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EDITORIAL

Gauging rheumatoid arthritis

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The ideal biomarker is a simple test that reliably recognises a disease when present, can predict the disease when not yet present, provides prognostic information, predicts response to treatment and reflects disease activity or actual response to treatment. In most instances, different biomarkers will be needed to cover the different aspects one wants to measure in a disease, such as for instance blood glucose, HbA1c and microalbuminuria in diabetes. Biomarkers are mostly thought of as biochemical measurements, but can equally well be clinical measurements or imaging features. In complex diseases such as rheumatoid arthritis, molecular and clinical biomarkers are often combined for the purpose of classification[1] or in order to create an index of (low) disease activity.[2] This editorial will briefly deal with molecular biomarkers of prediction, diagnosis and prognosis of rheumatoid arthritis, as measured in blood. For reasons of space, the emerging field of prediction of treatment response is left out.

Nowadays, quite a lot is known about the pathogenesis of rheumatoid arthritis. Acquiring this knowledge was facilitated by the easy accessibility of the main site of the pathology of this disease, the peripheral joints, and the accompanying immunological abnormalities that can be detected in the blood. That is a different situation compared with other immune diseases such as spondyloarthropathy or multiple sclerosis, which have a centrally located pathology and hardly any signs of autoimmunity. The discovery of the rheumatoid factor in the middle of the past century triggered an interest in pathophysiological research and even helped rheumatology to become a separate speciality. However, research on the rheumatoid factor has not led to a solution for rheumatoid arthritis, as it was also found in several other conditions involved with infections or tissue damage, and its pathogenic significance has remained uncertain.

Already in 1964, another serological hallmark of rheumatoid arthritis was discovered and named the anti-perinuclear factor.[3] It took more than 30 years to determine the antigen that these antibodies targeted, namely citrulline residues present in a large variety of intra- and extracellularly occurring proteins in the context of inflammation, e.g. due to infections or cigarette smoking.[4] The corresponding antibodies are collectively referred to as anti-citrullinated protein antibodies (ACPA). ACPA have clearly replaced rheumatoid factor as the main autoantibody in rheumatoid arthritis. ACPA not only have a higher diagnostic and prognostic value than rheumatoid factor,[5] they most likely play an important role in the pathogenesis of rheumatoid arthritis for several reasons, some of which are listed here: 1) ACPA production is linked to the presence of the strongest genetic risk factors for rheumatoid arthritis located on the HLA-DRB1 region, and the PTPN22 gene;[6] 2) ACPA appear earlier than rheumatoid factor in the asymptomatic phase, up to 15 years before the first symptoms;[7] 3) in persons at risk for rheumatoid arthritis, their presence is associated with an increase in the risk of the onset of clinical arthritis as the titre increases and a higher number of epitopes are recognised;[8,9] 4) they can exacerbate arthritis in animal models of arthritis. At present it is unknown why rheumatoid arthritis (pre-)patients develop an antibody response to the widely occurring citrulline. The practical value of ACPA testing is that a positive test greatly facilitates the early recognition of rheumatoid arthritis and at the same time defines the patient subset that has the highest likelihood of developing erosive disease, which in the long term is associated with functional deterioration. ACPA-positive early arthritis patients are thus the main candidates for early intervention. Naturally, ACPA testing should be restricted to persons with suspected rheumatoid arthritis.

The rheumatology research group from Leiden recently discovered a new group of autoantibodies in rheumatoid arthritis, called anti-carbamylated protein antibodies or anti-CarP, which they describe in this issue of the Journal in relation to ACPA.[10] The pathophysiology is similar to that of ACPA, in that the naturally occurring amino acid lysine is modified post-translationally – in an inflammatory environment – into homocitrulline, which

then acts as a neo-epitope for autoantibody formation, in the same manner as the amino acid arginine after conversion to citrulline in the case of ACPA. The reaction producing homocitrulline is a chemical one, however, whereas citrullination is an enzymatic process. Although homocitrulline is nearly identical to citrulline, the authors have shown that anti-CarP antibodies are not just cross-reacting ACPA. Furthermore, these are also found in some of the ACPA-negative patients and are associated with radiographic joint damage. The authors mention that the value of anti-CarP testing could be to recognise those ACPA-negative patients who have a poor prognosis. However, the question is whether we really need additional biomarkers for ACPA-negative rheumatoid arthritis, since even in the time before modern effective treatment of rheumatoid arthritis was the norm, the average radiographic damage was already extremely low in ACPA-negative patients.[11]

