIDS patients.[60,61]

HIV infection can also be complicated by autoimmune haemolytic anaemia. In this condition an increased risk of thromboembolic events, especially during infusion of red blood cells, was reported.[62,63]

## DISCUSSION

A number of case reports suggested that HIV infection was associated with an increased risk for venous thrombosis. In a systematic search of the literature, we retrieved ten relevant studies (*table 1*) that reported the incidence of venous thrombotic events in HIV-infected patients. These studies suggested that the incidence was probably increased two- to tenfold in comparison with a healthy population of similar age. The increased incidence was associated with various changes in blood coagulation in HIV-infected patients, increased risk of infections and autoimmune haemolytic anaemia.
However, the epidemiological studies were limited by some important factors. The power of the studies was limited by the generally low incidence of DVT. Endpoints and the diagnostics used varied greatly, which complicates comparison of the studies. Furthermore, most of the studies were retrospective cohort studies and included different study populations, including both hospital-based and population-based patient groups, making these studies prone to selection bias. Only three studies compared the results with control populations.[8,15,58] However, these control populations were not always fully described, confounding factors, such as interfering malignancies, were not always mentioned, the diagnostic work-up was not always clear, data may have been incomplete and endpoints included phlebitis in two studies.[9,15]

A considerable number of studies in HIV-infected patients described various haemostatic changes that are associated with the risk of developing venous thrombosis. Procoagulant factors, such as endothelial TF expression and thrombogenic properties of microparticles, were upregulated, whereas anticoagulant factors, including AT, HC II and the protein C pathway, were downregulated. In addition, fibrinolytic proteins were present in elevated concentrations and endothelial sTM production was increased. Taken together, these changes represented a general hypercoagulable state in HIV-infected patients and this state could be responsible for the increased risk of venous thrombosis (*figure 1*).

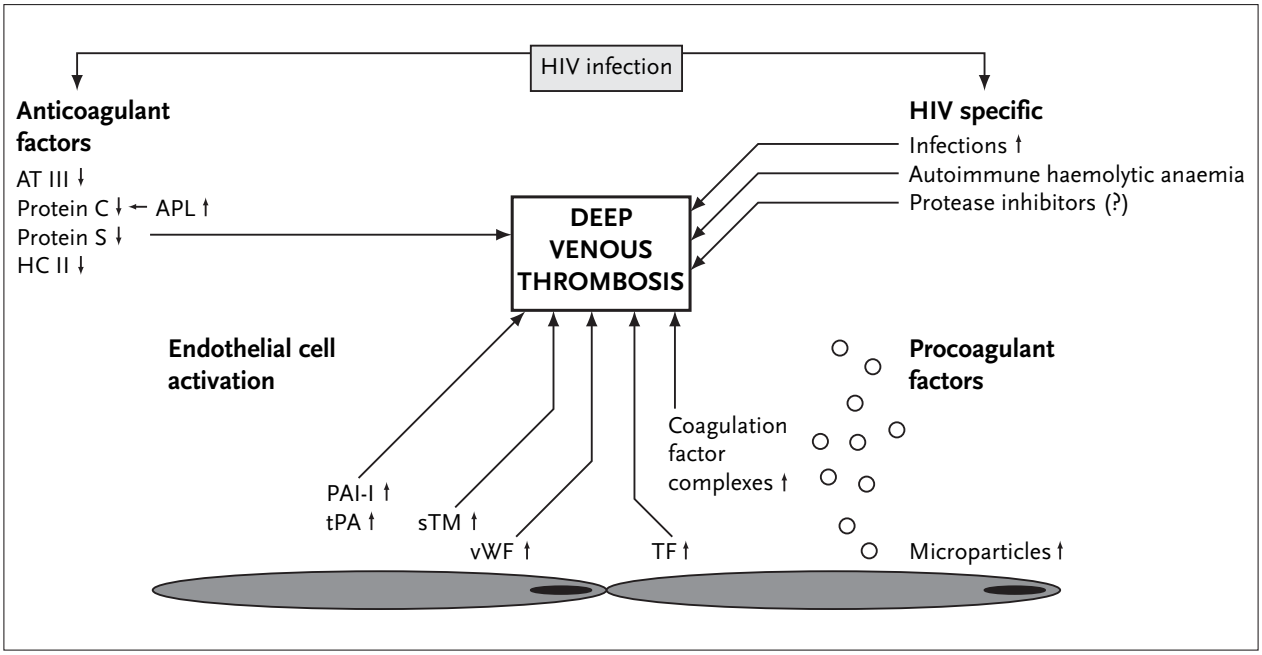


**Figure 1**
*Diagram summarising the hypercoagulable state in HIV-infected patients*

**The hypercoagulable state is associated with an increased risk for developing deep venous thrombosis. Changes in levels of several factors are indicated by arrows upwards and downwards. Continuous lines represent stimulation and dashed lines inhibition. The effect of protease inhibitors is not certain (see text).**

**HIV = human immunodeficiency virus; AT III = antithrombin; APL = antiphospholipid antibodies; HC II = heparin cofactor II; PAI-I = plasminogen activator inhibitor I; tPA = tissue plasminogen activator; sTM = soluble thrombomodulin; vWF = van Willebrand factor; TF = tissue factor.**

In many cases, the origin and mechanism of the haemostatic changes were not clear. The mechanism will probably be related in general to direct triggering of the immune system by HIV and the subsequent stimulation of common pathways involving the inflammatory response and the coagulation system.[64] The hypercoagulable state in HIV patients is also related to the increased risk of other infections. Superimposed infections triggered acquired deficiencies of protein C and protein S and were associated with increased levels of APL. Furthermore, two epidemiological studies showed higher risks for patients with AIDS or with a CD4 count <200/mm$^3$. Thus, the increased risk of DVT in HIV-infected patients is probably caused by active ongoing triggering of the immune system by both HIV infection and superimposed infections.
HAART was associated with an increased risk of DVT by some investigators but the evidence was weak. Considering the rather consistent evidence that HIV infection in itself is related to DVT, both epidemiological and haematological, and that it is likely to be mediated by direct triggering of the immune system, it seems prudent at the moment not to consider DVT as a direct complication of HAART. However, the evidence that HAART is not associated with DVT is not very strong either and is, in fact, limited to one epidemiological study showing a non-significant increase in risk of DVT by HAART. Because of the potential serious consequence of DVT, further studies are strongly recommended to establish whether or not HAART adds to the risk of DVT in HIV-infected patients.
In conclusion, currently available epidemiological evidence suggests that chronic HIV infection is associated with a two- to tenfold increased risk of venous thrombosis in comparison with a general population of the same age. However, these data lack reliability, because the incidence rates were often derived from retrospective cohort studies that were limited by low absolute risk numbers, and because the studies were susceptible to selection bias and they frequently lacked proper control groups. Because of the low absolute risk numbers, it will be difficult to organise adequately powered prospective cohort studies. Well-designed case-control studies could be a suitable alternative to determine the incidence of DVT. In these studies, proper attention should be paid to avoidance of selection bias and to analysis and correction of possible confounding factors, including age and gender, travel history, malignancies, infections, intoxications, PI and other HAART medication use, and inherited and acquired changes in levels of proteins involved in haemostasis.
After all, the multiple evidence of a hypercoagulable state in HIV-infected patients renders it likely that an association between DVT and HIV infection does exist. Finally, if the relationship were to be confirmed in well-designed case-control studies, the option of thrombosis prophylaxis should be considered in HIV-infected patients.

## ACKNOWLEDGEMENT

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## ERRATUM

In the article 'PR and QTc interval prolongation on the electrocardiogram after binge drinking in healthy individuals' by A. Lorsheyd *et al.* all percentage symbols (%) for the amount of alcohol should be read as permillage symbols (‰).

Lorsheyd A, de Lange DW, Hijmering ML, Cramer MJ, van de Wiel A. PR and OTc interval prolongation on the electrocardiogram after binge drinking in healthy individuals. Neth J Med 2005;63(2):59-63.