Altogether, we now have three autoantibody systems associated with the (prediction of the) more severe forms of rheumatoid arthritis: rheumatoid factors,[12] ACPA[5] and anti-CarP antibodies.[13] A common pathogenic denominator may be their local production in the inflamed synovium and their ability to bind complement and thereby enhance the level of inflammation. However, it is likely that this will not be the end of the rheumatoid arthritis autoimmunity story. Various proteases are active in the inflamed synovium, which through cleaving proteins might produce new epitopes with a potential for further autoantibody formation, and thereby further activation of the inflammatory cycle. Indeed, antibodies to Fab fragments of IgG molecules cleaved at the hinge region between the Fab and Fc portions were recently described in the serum of rheumatoid arthritis patients.[14]

Looking beyond autoantibodies, what other molecular biomarkers might be useful in rheumatoid arthritis? There are now over 30 confirmed genetic susceptibility loci for rheumatoid arthritis. However, even when combined these have only low predictive ability by themselves.[6] One can also study general inflammation markers such as acute-phase reactants and cytokine profiles. Acute-phase reactants, mainly C-reactive protein, are well established as markers of disease activity and perform well in composite measures of disease activity or remission in rheumatoid arthritis.[2] In the preclinical phase of the disease, levels become elevated from the appearance of ACPA onwards;[15] however, they do not provide additional predictive ability for future rheumatoid arthritis.[16] Similarly, various cytokines are elevated before clinical rheumatoid arthritis appears, which probably reflects increasing inflammation in this phase, rather than an initiating pathogenic event.[17,18] Recently, 14-3-3 proteins reflecting activated signal transduction pathways were identified as another specific biomarker of rheumatoid arthritis disease activity.[19]

A relatively novel approach is to analyse gene expression activity as opposed to the mere presence or absence of specific genetic traits. Using this approach, an increased expression of a number of interferon-related genes in combination with low activity of B-cell genes was discovered in the blood of persons at risk for rheumatoid arthritis,[20] which was also present in patients with active rheumatoid arthritis.[21] The so-called 'interferon signature' was predictive of future rheumatoid arthritis, independent of ACPA positivity.[20] A drawback of this technique is that it is dependent on qPCR, which is not readily available. Obviously, the new findings on biomarkers such as anti-CarP antibodies and the interferon signature need to be validated in other populations and tested in different phases of the disease. In parallel, in order to be clinically useful they will have to demonstrate additional value over testing of ACPA alone as the most potent single biomarker in rheumatoid arthritis to date.

Emerging technology will soon allow the testing of a large number of biomarkers in only a few drops of blood, essentially measuring rheumatoid arthritis-specific autoimmunity, biochemical inflammation and possibly also genetic susceptibility. In combination with a careful clinical examination and imaging results, the properties of such a blood-based biomarker set may prove to be better than the current evaluation in the following situations: prediction of future rheumatoid arthritis in persons at increased risk for rheumatoid arthritis, and prediction of the disease course in patients newly diagnosed with rheumatoid arthritis, including the preferred treatment regime. In order to be useful for the monitoring of treatment, a blood-based biomarker set should be cheaper than a visit to the clinic and be able to reliably predict or reflect a state of remission or minimal disease activity, which is the present goal of treatment. In spite of the usual proclamation of another step towards 'personalised medicine' for every new association of a single biomarker with a measure of disease, all these wishes are still far from being fulfilled at present. For quite some time now, molecular biomarker sets for rheumatoid arthritis have been more of a promise than a reality, and it will take some more time before we have a good 'rheumachip' for use in the clinic.

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REVIEW

New biomarkers in rheumatoid arthritis

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ABSTRACT

Rheumatoid arthritis (RA) is a common autoimmune disease affecting around 1% of the population. Although major advances have been made in the treatment of RA, still relatively little is known on disease pathogenesis and aetiology. From treatment studies it has become clear that treating patients early in their disease course will provide the best results. However, especially in the early phase of arthritis, in particular when the patients do not yet fulfil the criteria for RA, it is difficult to decide which patients would benefit most from an early and aggressive intervention. Good biomarkers are important to guide decisions in the clinical management of RA. Next to the well-known rheumatoid factor (RF) and the anti-citrullinated protein antibodies (ACPA), several new markers are now likely to become available with interesting potential. Besides antibody responses directed against citrullinated proteins, also antibodies against carbamylated proteins (anti-CarP) have recently been shown to be present in RA. Interestingly these anti-CarP antibodies are also present in around 20% of the ACPA-negative RA patients and are associated with more severe joint damage in this group. Apart from the antibodies that help in establishing the diagnosis and prognosis, also novel biomarkers that reflect clinical disease activity scores are being discovered. The development of biomarker-based disease activity scores might allow easy and frequent monitoring of patients to rapidly adjust treatment.

KEYWORDS

Rheumatoid arthritis, ACPA, anti-CarP, biomarkers, MBDA

INTRODUCTION

Rheumatoid arthritis (RA) is one of the most common autoimmune diseases, affecting up to 1% of the Dutch population. RA is characterised by persistent synovitis, systemic inflammation and the presence of several autoantibodies.[1] RA is a highly heterogeneous disease and therefore classification criteria were developed. These criteria were developed to arrive at relatively homogenous patient groups to be able to better compare studies performed across the world. In the development of the American College of Rheumatology (ACR) 1987 criteria, clinical cases of classic RA patients were included, which resulted in the inclusion of patients with conceivably different disease mechanisms.[2] Therefore, using this classification system there was still considerable variation in disease course and treatment responses among the RA patients. Given the fact that the sensitivity of the 1987 ACR criteria for RA is relatively low for recent-onset RA, the ACR/European League against Rheumatism 2010 criteria were developed, which display a higher sensitivity but a somewhat lower specificity for RA. Recent advances in biomarker discovery have been included in the new 2010 criteria for RA by inclusion of a set of autoantibodies, directed against citrullinated proteins (ACPA) (*figure 1*).[3,4] ACPA have a very high specificity for RA.[3]

Both genetic and environmental risk factors influence the development of RA. An estimated 50% of the risk to develop RA is mediated via genetic risk factors.[1] Of the genetic risk factors, the strongest predisposing variants are found in the human leucocyte antigen (HLA) alleles. These HLA molecules are essential for antigen presentation in the immune response. Although the association between HLA and RA is still incompletely understood, the association suggests that antigen presentation and the type of immune activation that leads to (auto)antibody formation are important in RA. Of the environmental risk factors, smoking is the most prominent with its largest effect in autoantibody positive patients.

Recent data have clearly shown that treatment (very) early in the disease course will provide the best results and will largely prevent irreversible damage to the joints. With these new approaches the classical picture of massive erosions,

Figure 1. *ACR 1987 criteria and ACR/EULAR 2010 criteria: Classification criteria for rheumatoid arthritis*

ACR 1987 criteria

- Morning stiffness (at least 1h)
- Arthritis of three or more joint areas
- Arthritis of hand joints (>1 swollen joints)
- Symmetric arthritis
- Rheumatoid nodules
- Serum rheumatoid factor
- Radiographic changes (erosions)

Four of seven criteria must be present. Criteria 1-4 must have been present for at least 6 weeks.

ACR/EULAR 2010 criteria

	Score
• Joint involvement	
1 large joint	0
2-10 large joints	1
1-3 small joints (large joints not counted)	2
4-10 small joints (large joints not counted)	3
> 10 joints (at least one small joint)	5
• Serology	
Negative RF and negative ACPA	0
Low-positive RF or low-positive ACPA	2
High-positive RF or high-positive ACPA	3
• Acute-phase reactants	
Normal CRP and normal ESR	0
Abnormal CRP or abnormal ESR	1
• Duration of symptoms	
<6 weeks	0
≥6 weeks	1

A score of ≥6 is the cutpoint for rheumatoid arthritis. Patients can also be classified as having rheumatoid arthritis if they have: 1) erosive disease typical for rheumatoid arthritis, 2) long-standing disease previously satisfying the classification criteria.

ACR = American College of Rheumatology; EULAR = European League Against Rheumatism; RF = rheumatoid factor; ACPA = anti-citrullinated peptide antibodies; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate. Adjusted from Scott *et al.*[1]

deformities and disability can be prevented in a large proportion of patients. However, having the opportunity to treat patients at a (very) early phase of their disease course also poses a problem. Many patients with early RA and especially those suffering from arthralgia or undifferentiated arthritis may undergo spontaneous remission, whereas patients may also progress to full-blown RA. A recent study showed that about a third of the undifferentiated arthritis patients go into remission, pointing to the relevance of biomarkers for diagnosis.[4] Thus, the aim is to identify those people who will require early (and aggressive) intervention and at the same time prevent over-treatment in those who do not need such an intervention. Next to clinical parameters, biomarkers are also helpful in making such decisions. In this review we will describe several new biomarkers which are now available or are currently being developed to guide such decisions.