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# Microscopic colitis: prevalence and distribution throughout the colon in patients with chronic diarrhoea

**W.J. Thijs[1-3*], J. van Baarlen[4], J.H. Kleibeuker[2], J.J. Kolkman[1]**

Departments of [1]Gastroenterology and [4]Pathology, Medisch Spectrum Twente, Enschede, the Netherlands, [2]Department of Gastroenterology, University of Groningen Medical Centre, the Netherlands, [3]Department of Internal Medicine/Gastroenterology, Scheperziekenhuis, Emmen, the Netherlands, tel.: +31 (0)59-169 13 60, fax : +31 (0)59-169 13 61, e-mail: w.thijs@sze.nl, [*]corresponding author

## ABSTRACT

**Background: Microscopic colitis presents with chronic diarrhoea with or without abdominal pain. Microscopic colitis is an important cause of chronic diarrhoea. It can be distributed throughout the colon, as well as limited to the right colon. Microscopic colitis is associated with coeliac disease. We studied the prevalence and distribution of microscopic colitis in patients with diarrhoea and normal colonoscopy and we studied the association with coeliac disease.**

**Methods: Colonoscopy was performed. Biopsies were taken from every segment of the colon. Lymphocytic colitis was defined as the presence of more than 20 lymphocytes per 100 epithelial cells and collagenous colitis was defined as thickening of the basal membrane of more than 10 μm. Upper endoscopy was performed if upper intestinal symptoms were present. If this was the case, small bowel biopsies were taken.**

**Results: Microscopic colitis was found in 13 out of 103 patients. The distribution was diffuse throughout the colon in ten and restricted to the right colon in three patients. In seven patients, upper endoscopy was performed. Marsh I/II lesions were found in six out of seven patients.**

**Conclusion: Microscopic colitis was limited to the right colon in 23% of patients. Biopsies of macroscopically normal colonic mucosa in patients with diarrhoea is mandatory.**

## KEYWORDS

Colonoscopy, diarrhoea, distribution, microscopic colitis

## INTRODUCTION

Microscopic colitis is an entity that presents with chronic, watery diarrhoea and with a macroscopically normal looking colonic mucosa.[1,2] In addition to diarrhoea, cramping abdominal pain and weight loss may occur. The peak incidence of microscopic colitis is in the fifth and sixth decade of life and the disorder has a female preponderance.[3] Three forms of microscopic colitis have been identified based on histopathology: lymphocytic colitis, characterised by the presence of a lymphocytic infiltrate in the epithelium; collagenous colitis, in which the subepithelial collagen layer is thickened (>10 μm), and a mixed form.[4-6] Microscopic colitis is recognised as an important cause of chronic diarrhoea. A large study found a frequency of 9.5 per 100 patients with chronic diarrhoea and normal-looking colonoscopies.[7] Shah *et al.* found microscopic colitis in 13 of 168 patients (7.7%) with chronic diarrhoea in a study, which also included patients with endoscopic abnormalities in the colon.[8]

The presence of histopathological changes diffusely throughout the colon has been described, but the microscopic abnormalities may be limited to the transverse and right colon.[9] The distribution of the histopathological abnormalities obviously has direct consequences for the diagnostic tool that should be used. Tanaka *et al.* found a 27% false-negative rate in a group of patients with collagenous colitis if only biopsies were taken within the reach of a 60 cm sigmoidoscope.[10] Another study reports that in even 40% of patients the diagnosis of microscopic colitis would not have been made if sigmoidoscopy were to have been performed instead of colonoscopy, implying that sigmoidoscopy is inferior to colonoscopy in diagnosing this disease.[11] However, others found a false-negative rate

of only 0.2% using sigmoidoscopy in a group of patients with a variety of colonic diseases presenting with chronic diarrhoea claiming sigmoidoscopy to be a suitable and cost-effective diagnostic tool in the evaluation of chronic diarrhoea.[12]

In patients with microscopic colitis, a high frequency of coeliac disease has been reported[13-15] and persisting diarrhoea in coeliac disease despite adherence to a gluten-free diet warrants a search for microscopic colitis.[16,17]

The goals of the present study were 1) to assess the prevalence of microscopic colitis in patients with chronic diarrhoea and normal looking colonic mucosa, 2) to determine the distribution of microscopic colitis in the colon to investigate whether sigmoidoscopy would suffice in these patients and 3) to investigate the association with coeliac disease.

## MATERIALS AND METHODS

During a period of two years (1999-2000), all patients who presented with chronic diarrhoea underwent a total colonoscopy using a standard colonoscope after giving informed consent. The study took place in a large general hospital. Chronic diarrhoea was defined as loose, frequent bowel movements existing for more than three months. All patients were given midazolam intravenously for sedation and they were monitored with pulse oximetry during the procedure. If no endoscopic abnormalities were found, patients were included in the study. Two mucosal biopsies were taken from every segment of the colon (caecum, ascending colon, transverse colon, descending colon and sigmoid colon) and from the rectum. These biopsies were oriented on a methylcellulose strip paper (Sartorius AG, Germany) using two little wooden sticks. The biopsies were oriented on the strip in the same order as they were taken. Thus, the first biopsies on the strip were from the caecum and the last ones were from the rectum.

Histopathological examination was performed. The biopsies were fixed in 10% (v/v) neutral buffered formalin and embedded in paraplast. Thereafter, 2 μm thin sections were prepared, which were routinely stained, using haematoxylin and eosin. When an increase in intra-epithelial lymphocytes was suspected, the exact number of intra-epithelial lymphocytes per 100 surface epithelial cells was determined by histomorphometry, counting an area of maximally 400 epithelial cells, using a Leica Imaging System (Cambridge, United Kingdom; Software Qwin 2.1 with a locally designed programme) after immunohistochemical staining of the lymphocytes (CD3 clone F7.2.38, DAKO, Glostrup, Denmark; detection system Power Vision HRP-mono, Labvision, Fremont CA, USA). If there was suspicion of collagenous colitis, the basal membrane was also visualised using the AZAN staining and measured using the Leica Imaging System. Lymphocytic colitis was defined as the presence of more than 20 lymphocytes per 100 epithelial cells and collagenous colitis was defined as thickening of the basal membrane of more than 10 μm. Mucosal biopsies from all segments of the colon as mentioned above were examined and the distribution of microscopic colitis along the colon was assessed.

Duodenal biopsies were obtained in patients with an indication for upper endoscopy because of symptoms. In these duodenal biopsies histopathology and histomorphometry were performed. The exact number of intra-epithelial lymphocytes per 100 surface epithelial cells was determined by histomorphometry, counting an area of maximally 400 epithelial cells, after immunohistochemical staining of the lymphocytes as described above. Histopathology of duodenal biopsies was expressed according to the Marsh classification.[18]

An increased number of intraepithelial lymphocytes of >30 per 100 epithelial cells with otherwise normal histology was defined as Marsh I.[19,20] An increased number of intraepithelial lymphocytes in combination with crypt hyperplasia was defined a Marsh II lesion and partial/total villous atrophy as Marsh III.

## RESULTS

In a two-year period, 103 patients (66 female, mean age 45 years, range 17-82 years) with chronic diarrhoea and normal colonoscopy were studied. The caecum was reached in all patients. No complications due to the procedure or sedation occurred. Microscopic colitis was diagnosed in 13 (11 female) patients in total (13%); lymphocytic colitis was diagnosed in 12 patients and collagenous colitis in one patient. The mean age of these patients was 52 years (range 17-82). The presenting symptoms were diarrhoea in all patients, abdominal cramps in 11 (85%) and weight loss in 8 (62%) patients.

The distribution of histopathological abnormalities was diffuse throughout the colon in ten (77%) patients with lymphocytic colitis while the inflammatory changes were located only in the transverse colon and ascending colon in three patients, among whom the patient with collagenous colitis. In these three patients (23%) the diagnosis would have been missed if only sigmoidoscopy had been performed.