ANTI-CITRULLINATED PROTEIN ANTIBODIES (ACPA)

The first RA-associated antibody, rheumatoid factor (RF), was discovered 1940,[5] and was later found to be directed to the Fc region of IgG. In the past two decades, it has been shown that the autoantibodies with the highest specificity for RA are antibodies directed against proteins containing citrulline epitopes. These antibodies are called ACPA and were first described as anti-perinuclear factor antibodies (APF).[6] ACPA can recognise the non-classical amino acid citrulline, embedded on a protein backbone.[7,8] Citrulline is a non-encoded amino acid, generated by a post-translational modification of arginine mediated by protein-arginine deiminase (PAD) enzymes.[9] This modification takes place during a variety of biological processes, including inflammation. In 1998, the first ELISA using citrullinated peptides was developed[8] and was followed, in 2000, by the first ELISA based on artificial cyclic citrullinated peptides (CCP).[10] The first commercial version of this test, the CCP2 assay, became available in 2002 and enabled routine testing for antibodies directed against citrullinated epitopes as a biomarker for RA.[10-14] As well as the CCP2 assay, a few other assays for ACPA, such as CCP3 and MCV (mutated citrullinated vimentin), have made their way into the clinic. These assays differ slightly in terms of specificity and sensitivity.[15] As anti-CCP2 antibodies recognise a variety of citrullinated peptides and proteins, these antibodies are now called ACPA.[16]

ACPA can recognise a variety of citrullinated antigens, including citrullinated fibrinogen,[17,18] citrullinated vimentin (which is also known as the Sa antigen),[19] citrullinated type II collagen,[20] citrullinated α-enolase[21] and many more citrullinated proteins. An increase or shift in the antigen recognition profile (a phenomenon known as epitope spreading) can have important pathophysiological consequences, as has been described in, for example, systemic lupus erythematosus[22] and pemphigus. Autoantibodies such as anti-desmoglin antibodies present in patients with pemphigus vulgaris have been convincingly shown to mediate a pathogenic

effect, through transfer into experimental animals.[23] Furthermore, in pemphigus, reactivity against different desmoglein epitopes is associated with different outcomes.[24] Nonetheless, current evidence indicates that epitope spreading to defined antigens does not correlate with clinical phenotype.[25-27] In contrast, an overall broadening of the ACPA-recognition profile is noted before disease precipitation after which a stabilisation occurs.[28,29] Isotype switching is another event involved in enhancing the efficacy of (auto)antibodies, and leads to an increase in the diversity of antibody response that enables the activation of more immune effector mechanisms. ACPA can be present in different forms, including IgG, IgA, IgM and IgE.[30-33] Healthy family members of patients with RA display fewer ACPA isotypes than the patients.[30] Nonetheless, multiple ACPA isotypes are present before the onset of RA.[31] Likewise, the ACPA isotype distribution does not seem to significantly expand anymore during disease progression from undifferentiated arthritis to RA, indicating that most of the expansion of isotype usage by ACPA, like the epitope recognition profile, takes place before the onset of arthritis.

ACPA are strongly associated with RA, which suggests they have a prominent role in disease pathogenesis.[34-36] Selective B-cell depletion has been shown to be effective in the treatment of RA, providing evidence for the involvement of B cells and possibly autoantibodies in its pathogenesis.[37,38] Furthermore, most ACPA-positive patients with RA seem to be ACPA positive years before the onset of disease,[39] although the extent of the ACPA repertoire seems to be limited at this preclinical stage. Likewise, in patients with arthralgia, the development of arthritis is predicted not only by the presence of ACPA, but also by their levels.[40,41] High titres of ACPA are associated with the recognition of more citrullinated epitopes. Indeed, patients with arthralgia who have an extended ACPA repertoire are at higher risk of developing arthritis.[41] Similarly, ACPA-positive patients with early arthritis who do not fulfil the American College of Rheumatology (ACR) classification criteria for RA are more likely to develop RA if their ACPA response is reactive to more citrullinated epitopes.[29] These findings are consistent with the notion that a 'broader' ACPA recognition profile is associated with the transition towards (persistent) disease, and resemble the observations made in pemphigus with the exception that, thus far, no specific anti-citrullinated epitope or protein reactivity has been identified that would predict disease course in RA.