Seven patients had upper intestinal symptoms. Upper endoscopy was performed in these patients and small bowel biopsies were obtained. In six of these patients (86%) histopathological investigation was abnormal. In three patients, biopsies showed an increased number of intraepithelial lymphocytes with normal villous architecture (Marsh I), and in three patients an increased number of intraepithelial lymphocytes and crypt hyperplasia was seen (Marsh II). Partial or total villous atrophy (Marsh III) was not found in any of the patients.

## DISCUSSION

Microscopic colitis is an important cause of chronic, watery diarrhoea. The pathogenesis of microscopic colitis is not completely understood. An autoimmune basis has been suggested. Also, microscopic colitis may be caused by several drugs. PPIs may cause microscopic colitis,[21] as well as NSAIDs.[22]

Several studies found a prevalence of 5 to 10% in populations with chronic diarrhoea and normal looking mucosa.[7,23] In our study we investigated 103 patients with total colonoscopy. All patients had normal looking mucosa. In 13 patients (13%) microscopic colitis was diagnosed. Some reports have suggested that in microscopic colitis the histopathological abnormalities are not distributed evenly throughout the colon, but may be located mainly in the right and transverse colon.[10,11] Our results show that in our group of patients the distribution of the disease was diffuse throughout the colon in most of the patients. However, in a considerable proportion of patients the histopathological abnormalities were located solely in the right and transverse colon, not within reach of a sigmoidoscope. In these patients the diagnosis microscopic colitis would not have been made if a sigmoidoscopy had been performed instead of a total colonoscopy. This finding is in line with other studies.[9-11] Our results imply that a diagnosis of microscopic colitis cannot be excluded without a total colonoscopy being performed. To orient our biopsies, we used a simple and reliable method using a methylcellulose strip paper and two little wooden sticks. This simple method allows good orientation of biopsies. Optimal orientation of biopsies is advocated in the diagnosis of coeliac disease[24,25] and we hypothesised that also in microscopic colitis it may facilitate pathological examination, especially in collagenous colitis, where the measurement of the thickness of the basal membrane may depend on the quality of the orientation.

The association of microscopic colitis, lymphocytic as well as collagenous colitis, with coeliac disease is well established.[13,14,26] In our study, we also found abnormal duodenal histology in a high percentage of patients.

It should be mentioned that not all patients with microscopic colitis underwent upper endoscopy, but only those patients with upper abdominal symptoms. We found an increased number of intraepithelial lymphocytes (Marsh I) in three patients and increased intraepithelial lymphocytes with crypt hyperplasia (Marsh II) in another three. The significance of Marsh I-II lesions has not yet been fully elucidated. Currently it may be regarded as latent coeliac disease and is usually not treated with a gluten-free diet.[27] However, some recent studies indicate that a significant proportion of these patients may indeed respond to a gluten-free diet.[28-30] However, these studies were small and uncontrolled and the clinical relevance of Marsh I lesions needs to be further clarified.

Nevertheless, if a patient with microscopic colitis does not respond to treatment, one should be suspicious of coexisting coeliac disease. Alternatively, a patient with coeliac disease and persisting diarrhoea despite strict adherence to a gluten-free diet should undergo evaluation for microscopic colitis.[16,17]

In conclusion, our study confirms that microscopic colitis is an important cause of chronic diarrhoea. The distribution of this disease throughout the colon can be diffuse but the histopathological abnormalities are located mainly in the ascending colon in a considerable proportion of patients. Therefore, patients with chronic diarrhoea should undergo a total colonoscopy with biopsy sampling from both right and left colon.

## NOTE

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ORIGINAL ARTICLE

# The importance of corpus biopsies for the determination of *Helicobacter pylori* infection

**M.C. van IJzendoorn[1], R.J.F. Laheij[1*], W.A. de Boer[1,2], J.B.M.J. Jansen[1]**

[1]Department of Gastroenterology, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB Nijmegen, the Netherlands, fax: +31 (0)24-361 03 83, e-mail: r.laheij@mdl.umcn.nl,
[2]Department of Internal Medicine, Bernhoven Hospital, Oss, the Netherlands,
[*]corresponding author

## ABSTRACT

**Background: The aim of this study was to determine whether an antral biopsy alone represents an adequate tissue sample to diagnose the presence of *Helicobacter pylori* on the mucosa. Furthermore, we explored the conditions associated with the presence of *H. pylori* in the corpus.**
**Methods: Consecutive patients who underwent an upper gastrointestinal endoscopy at a single centre between January 1995 and May 1997 were studied. Biopsies were taken at each endoscopy to assess the presence of *H. pylori*: two antral and two corpus biopsies for histological examination and one antral and one corpus biopsy for the CLO test.**
**Results: A total of 620 patients underwent an upper gastrointestinal endoscopy, 307 (50%) were *H. pylori* infected. In 80% of the endoscopies there was total agreement between the performed biopsy tests. The addition of corpus biopsies increases the diagnostic yield by 10% in *H. pylori*-positive patients. Patients with only corpus infection more often showed atrophy and intestinal metaplasia compared with patients with both antral and corpus infection, 37 *vs* 20%, respectively (OR 2.2, 95% CI 1.1-4.4).**
**Conclusion: One biopsy from the antrum or corpus seems to be inadequate to diagnose the presence of *H. pylori* on the mucosa. Patients with an infection exclusively in the corpus more often had worse mucosa pathology.**

## INTRODUCTION

*Helicobacter pylori* is a gram-negative bacterium involved in the pathogenesis of peptic ulcer disease, nonulcer dyspepsia, gastric carcinoma and lymphoma.[1-4] The prevalence of *H. pylori* colonisation ranges from around 25% in developed countries to over 80% in developing countries. Therefore, diagnosis and subsequent eradication of *H. pylori* may be responsible for a reduction in morbidity and mortality. Several invasive and noninvasive diagnostic tests can be used to determine *H. pylori* status. Of all the available tests, invasive tests (histology, culture, and rapid urease tests) are considered the most accurate. However, there is no established gold standard for diagnosing *H. pylori* status. Invasive tests are mainly limited by their proneness to sampling error, because of the patchy distribution of the bacteria throughout the stomach.[5,6] Furthermore, the relative distribution of the bacteria may be altered due to the development of gastric atrophy or metaplasia and after acid suppression or antibiotic therapy.[7-9] These circumstances yield the possibility of false-negative results if only the antrum or only the corpus is used as biopsy site.
The guidelines of the European *Helicobacter pylori* Study Group recommend that prior to treatment two antral biopsies should be taken for histological examination, for rapid urease testing, and for culture.[10] The biopsy site mentioned in these guidelines is only partly evidence based. This is reflected by the results of several studies which investigated the most suitable biopsy sites for histology to detect *H. pylori* status. Hazell *et al.* found it necessary to take antral and corpus biopsies,[11] while Genta *et al.* reported that it was sufficient to take only antral biopsies.[12] Satoh *et al.* found that in their Japanese study population it was best to take at least one corpus biopsy.[13] However, these studies were limited by relatively small patient populations or by patient selection. As a consequence, it is unclear how often the presence of *H. pylori* on the

mucosa may not be identified if only antral or corpus biopsies are collected. Most endoscopists use only antral biopsies. Therefore, the aim of this study was to examine how many patients with *H. pylori* present on their mucosa would be misdiagnosed by only taking antral biopsies for *H. pylori* testing. Furthermore, we investigated in which patients *H. pylori* was only present in the corpus.

## METHODS

### Study population

The study population consisted of consecutive patients undergoing routine upper gastrointestinal endoscopy at the Bernhoven Hospital in Oss between January 1995 and May 1997. All patients were symptomatic and had been referred either to the outpatient clinic for evaluation by a gastroenterologist, or to the open-access endoscopy by general practitioners for diagnostic upper gastrointestinal endoscopy. Patients were investigated by one of three experienced endoscopists. A standard biopsy protocol, which consisted of taking three biopsies from the antrum and three biopsies from the corpus, was used for *H. pylori* diagnosis at all times. Patients were asked not to take any acid secretion inhibitory therapy in the week before the upper gastrointestinal endoscopy. Patients with a history of *H. pylori* eradication therapy and patients in whom not all six test results for the presence of *H. pylori* were available were excluded from the study.

### Investigations

At baseline, gender and age of the patient were noted. For each endoscopy gastrointestinal conditions and histopathological findings were recorded by the endoscopist and by the pathologist, respectively. Antral and corpus biopsies were assessed for *H. pylori* by histological examination and by rapid urease testing. Test outcome for each method was assessed independently from the other test results. For histological examination, two antral and two corpus specimens were fixed in neutral buffered 4% formaldehyde. *H. pylori* identification was performed on Giemsa-stained sections of paraffin-embedded tissue. To measure urease activity, we performed the CLO-duo test (Delta West, Bentley, WA, Australia), which contains two wells. Two mucosal biopsies, one from the antrum and one from the corpus, were placed in the two separate wells containing the test reagent, which enabled us to document the presence of urease activity separately for the two biopsy sites. The reaction was analysed after 24 hours.

### Data analysis

The positive or negative occurrence of *H. pylori* for each of the tests was noted. Antrum and corpus were defined as being *H. pylori* positive if both histology and the CLO test were positive for antrum and corpus, respectively. Combining *H. pylori* status for antrum and corpus provides four possible outcomes. All scores were entered into a database. Statistical analysis was carried out using $\chi^2$ tests. Unadjusted analysis for age, gender, and gastroduodenal pathology, assessed endoscopically as well as histologically, were calculated in order to identify factors related to the distribution of *H. pylori* infection. In addition, an adjusted regression analysis was constructed by selecting patient characteristics found to be significantly associated with *H. pylori* present only in the corpus. All analyses were performed using SAS statistical software (SAS Institute, Inc., Cary, NC, USA). Statistical significance was determined by $p<0.05$.

## RESULTS

A total of 620 patients underwent an upper gastrointestinal endoscopy in which the standard biopsy protocol was followed. The mean age of the patients (±SD) was 53 ± 15 years; 258 (42%) were women. None of the patients were diagnosed with gastric carcinoma, 57% had functional dyspepsia, 20% gastroduodenal ulcers, 19% reflux oesophagitis and in 4% other diagnosis were found. *H. pylori* infection was identified in 307 (50%) of the patients. In 80% of the endoscopies there was total agreement between the performed diagnostic tests for *H. pylori* status (*table 1*). Histology of the corpus was the

**Table 1**

*Frequency of the different combinations of H. pylori status for each of the biopsy tests examined in antrum and corpus*

| HISTOLOGY ANTRUM | CLO TEST ANTRUM | HISTOLOGY CORPUS | CLO TEST CORPUS | FREQUENCY N=620 | % |
|---|---|---|---|---|---|
| + | + | + | + | 230 | 37.1 |
| - | - | - | - | 268 | 43.2 |
| - | + | + | + | 13 | 2.1 |
| + | - | + | + | 8 | 1.3 |
| + | + | - | + | 37 | 6.0 |
| + | + | + | - | 5 | 0.8 |
| - | - | + | + | 10 | 1.6 |
| - | + | - | + | 18 | 2.9 |
| - | + | + | - | 0 | 0 |
| + | - | - | + | 0 | 0 |
| + | - | + | - | 2 | 0.3 |
| + | + | - | - | 4 | 0.6 |
| + | - | - | - | 1 | 0.2 |
| - | + | - | - | 3 | 0.5 |
| - | - | + | - | 0 | 0 |
| - | - | - | + | 21 | 3.4 |

only negative test in 6%. Both histology tests were negative and both CLO tests were positive in 3%. CLO test of the corpus was the only positive test in 3%.
With the chosen definition for *H. pylori* status in antrum and corpus, combined antral and corpus biopsies increased the yield compared with antral biopsies alone by 31(5%) and compared with corpus biopsies alone by 46 (7%) (*table 2*). In patients with positive test results for antrum and/or corpus *H. pylori* was identified from only antral biopsies in 46 out of 307 (15%) patients, and from only corpus biopsies in another 37 patients (10%).
Defining *H. pylori* status in the antrum and corpus as positive if only one of the two tests instead of both tests were positive resulted in more positive results.
Furthermore, changing the definition showed a considerable decrease in the number of patients who were considered positive for antrum and negative for corpus (from 46 to 8 patients), whereas there was no difference in the number of patients who were considered negative for antrum and positive for corpus (31 patients).
Atrophy and/or intestinal metaplasia, revealed by histological examination, were found significantly more often in the antrum than in the corpus (18 *vs* 2% of all patients, respectively, p<0.05). Patients with *H. pylori* present in only corpus biopsies more often showed atrophy and metaplasia compared with the other possible outcomes, 39 *vs* 17%, respectively (adjusted odds ratio 3.0, 95% CI 1.4-6.1).

## DISCUSSION

In current practice *H. pylori* detection is often based on antral biopsies alone, as recommended by the European *Helicobacter pylori* Study Group.[10] Our results demonstrate that if *H. pylori* status was based on only antrum biopsies, 10% of all *H. pylori*-positive patients would be misdiagnosed. The addition of corpus biopsies increases the diagnostic yield of invasive tests in a group of patients that seems to be of great importance because of the underlying mucosa pathology.
A number of studies have investigated whether it is necessary to take both antral and corpus biopsies for the diagnosis of *H. pylori*. Laine *et al.* reported that prior to treatment a single antral biopsy for detection of *H. pylori* provided excellent sensitivity.[14] Genta *et al.* assessed 12 biopsy sites of the stomach for the presence of *H. pylori* by histological examination and found that performing two antral biopsies provides the detection of *H. pylori* in virtually all infected patients.[12] Patients having extensive gastric atrophy with intestinal metaplasia were not enrolled in this study.
Our results indicate that patients with *H. pylori* present only in corpus biopsies showed atrophy and intestinal metaplasia significantly more often. This is explained by the fact that in our study the antrum was the predominant site for atrophy and intestinal metaplasia and the prevalence

**Table 2**
*Biopsy test outcomes for antrum and corpus by patient characteristics*

| | N | ANTRUM + CORPUS + N=230 (37%) | ANTRUM - CORPUS - N=313 (51%) | ANTRUM + CORPUS - N=46 (7%) | ANTRUM - CORPUS + N=31 (5%) |
|---|---|---|---|---|---|
| **Gender** | | | | | |
| Male | 362 | 59% | 59% | 61% | 48% |
| Female | 258 | 41% | 41% | 39% | 52% |
| **Age** | | | | | |
| 0< years ≤45 | 192 | 31% | 32% | 30% | 22% |
| 45< years <60 | 213 | 33% | 36% | 37% | 26% |
| ≥60 | 215 | 36% | 32% | 33% | 52% |
| **Macroscopic diagnosis** | | | | | |
| Peptic ulcer disease | 125 | 33% | 9% | 37% | 19% |
| Gastritis/duodenitis | 215 | 37% | 32% | 43% | 32% |
| Oesophagitis | 118 | 14% | 24% | 20% | 3% |
| Normal | 137 | 14% | 30% | 7% | 26% |
| **Microscopic diagnosis** | | | | | |
| Atrophy/metaplasia in antrum | 113 | 16% | 20% | 7% | 35% |
| Atrophy/metaplasia in corpus | 12 | 2% | 2% | 0% | 10% |
| Atrophy/metaplasia | 115 | 16% | 20% | 7% | 39% |

*Biopsy site (antrum or corpus) + : both histology and CLO test give positive test results for H. pylori status at that biopsy site.*

of *H. pylori* decreases from gastric mucosa with atrophy and intestinal metaplasia.[7] There is considerable variation in the prevalence of atrophy, intestinal metaplasia and gastric cancer. Our study was performed in the Dutch population and the results are probably applicable for the Western population. The study by Satoh *et al.* was performed in Japan, where the prevalence of gastric atrophy, intestinal metaplasia and gastric cancer is much higher compared with the Western world.[13] They reported that a corpus biopsy would be the most important site to determine *H. pylori* status. Therefore, the preferential biopsy site probably depends on local prevalence of atrophy or metaplasia.