During a B-cell response against recall antigens, isotype switching and affinity maturation typically occur in germinal centres. Following somatic hypermutation, different B-cell clones will compete for antigens presented on follicular dendritic cells. B cells that express immunoglobulins of sufficiently high avidity will acquire the signals necessary for survival and proliferation. As a result, the total avidity of the immune response—defined as the overall binding strength of polyclonal antibodies to a multivalent antigen—increases, because low avidity B cells will not be stimulated and will eventually disappear from the population. The avidity maturation of antibody responses against recall antigens, mostly following vaccination, has been studied extensively, but autoantibody responses seem to behave differently.[42-44] For example, the avidity of ACPA is significantly lower than the avidity of antibodies to the recall antigens tetanus toxoid and diphtheria toxoid, pointing to a different regulation of autoantibody responses as compared with recall antigens. In individual patients with RA, ACPA do not show avidity maturation during longitudinal follow-up and even in patients who displayed extensive isotype switching, ACPA avidity was relatively low,[45,46] indicating that these two maturation processes are uncoupled in the ACPA response.

ACPA AND TREATMENT OUTCOMES

Diseases with distinct pathogeneses might benefit from different treatment strategies; therefore, ACPA-positive and ACPA-negative disease are treated differently. Methotrexate is the most prominent disease-modifying antirheumatic drug (DMARD). A few years ago, we performed a double-blind placebo-controlled randomised trial comparing two treatment strategies in patients with undifferentiated arthritis. Interestingly, the outcome of this study indicated that ACPA-positive patients with undifferentiated arthritis treated with methotrexate are less likely to progress to RA, and do so at a later time point as compared with a placebo-control group. Unexpectedly, no effect of methotrexate therapy on progression to RA in the ACPA-negative group was observed.[47] Interestingly, among patients with undifferentiated arthritis, those with low or intermediate ACPA levels respond better to methotrexate than patients with high ACPA levels.[48] The data from this randomised trial not only indicate that the two ACPA subgroups respond differently to methotrexate treatment, but also that in patients with high APCA levels methotrexate monotherapy might be insufficient. Indeed, the presence of ACPA and IgM RF together with elevated levels of C-reactive protein (CRP) is predictive of more rapid radiographic progression in patients with RA. Patients with ACPA and IgM RF are also more likely to respond insufficiently to methotrexate monotherapy for recent-onset RA.[49]

It is not only in regard to response to DMARDs that ACPA status seems to matter. In a trial published in 2011,[50] 208

patients with RA refractory to therapy with TNF blockers were treated with rituximab. Rituximab is a monoclonal antibody directed towards the B-cell marker CD20, and has been shown to be an effective treatment in RA. In these patients, the presence of ACPA predicted a better EULAR response to rituximab at 24 weeks. Thus, rituximab might have a greater role in ACPA-positive RA patients than in ACPA-negative individuals.[50] The mechanisms of rituximab efficacy, and of B-cell involvement in RA, are incompletely known; the basis for these differing outcomes remains to be elucidated.

The absence of ACPA and IgM RF are independent predictors of drug-free remission.[51] The course of ACPA-positive disease seems to be characterised by more persistent inflammation than its ACPA-negative counterpart. Together, these data indicate that treatment decisions in RA can be guided by ACPA status. Seroconversion is uncommon among ACPA-positive and ACPA-negative patients; therefore, it does not seem to be useful to repeat ACPA measurements in daily practice.[52,53] Thus, these data support the hypothesis that RA can be classified into two different disease subsets, and suggest that developing different classification criteria for ACPA-positive and ACPA-negative RA might help to further optimise treatment strategies.