*H. pylori* infection is known to play an important role in the development of gastric atrophy, intestinal metaplasia,[15] and gastric carcinoma.[3,16,17] Uemura *et al.* found in their study that among patients with *H. pylori* infection, those with corpus predominant gastritis, severe gastric atrophy, and intestinal metaplasia are at particularly high risk for gastric cancer.[3] Therefore, our findings that patients with *H. pylori* only present in corpus biopsies showed gastric atrophy and intestinal metaplasia significantly more often, suggest that patients at high risk for gastric cancer might be misdiagnosed for *H. pylori* infection if only antrum biopsies are taken. Since the antrum is the predominant site for gastric carcinoma, our finding that the antrum showed more atrophy and intestinal metaplasia further supports the role of *H. pylori* in the development of gastric carcinoma as suggested by others.[18] Although some studies reported improvement of preneoplastic gastric lesions after the cure of *H. pylori* infection,[19,20] there is still debate whether eradication of *H. pylori* results in regression of atrophy and intestinal metaplasia.

A limitation of our study was that, although we asked patients not to take acid secretion inhibitory therapy, we did not assess whether they really did so. Especially proton pump inhibitors are known to shift the distribution of *H. pylori* proximally, and could at least in part account for the ones with *H. pylori* present only in corpus biopsies. However, when this study was performed proton pump inhibitory therapy was a rarity before an endoscopy was performed, quite the reverse from current practice.

Furthermore, the definition of *H. pylori* infection used in this study might be disputable. For a patient to be considered *H. pylori* positive at a biopsy site both tests needed to be positive as compared with only one of the two tests, potentially resulting in a higher occurrence of false-negatives. However, the diagnostic yield for corpus biopsies remained unchanged while the yield for antral biopsies decreased considerably if only one of the tests instead of both tests needed to be positive. This further supports the importance of obtaining corpus biopsies.

Finally, we distinguished two categories: metaplasia present or absent and ignored the intensity of inflammation. A biopsy specimen may have one goblet cell or consist entirely of intestinal metaplasia. The extent and the grade of metaplasia, and in particular of atrophy, are quite important in determining the type of gastritis.

In conclusion, the combination of antral and corpus biopsies instead of antrum biopsies alone significantly increases the diagnostic yield of invasive *H. pylori* testing. Adequate tissue sampling results in patients being discovered with an infection exclusively in the corpus, the corpus-predominant gastritis with more ominous prognostic implications.

## ACKNOWLEDGEMENTS

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ABOUT THE COVER

# 'Untitled'

## Jadranka Njegovan

Jadranka Njegovan was born in Zadar, Croatia in 1959. Since 1990 she has been living in the Netherlands. At the moment she lives in The Hague where she works as a visual artist, graphic designer and scientific illustrator. Searching for the essence of an image, she came spontaneously to elements such as line, tone, repetition, rhythm and structure. The number of elements is kept to a minimum in her work, so that purity dominates. She focuses on lithography, a graphic technique that can express the variation of tone really well. Studies of line and tone, the hard contrast between black ink and white paper, and soft tone variations play such an important role, so that the absence of colour seems perfectly normal. The images are not based on observations of reality, but on basic elements: line and tone. Lines are freely drawn on the lithographic stone and once they are given a shadow, suddenly become wires, branches or 'something' lying on a surface. A space is created. An abstract drawing becomes figurative.
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CASE REPORT

# Severe hypokalaemic paralysis and rhabdomyolysis due to ingestion of liquorice

**A.E. van den Bosch[1], J.M. van der Klooster[2], D.M.H. Zuidgeest[3], R.J.Th. Ouwendijk[1], A. Dees[1*]**

Departments of [1]Internal Medicine, [2]Intensive Care and [3]Neurology, Ikazia Hospital, Rotterdam, the Netherlands, [*]corresponding author: Montessoriweg 1, 3083 AN Rotterdam, the Netherlands, tel.: +31 (0)10-297 51 36, e-mail: ADees@planet.nl

## ABSTRACT

**Chronic ingestion of liquorice induces a syndrome with findings similar to those in primary hyperaldosteronism. We describe a patient who, with a plasma $K^+$ of 1.8 mmol/l, showed a paralysis and severe rhabdomyolysis after the habitual consumption of natural liquorice. Liquorice has become widely available as a flavouring agent in foods and drugs. It is important for physicians to keep liquorice consumption in mind as a cause for hypokalaemic paralysis and rhabdomyolysis.**

## KEYWORDS

Hypokalaemia, liquorice consumption, paralysis, rhabdomyolysis

## INTRODUCTION

Chronic ingestion of liquorice or liquorice-like compounds induces a syndrome with findings similar to those in primary hyperaldosteronism. This syndrome is characterised by sodium retention, hypertension, hypokalaemia, metabolic alkalosis and low plasma renin activity. The hypokalaemia is usually mild; nevertheless it could become extremely severe and even life threatening. A frequently undiagnosed serious complication of hypokalaemia is rhabdomyolysis.[1,2] Here, we describe a patient who showed a plasma $K^+$ of 1.8 mmol/l, paralysis and severe rhabdomyolysis after the habitual consumption of natural liquorice.

## CASE REPORT

A 59-year-old Caucasian man presented to the neurological outpatient department with muscular weakness that progressed to paralysis involving all extremities. He was unable to stand up from the sitting position (Gower's phenomenon). He denied nausea, vomiting, diarrhoea or the use of drugs, including diuretics. However, he had been eating nearly 200 g of liquorice a day during the last four weeks after quitting smoking. His family and past medical histories were unremarkable. He did not use alcohol.

On physical examination, his blood pressure was 187/87 mmHg, heart rate 83 beats/min, respiratory rate 15/min, and body temperature 37.9°C. His thyroid gland was not enlarged. Cardiopulmonary examination was unremarkable. There was a symmetric flaccid paralysis with areflexia in the lower and upper extremities. Fasciculations, myoclonus and muscular atrophy were not observed. The remainder of the physical examination was normal. The major biochemical abnormalities are shown in *table 1*. Laboratory tests showed severe hypokalaemia (1.8 mmol/l), metabolic alkalosis and extreme enzyme abnormalities (CK 35.063 U/l) compatible with rhabdomyolysis. Urinary potassium excretion was low. Plasma renin activity and aldosterone levels were far below the plasma level of normal. As a conformation of our diagnosis, we found a very high plasma level of glycyrrhetic acid at 257 μg/l (normal range <5 μg/l). Hypokalaemia was associated with typical electrocardiographic changes. Computed tomography scanning showed normal adrenal glands.

**Table 1**
*Laboratory data on admission*

| PLASMA | |
|---|---|
| $Na^+$ (mmol/l) | 147 |
| $K^+$ (mmol/l) | 1.8 |
| $HCO_3^-$ (mmol/l) | 40 |
| pH | 7.54 |
| Magnesium (mmol/l) | 1.00 |
| Urea (mmol/l) | 3.6 |
| Creatinine (µmol/l) | 83 |
| Creatinine kinase (u/l) | 35,063 |
| Renin activity (ng/ml/h) | 3.9 (10-60)* |
| Aldosterone (ng/dl) | <0.04 (0.04-0.35)* |
| Glycyrrhetic (µg/l) | 257 (<5)* |

| URINE | |
|---|---|
| Potassium (mmol/l) | 12 |
| Osmolality (mOsm/kg) | 350 |

**Normal range for laboratory data.*

His initial therapy included 10 mmol of potassium chloride (KCl) per hour given by the intravenous route, and a potassium-sparing diuretic (spironolactone 100 mg/day) was prescribed. Within one week, serum levels of potassium normalised and all clinical symptoms improved. Nevertheless, potassium chloride supplementation was needed for several weeks. The serum creatine kinase isoenzymes (CK total and MB) returned to normal during a prolonged period (*figure 1*).

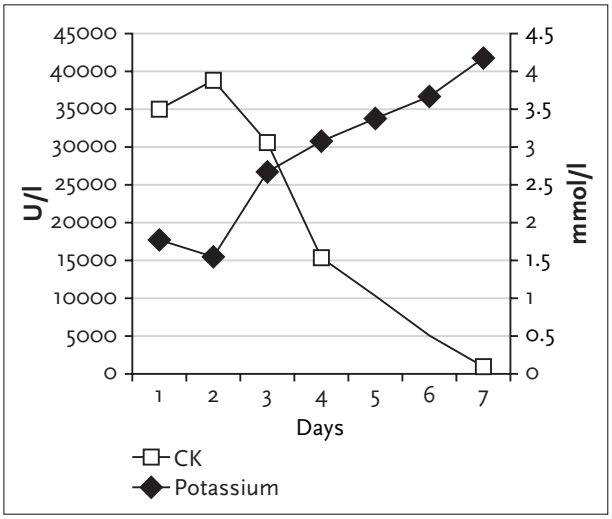


**Figure 1**
*Time course, in days, of the patients' serum creatine kinase and potassium*

## DISCUSSION

Hypokalaemic paralysis and rhabdomyolysis due to liquorice consumption are rare. The potential dangers of ingesting liquorice, even in small amounts such as 50 g daily after only two weeks, are discussed here.[3] Liquorice-induced hypokalaemia is a rare disorder first described by Revers in 1946; since that time the potential neuromuscular complications of severe hypokalaemia have been demonstrated in a number of case reports.[4-7]
To mention just some examples: regular ingestion of natural liquorice, flavoured tea, white beer, grapefruit or even alternative medications has been complicated by severe hypokalaemia.
Our patient had an extreme degree of hypokalaemia and rhabdomyolysis in combination with metabolic alkalosis. This, together with the mild hypertension found on physical examination, suggested that he had a condition with high mineralocorticoid activity. Measurements of renin and aldosterone plasma levels are helpful at this point. Low plasma renin and aldosterone levels suggested an apparent mineralocortoid excess-like disorder. Diagnosis depends on elicitation of a thorough history and laboratory evidence of hypokalaemia. In this patient, it quickly became evident that this was caused by liquorice consumption.
The mechanism behind liquorice-induced hypokalaemic hypertension was briefly reviewed in this journal.[8] Liquorice causes hypokalaemia through its active metabolite, glycyrrhetic acid, which inhibits the renal enzyme 11β-hydroxysteroid dehydrogenase. This enzyme is responsible for renal conversion of cortisol to cortisone, which is inactive and does not bind to the mineralocorticoid receptor.[9] The acquired dehydrogenase inhibition due to liquorice thus leads to activation of renal mineralocorticoid receptors by cortisol, resulting in a state of apparent mineralocorticoid excess. Hypokalaemia is caused by renal and extrarenal loss of potassium or by an acute shift of potassium into the cells. Hypokalaemia is associated with typical electrocardiographic changes and marked acid-base disturbance. Incidentally paralysis and even rhabdomyolysis have been reported. The most frequent symptoms of rhabdomyolysis are fatigue, weakness, muscular pain and swelling, although it is possible that some patients are completely asymptomatic.[6,7] The severity of neuromuscular disorders tends to be proportionate to the rate at which hypokalaemia develops. Muscle destruction due to rhabdomyolysis causes the release of large amounts of potassium into the circulation. Consequently, when the clinical syndrome of hypokalaemia and rhabdomyolysis develops, the absolute concentration of potassium is far below the normal limits. Large amounts of KCl supplementation are necessary.

A liquorice-induced excessive mineralocorticoid effect usually responds to spironolactone and is reversible upon cessation of liquorice ingestion.[10] In addition to KCl supplementation and spironolactone, the administration of dexamethasone should be considered. Dexamethasone causes suppression of endogenous cortisol production and thus reduces the cortisol-mediated stimulation of the mineralocortcoid receptor.

After liquorice consumption was discontinued and KCl and spironolactone were taken, the plasma $K^+$ of our patient rose to normal levels and hypertension resolved, but the time required for these to occur was more than two weeks. This can be explained by glycyrrhetic acid having a large volume of distribution, a long biological half-life, and undergoing substantial enterohepatic circulation. Therefore, physicians should anticipate that the effects might take a considerable time to abate as documented in our patient.

## CONCLUSION

In conclusion, severe hypokalaemia with paralysis and rhabdomyolysis is a potentially life-threatening medical emergency. Besides KCl supplementation, a vigorous search for the underlying cause is necessary to avoid missing treatable causes – in this case, liquorice consumption. Given the diagnosis, large doses of KCl supplementation for weeks are necessary because of the long half-life of glycyrrhetic acid. Liquorice has become widely available as a flavouring agent in foods and drugs. It is important for physicians to keep liquorice consumption in mind as a cause for hypokalaemic rhabdomyolysis.

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CASE REPORT

# The old lady who liked liquorice: hypertension due to chronic intoxication in a memory-impaired patient

**A. Janse, M. van Iersel, W.H.L. Hoefnagels, M.G.M. Olde Rikkert***

Department of Geriatrics (318), Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB Nijmegen, the Netherlands, tel.: +31 (0)24-361 67 72, fax: +31 (0)24-361 74 08, e-mail: M.Olde-Rikkert@ger.umcn.nl, *corresponding author

## ABSTRACT

**The authors report an 85-year-old patient admitted because of cognitive impairment. During examination hypertension and hypokalaemia were found. After some time it was discovered that the patient was eating too much liquorice. The case demonstrates that liquorice intoxication should be considered as a cause of hypertension in old age. Furthermore the case demonstrates that missing an intoxication is a pitfall for medical history taking of patients with cognitive impairment.**

## INTRODUCTION

Ingestion of liquorice is a well-known reason for a syndrome mimicking mineralocorticoid excess, of which the pathophysiology is completely clarified.[1] The characteristics of this syndrome are hypertension, hypokalaemia, alkalosis, low renin activity and hypoaldosteronism.[2] Most of the recently published literature consists of case reports of patients suffering from paralysis because of liquorice-induced hypokalaemia. Most of these case histories report on younger or middle-aged patients. As far as we know, self-induced licorice intoxication has not been reported before in the very elderly (over 80) as we only found one case of an elderly woman (90) with liquorice-related hypertension caused by medication.[3]

## CASE HISTORY

Recently an 85-year-old woman was referred to our outpatient clinic because of progressive cognitive impairment for five years. Her performance in activities of daily living was clearly impaired. Her single comorbid condition was hypertension, which she was known to have had for many years. The hypertension was diagnosed as essential hypertension and treated with a thiazide/amiloride diuretic. After her medical history had been taken (and also her son's), and after physical examination (no focal general or neurological pathology), assessment of cognition by Mini Mental State Examination (score 15, normal 30), CAMCOG (Cambridge cognitive examination: score 48, maximum 106; lower than 76 substantially increases the likelihood of dementia), and laboratory examination, we concluded she fulfilled the criteria for a dementia syndrome, probably due to Alzheimer's disease (according to the NINDS-ADRDA criteria) in a moderately severe stage.[4] She refused neuro-imaging, so we could not completely rule out cerebrovascular disease, though her family did not report acute neurological events.

During physical examination we found a high systolic blood pressure (180/82 mmHg) in both supine and upright position. Routine laboratory investigations showed a serum sodium concentration of 144 mmol/l (normal 137-144), potassium 2.4 mmol/l (normal 3.5-5.0), bicarbonate 34.1 mmol/l (normal 24-30), and creatinine concentration 106 μmol/l. Because of the hypokalaemia, we discontinued diuretics and advised the patient's family to ensure that she eats potassium-rich food. After a diuretic-free period of three weeks the hypertension and hypokalaemia persisted (RR 210/100 mmHg, K 2.5 mmol/l), and we advanced the hypotheses of primary or pseudohyperaldosteronism. Additional laboratory investigations were performed. The plasma aldosterone was 0.03 nmol/l (normal 0.08-069) and the plasma renin concentration (upright) was 12 mE/l (normal 5-75). These findings supported the hypothesis of pseudohyperaldosteronism.