ACPA AND DISEASE OUTCOME

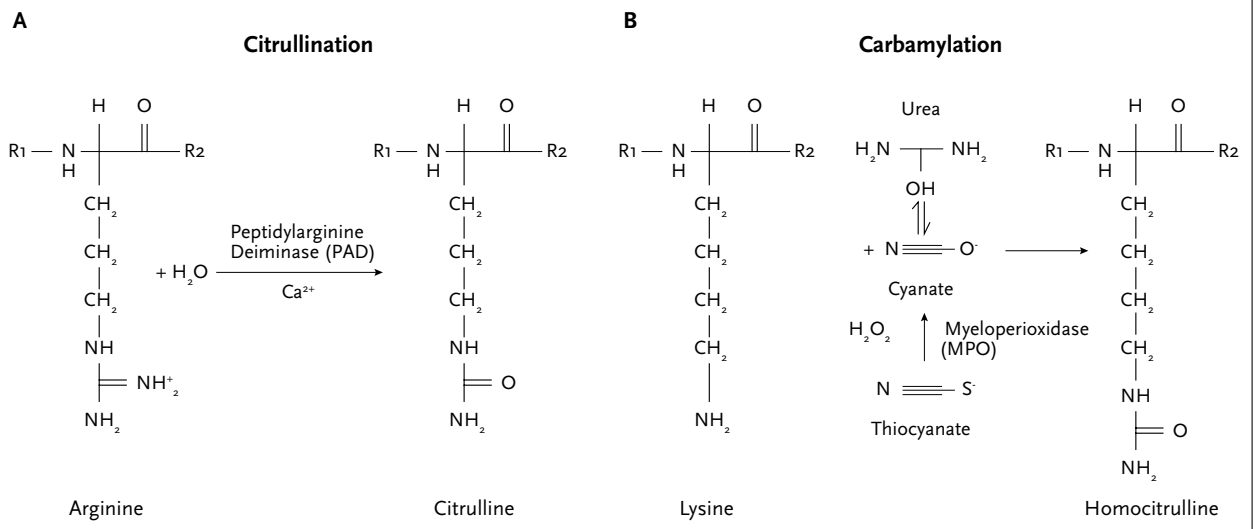
The emerging relevance of ACPA status to treatment decisions is not only based on differential treatment efficacies, but is also supported by differences in disease outcome. Typically, 50 to 70% of the patients with RA are ACPA positive.[54] Although ACPA-positive and ACPA-negative patients with RA show a very similar clinical presentation in the early phase of the disease,[55,56] their subsequent disease course is different: extra-articular manifestations are clearly associated with ACPA status. For example, ACPA positivity is associated with an increased risk of developing ischaemic heart disease[57] or lung pathology.[58] Likewise, ACPA-positive patients have more destructive joint disease than ACPA-negative patients.[56,59-61] ACPA-positive patients develop erosions earlier and more abundantly than patients without ACPA.[62] Owing to their more severe disease course, ACPA-positive patients require a more aggressive treatment regimen than ACPA-negative patients.[63] Indeed, in the BeSt study, ACPA-positive patients initially treated with DMARD monotherapy displayed greater radiographic joint destruction after two years than ACPA-negative patients.[63] In patients initially treated with combination therapy, by contrast, no difference with respect to joint destruction was observed between ACPA-positive and ACPA-negative patients. These observations suggest that effective treatment with combination therapy, together with steering treatment according to disease activity, can prevent radiographic progression, even in patients with risk factors for severe damage, such as ACPA-positive patients.

Most ACPA characteristics, such as the fine-specificity profile, are not associated with the rate of joint destruction once RA is established.[25,26] The ACPA isotype profile seems, however, to be an exception—in two cohorts ACPA isotype diversity has been associated with a higher risk of radiographic progression, equating to a 1.4-fold increase in risk per isotype used in the ACPA response, illustrating that an extended isotype usage is associated with a worse outcome.[64] Altogether, evidence is emerging that ACPA-positive and ACPA-negative RA represent two different disease entities with different outcomes, and, possibly, different responses to medication. The latter notion is especially important as it indicates that treatment regimes can be further optimised by guiding their development by ACPA status.

ANTI-CARBAMYLATED PROTEIN (ANTI-CARP) ANTIBODIES

ACPA recognise proteins only after the enzymatic conversion of the amino acid arginine by PAD enzymes to become the amino acid citrulline. Next to citrullination, also other post-translational modifications are known to occur. Therefore, it is likely that proteins that have undergone a different type of post-translational modification are also recognised by autoantibodies. One of these other post-translational processes is the process of carbamylation. In this chemical reaction, mediated by cyanate, the amino acid lysine is changed to become the amino acid homocitrulline. Cyanate, necessary for such carbamylation, is naturally present in the body and in equilibrium with urea.[65] In the healthy situation the concentration of urea is rather low. It is likely that under such conditions especially long-lived proteins, such as matrix molecules, become carbamylated. Renal failure, a condition with increased urea concentrations, is known to be associated with enhanced protein carbamylation.[66] Also smoking, the most prominent environmental risk factor for RA, enhances carbamylation by increasing the cyanate concentration.[67] Extensive carbamylation is especially thought to occur during (chronic) inflammation, when myeloperoxidase is released from neutrophils as this enzyme shifts the equilibrium of thiocyanate towards cyanate.[67] As smoking and chronic inflammation are important in the context of RA, it is likely that in the inflamed synovium carbamylation is taking place. The post-translationally modified amino acids citrulline and homocitrulline are very similar structures (*figure 2*). In

Figure 2. *Illustration of citrullination and carbamylation. Citrullination (A) and carbamylation (B) occur on different amino acids via different mechanisms, but yield similar end-products*



both cases a positively charged amino acid is replaced by a neutral one. The only structural difference is the difference in length; homocitrulline is one CH_3 group longer. The resemblance between the two modifications and the likely presence of carbamylated proteins in the joint prompted us to test for the presence of antibodies directed against carbamylated proteins.

Therefore, we developed a novel assay that specifically detects the presence of antibodies directed against carbamylated proteins (anti-CarP).[68] Using this assay we could show that RA patients indeed also harbour antibodies directed against carbamylated proteins. Importantly we observed these anti-CarP antibodies not only in ACPA-positive but also in ACPA-negative RA patients. This suggests that antibodies recognising one modification do not necessarily cross-react with the other modification. This notion was further supported experimentally by inhibition studies and Western blotting.[69] When analysing the clinical status of anti-CarP antibody positive RA patients, we observed that the presence of anti-CarP antibodies was associated with a higher rate of joint damage. This phenomenon was especially observed in the ACPA-negative subgroup. Especially for these ACPA-negative patients no prognostic markers were available and the identification of anti-CarP antibodies in this group may be useful clinically. These observations suggest that the population of RA patients is more heterogeneous than initially thought as, perhaps, besides ACPA-positive disease also anti-CarP-positive RA might represent an additional disease entity with its own genetic/environmental contributions. However, conformational studies are required to support this notion. Several major questions are still open regarding anti-CarP such as: why do some people make these antibodies? Do they contribute to the disease process and if so, how? What are the possibilities to interfere with these putative processes? Does the presence of anti-CarP antibodies predict progression towards disease in subjects at risk to develop arthritis, and if so, will it have added value as a biomarker next to ACPA testing? As indicated above international replication studies are needed to confirm and expand the presented observations. Interestingly, the anti-CarP immune response is not only restricted to humans, but can also be induced in mice and rabbits by vaccination with carbamylated proteins.[70,71] This might allow to study the driving mechanisms underlying the anti-CarP response and if and how it contributes to arthritis.

BIOMARKER-BASED DISEASE ACTIVITY INDICES

Accurate and frequent assessment of RA disease activity is critical for optimal treatment planning. In numerous studies it has been shown that treatment guided by disease activity improves outcome in RA.[72,73] Moreover, several studies have suggested that frequent measurement of disease activity and subsequent tight control of disease activity is associated with good clinical outcome. In a study

from Utrecht, monthly assessment of disease activity was proposed.[74] However, in daily clinical practice it is often not feasible to schedule monthly patient visits to assess disease activity. As such, a 'simple' biomarker that replaces the clinical assessment of disease activity is relevant. To this end a novel algorithm has been developed to determine a multi-biomarker disease activity (MBDA) score based upon measurement of the concentrations of 12 serum biomarkers (SAA, IL-6, TNF-RI, VEGF-A, MMP-1, YKL-40, MMP-3, EGF, VCAM-1, leptin, resistin and CRP).[75] This MBDA score was significantly associated with conventional disease activity measured, the so-called disease activity score, which is a composite index of the number of swollen and painful joints, the assessment of the patient on disease activity and an acute phase response. Importantly, this association has been observed in several Dutch and American cohorts from RA patients.[76,77] Moreover changes in MBDA scores were able to discriminate clinically relevant reductions in disease activity scores, suggesting that this test has the potential to be used to evaluate disease activity measures.

CONCLUSION

Both with regard to the prognosis as well as follow-up of patients, biomarkers have been developed that can help the clinical management of RA patients.