We re-questioned the possibilities of intoxication and repeated medical history taking. At this stage, her son told us she had been very fond of liquorice her whole life. At home he had found a stock of liquorice, and he now realised that he had seen his mother eating it, whatever the time of day he visited her. Reconsidering her liquorice intake, we estimated her intake of liquorice at about 500 g a day. The patient herself denied eating liquorice.
We advised her children not to buy her any more liquorice. We also prescribed her potassium tablets; subsequently, the plasma potassium concentration normalised within two weeks. After a period of three weeks, we terminated the potassium supplementation. This did not lead to a renewed decrease in her plasma potassium level. This is in line with the hypothesis of pseudohyperaldosteronism by liquorice abuse. Because of persistent (systolic) hypertension one month after cessation of the liquorice ingestion, we started her on β-blockers. According to the literature, normalisation of the renin-aldosterone axis and blood pressure takes up to four to six months.[5] After four months, the blood pressure, influenced by a low dose of a β-blocker (metoprolol 50 mg /day), was 142/68 mmHg. The hypertension returned after discontinuing medication (180/84 mm Hg), but was less high than during the liquorice abuse, so the low-dose β-blocker was re-started.

## DISCUSSION

This case of a very old patient with liquorice-induced hypokalaemia and hypertension partially caused by liquorice abuse is instructive for more than one reason. First, this case is an alert for the possibility of secondary hypertension due to pseudohyperaldosteronism in the very old. Eating liquorice is mainly a habit in Northern European countries. On a worldwide scale, liquorice is used as an additive in foods and drinks because of a very sweet constituent called glycyrrhetinic acid, which is the substance that causes hypertension and hypokalaemia. Furthermore liquorice is used in herbal drugs. Although the concentration of glycyrrhetinic acid is the highest in candies, intoxication from other causes is well known. Liquorice should not be overlooked as a cause of hypertension and hypokalaemia in old age, especially in cases of diuretic drug use and a history of primary hypertension. Not recognising this cause of hypertension may easily lead to frequent hospital contacts and polypharmacy of more than one antihypertensive agent and potassium suppletion, as illustrated in the case presented by Farese *et al.*[1] It is possible to distinguish primary hypertension and hypertension due to glycyrrhetinic acid by measuring the cortisol/cortisone ratio in arterial plasma or saliva.[6] This ratio is sharply raised in pseudohyperaldosteronism, because glycyrrhetinic acid inhibits the conversion of cortisol into cortisone by 11β-hydroxysteroid dehydrogenase. In these higher concentrations, cortisol acts as the major endogenous mineralocorticoid compound. Together, the history of liquorice abuse, the low potassium, renin and aldosterone plasma levels, combined with the lowering of the hypertension and normalisation of the serum potassium after cessation of the liquorice abuse are sufficient evidence for the diagnosis of pseudohyperaldosteronism by liquorice abuse. Moreover, the patient probably suffers from essential (systolic) hypertension because of arterial wall stiffening, as is common in elderly patients.
In our case we cannot rule out that the liquorice-related hypertension also caused cerebral damage and therefore may also be related to her cognitive decline. Russo *et al.* reported acute hypertensive encephalopathy in cases of more severe liquorice-induced hypertension.[7]
An even more important lesson to be drawn from this case is that, if we want to rule out intoxications, we should not limit medical history taking of elderly patients to merely questioning them about their intake of alcohol and nicotine. The case clearly illustrates that also in the very old one needs to be alert for the possibility of other intoxications. Moreover, patients with memory impairments are at risk to overeating of potentially toxic nutrients or drugs, by forgetting what and how much they eat. This is not limited to the Kluver-Bucy symptoms in frontal lobe dementia. In conclusion, because geriatric patients may forget what they eat or that they are overeating (or undernourishing) themselves, it is the task of geriatricians to actively search for these data, and to remember that not all hypertension in old age is essential hypertension.

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# A diagnosis not to be missed

**A.M.M. Kotsopoulos, F. Huvers***

Department of General Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands, tel.: +31(0)24-361 72 76, fax: +31(0)24-354 17 34, e-mail: f.huvers@aig.umcn.nl, *corresponding author

## CASE REPORT

A 78-year-old woman was admitted to the hospital because of severe abdominal pain and vomiting. During the past weeks she had not been feeling well, and she was anorectic. She had not passed any stools in the previous four days. For three days she had had diffuse dull abdominal pain, radiating to the lumbar region. Shortly before presentation the abdominal pain aggravated and was accompanied by nausea and vomiting. Her daughter noticed some blood in the vomit. One hour before admission the patient collapsed.

There was no history of recent melaena, diarrhoea, fever, chills, angina pectoris or coughing.

Three years ago a left-sided ovariectomy and uterus extirpation due to a Brenner tumour was performed, which was complicated by ileus. Furthermore, she had documented hypertension and hyperlipidaemia. Finally, a silent myocardial infarction had occurred one year ago.

The patient's medication comprised aspirin, lisinopril, atenolol, triamtereen/epitizide and temazepam.

On physical examination, the patient appeared ill and was perspiring. Her body temperature was 36 °C, pulse rate 90 beats/min, blood pressure 165/90 mmHg and respiration rate was 14/min. The jugular venous pressure was not elevated. Her hands and feet were cold. The abdomen was distended, diffusely tender, without rebound tenderness, but with diminished bowel sounds. Rectal examination was unremarkable.

The laboratory results were as follows: haemoglobin 6.7 mmol/l, haematocrit 0.32, white-cell count 23.6 x $10^9$/l, thrombocytes 259 x $10^9$/l, creatinine 79 μmol/l, urea 6.9 mmol/l, potassium 3.4 mmol/l, sodium 125 mmol/l, ASAT 23 U/l, ALAT 14 U/l, alkaline phosphatase 69 U/l, γ-glutamyltransferase 20 U/l, lactate dehydrogenase 373 U/l, amylase 85 U/l and lactic acid 4.2 mmol/l. The blood gas analysis revealed a metabolic acidosis (pH 7.33, $pO_2$ 17.7 kPa, $pCO_2$ 3.8 kPa, bicarbonate 14.5 mmol/l, base excess -10.0 mmol/l). The ECG showed sinus rhythm and an old inferoposterior infarction. A computerised tomographic angiography (CTA) of the abdomen was performed.

## WHAT IS YOUR DIAGNOSIS?

See page 153 for the answer to this photo quiz.

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# Pneumococcal polysaccharide vaccines

*In their editorial for the February 2004 issue of this journal Lipsky and Hirschman take a clear stand on the lack of protection against pneumococcal infections by pneumococcal polysaccharide vaccines in elderly people.[1] Their conclusion might have been different if the results of two studies, which demonstrated that in more than 25% of aged people the splenic function is impaired (4 to 14% 'pitted' erythorcytes), had been taken into account.[2,3] The evidence for an impaired splenic function was evident in spite of the limited number of patients tested and the existence of comorbidity from diseases not known to be associated with hyposplenism. This finding in conjunction with the fact that the presence of more than 3.5% 'pitted' erythrocytes correlates strongly with functional hyposplenism suggests that the editorial conclusion does not apply to elderly people with an impaired function of the spleen.[4]*

*Therefore, we are convinced that attention should be paid to a possibly increased susceptibility to pneumocccal infections of aged individuals with an impaired function of the spleen before a prospective trial in the elderly Dutch population is designed.[5]*

**J.M. Werre[1*], L.D. de Haan[2]**
[1]Department for Blood Transfusion and Transplant Immunology, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB Niimegen, the Netherlands, [2]Department of Medicine, Scheper Hospital, Emmen, the Netherlands, [*]corresponding author

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ANSWER TO PHOTO QUIZ (ON PAGE 151)

# A DIAGNOSIS NOT TO BE MISSED

## DIAGNOSIS

There was a high suspicion of mesenteric ischaemia in this particular patient because of the discrepancy between the patient's poor clinical condition on presentation and the lack of alarm signs, especially from the abdomen, on physical examination. Furthermore, the laboratory tests supported our suspicion of intestinal ischaemia.
The CTA confirmed the suspected diagnosis of small intestinal ischaemia more specifically.
*Figures 1* and *2* demonstrate pneumatosis intestinalis, a typical radiological sign of bowel infarction, ascites and marked focal lack of wall enhancement of the small intestine, located on the right side. No thrombus is seen in the mesenteric trunk, superior or inferior mesenteric artery, the portal vein, and lienalic vein.

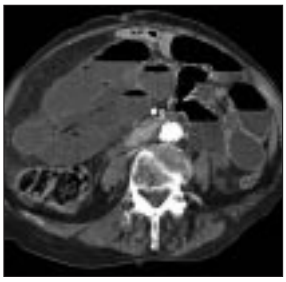

**Figure 1**
*Pneumatosis intestinalis*

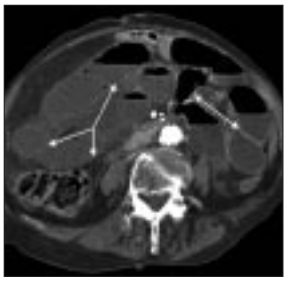

**Figure 2**
*No enhancement of bowel wall on the right side vs enhancement on left-hand side*

A laparotomy was performed. The small intestine was rotated, ischaemic and necrotic; 45 cm of the small intestine was resected. In the postoperative period the patient developed refractory septic shock accompanied by multiple organ failure. Three days after admission she died.
Mesenteric ischaemia in adults typically presents in the fifth to eighth decade and is idiopathic (15%) or secondary (85%) to a wide variety of gastrointestinal and nongastrointestinal illnesses.
Undiagnosed or delayed diagnosis has severe clinical consequences including sepsis, intestinal infarction or death, making it a diagnosis not to be missed. Mortality rates exceed 60%.[1]
Mesenteric ischaemia is caused by intestinal hypoperfusion. Reduced intestinal blood flow can be due to arterial or venous occlusion, such as thrombus, embolus or strangulation. Nonocclusive causes of intestinal hypoperfusion are vasospasm of the splanchic vasculature or hypotension.[2] The clinical presentation can be nonspecific, with absence of signs of rebound tenderness and guarding, making the diagnosis primarily based upon high clinical suspicion. Laboratory tests are not very helpful either. Metabolic acidosis and high levels of serum lactate are late features and are present in patients with established bowel infarction (transmural necrosis). The clinical diagnosis is confirmed by radiographic imaging as computerised tomographic angiography (CTA) or magnetic resonance angiography (MRA).[3] MRA is highly sensitive for both arterial and venous thrombosis, however it is not widely available and is expensive. CTA can provide detailed information about the arterial as well as the venous vessels, and is lower in costs. Laparotomy should be performed when intestinal ischaemia is suspected.

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# Richtlijn Behandeling van tabaksverslaving

Met jaarlijks meer dan 20.000 doden door tabaksgebruik, is roken in Nederland een volksgezondheidsprobleem van de eerste orde. Dertig procent van de bevolking rookt nog. De winst van stoppen met roken is niet alleen voor de maatschappij als geheel, maar ook voor elke individuele roker, aanzienlijk. De meeste rokers blijken graag te willen stoppen. Velen hechten daarbij grote waarde aan advies van een medicus.

De richtlijn sluit aan op het principe van eenmalige en korte ondersteunende interventies en op het model van 'stages of change'. De minimale-interventiestrategie (MIS) kent in ons land als toegepaste methode een zekere traditie waar het gaat om het bieden van 'stoppen met roken'-adviezen: diverse beschikbare programma's zijn hierop gebaseerd. In de richtlijn wordt de MIS echter als een methode gezien en wordt een andere indeling gehanteerd dan in de traditie van de MIS. Een belangrijk uitgangspunt in richtlijnen voor de medische praktijk is dat optimaal gebruik wordt gemaakt van beschikbare voorzieningen. In deze richtlijn is dat ook het geval waar het gaat om een consistente motiverende interventie bij rokers. Een bijzonder punt in deze richtlijn is echter het positioneren van gespecialiseerde voorzieningen. Deze, in het Verenigd Koninkrijk en de Verenigde Staten bekende, voorzieningen zijn in ons land op beperkte schaal voorhanden. Met het lanceren van deze richtlijn wordt bepleit dat zulke faciliteiten op grotere schaal beschikbaar komen. Ondanks het beperkte wetenschappelijk bewijs wordt hiervoor een argumentatie gegeven. Daarmee zal het realiseren van de inspanning om via individuele interventies het tabaksgebruik terug te dringen, aan slagkracht winnen.

In de gezondheidszorg doen zich op grote schaal contacten met individuele rokers voor. In deze richtlijn worden die momenten als aanleiding beschouwd om stoppen met roken aan de orde te stellen. Voor de medische praktijk en de daarbij betrokken hulpverleners biedt deze richtlijn mogelijkheden om tabaksverslaving effectief te behandelen. Hoewel primaire preventie met name voor jongeren van groot belang is, komt in deze richtlijn alleen de behandeling van tabaksverslaving in de zorg aan de orde.

**Richtlijn Behandeling van tabaksverslaving**
(met samenvattingkaart)

Partnership Stop met Roken in samenwerking met het Kwaliteitsinstituut voor de Gezondheidszorg CBO en de Orde van Medisch Specialisten

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# Richtlijn Polyneuropathie

Polyneuropathieen kunnen zeer veel oorzaken hebben. Bij gebrek aan een goede richtlijn leidt dit momenteel tot een zeer grote variabiliteit in de aard en de hoeveelheid diagnostiek/verrichtingen. Enerzijds is er sprake van 'overdiagnostiek' en zijn er problemen met de interpretatie van uitslagen. Anderzijds worden diagnoses gemist en wordt de patiënt behandeling onthouden. Ten slotte duurt de diagnostische fase nu veelal te lang.

De richtlijn 'Polyneuropathie' bestaat uit aanbevelingen betreffende de diagnostiek van polyneuropathieen bij volwassen patiënten in het algemeen en biedt aanknopingspunten voor bijvoorbeeld transmurale afspraken of lokale protocollen. Er wordt uitgebreid ingegaan op de minimale hoeveelheid diagnostiek die noodzakelijk is om een polyneuropathie te classificeren en tot een diagnose te komen. Dit is essentieel voor de prognose en de in te stellen therapie. Een belangrijk element van de richtlijn is het diagnostische stroomdiagram. Dit schema is naar verwachting goed toepasbaar in de dagelijkse praktijk van iedere neuroloog die volwassen patiënten beoordeelt met klachten en verschijnselen van een neuropathie. Ten slotte wordt de medicamenteuze en niet-medicamenteuze symptomatische behandeling besproken van positieve symptomen (pijn, pijnlijke paresthesieën, dysesthesie, hyperpathie, en dergelijke) die kunnen ontstaan in het kader van polyneuropathieën.

De richtlijn berust op de resultaten van wetenschappelijk onderzoek en aansluitende meningsvorming gericht op het expliciteren van goed medisch handelen en is geschreven voor en door de zorgverleners.

**Richtlijn Polyneuropathie**

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

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

*Subheadings* should not exceed 55 characters, including spaces.

*Abbreviations:* Measurements should be abbreviated according to SI units. All other abbreviations or acronyms should be defined on the first appearance in the text. Use a capital letter for proprietary names of substances and materials. At first mention of a chemical substance, use the correct chemical designation as well as the generic name.

The *Abstract*, not exceeding 200 words, should be written in a structured manner and with particular care, since this will be the only part of the article studied by some readers. In original articles, the abstract should consist of four paragraphs, labelled Background, Methods, Results, and Conclusion. They should briefly describe the problem being addressed in the study, how the study was performed and which measurements were carried out, the most relevant results, and what the authors conclude from the results.

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

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

The *Results* should be presented precisely without discussion.

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

*Acknowledgement:* All finding sources should be credited here. Also a statement of conflicts of interest should be put here.

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

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

Please note that the first six authors should be listed; when seven or more, list only the first three and add *et al*. Do not include references to personal communications, unpublished data or manuscripts either 'in preparation' or 'submitted for publication'. If essential, such material may be incorporated into the appropriate place in the text. Recheck references in the text against reference list after your manuscript has been revised.

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