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REVIEW

Prothrombotic disorders in abdominal vein thrombosis

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ABSTRACT

Abdominal vein thrombosis is a rare, but potentially life-threatening form of venous thrombosis. It mainly involves the hepatic veins (Budd Chiari syndrome, BCS), portal veins (PVT) and mesenteric veins. In recent years several large-scale studies have been performed to study the underlying aetiological factors in these thrombotic disorders. Both inherited and acquired thrombophilia factors are frequently observed in these patients. Factor V Leiden mutation is frequently found in patients with BCS and prothrombin gene variant is seen more frequently in PVT. Myeloproliferative neoplasms (MPNs), including polycythemia vera and essential thrombocythemia, are underlying disorders in 30-40% of patients with abdominal vein thrombosis. Other aetiological factors are paroxysmal nocturnal haemoglobinuria (PNH), autoimmune disorders and hormonal factors. Recently, several new risk factors have been reported and are discussed in this review. BCS and PVT are multi-factorial disorders. In nearly 50% of patients two, and in 16% even three prothrombotic risk factors were found at presentation. Because patients with abdominal vein thrombosis have a high risk of recurrence immediate anticoagulant treatment is necessary. The duration of treatment is still a matter of debate because these patients also have a high risk of bleeding, especially those with portal hypertension. For BCS patients life-long anticoagulant treatment is advised. In patients with PVT it is recommended to tailor treatment to the individual patient based on the presence of an underlying prothrombotic disorder and the risk of bleeding.

KEYWORDS

Portal vein thrombosis, Budd Chiari syndrome, thrombophilia, splanchnic vein thrombosis, thrombosis, myeloproliferative neoplasm

INTRODUCTION

Abdominal vein thrombosis or splanchnic vein thrombosis is a rare, but life-threatening form of venous thrombosis and includes hepatic vein thrombosis (Budd-Chiari syndrome, BCS), portal vein thrombosis (PVT) and mesenteric vein thrombosis (MVT). The splanchnic venous system consists of the portal vein and its branches that direct blood flow from the gastrointestinal organs to the liver. The portal vein is formed by the union of the superior mesenteric vein and the splenic vein, and subdivides into a left and right branch, which are segmentally distributed throughout the liver. The terminal portal venules drain into the sinusoids, after which the blood flows from the small to large hepatic veins, ultimately reaching the inferior vena cava. Thrombosis can occur in the hepatic veins or the portal veins and sometimes extends to the mesenteric veins. BCS and PVT are the two most frequent manifestations of superficial venous thrombosis (SVT), and simultaneous involvement of these venous systems may occur.[1]

BCS is defined as an obstruction of the hepatic venous outflow tract from the level of the small hepatic veins to the entrance of the inferior vena cava into the right atrium. BCS is considered primary when obstruction of the venous tract is the result of an endoluminal lesion, i.e. thrombosis, and secondary if the obstruction results from compression or invasion of the venous system.[2] BCS is a rare disorder with an incidence of around 1-2 per million inhabitants in the Western world. In the Netherlands an estimated number of 10-20 cases per year are seen, most of whom are young females.[3] The main complications of BCS are the result of portal hypertension and liver dysfunction. The classical triad of presenting symptoms in BCS consists of abdominal pain, ascites and hepatomegaly. In addition, alterations in liver biochemical tests can be observed. Clinical presentation may range from absence of symptoms, in case of preservation of hepatic veins

and/or formation of collaterals, to fulminant hepatic failure requiring liver transplant, with an acute or chronic development of symptoms ranging from weeks to months.[4] The survival rate is 87% at one year and 82% at two years.[5] In PVT, the obstruction is located in the extra-hepatic portal vein, but sometimes the intra-hepatic portal, superior mesenteric and splenic vein are also involved. PVT is considered a rare disorder, mainly occurring in individuals with pre-existent liver disease (cirrhosis) or hepato-biliary malignancies. However, it can also develop in patients without liver disease. Most patients present with gastrointestinal bleeding from oesophageal varices and splenomegaly as complications of portal hypertension.[4] Bowel infarction can occur when thrombosis extends into the mesenteric vein. Survival of patients with non-cirrhotic, non-malignant PVT can be considered good. Five- and ten-year survival rates are 90% and 80%, respectively. In patients with underlying cirrhosis or hepato-biliary malignancy the prognosis is poor. Recently, two reviews on the current management of hepatic and portal vein thrombosis have been reported in this journal.[3,6] In this article we will focus on the most current findings on the role of prothrombotic factors in the aetiology of abdominal vein thrombosis with special emphasis on inherited thrombophilia and myeloproliferative neoplasms.

Table 1. *Aetiological factors in Budd Chiari syndrome and portal vein thrombosis based on the result of the En-Vie study cohorts*[5,7]

Risk factor	BCS Frequency (%)	PVT Frequency (%)
Inherited thrombophilia	21	35
Acquired thrombophilia	44	19
Myeloproliferative neoplasm	49	21
JAK2 pos	29	16
Hormonal factors	38	44
Oral contraceptives	33	44
Pregnancy	6	0
PNH	19	0
Other systemic factors	23	n.d.
Local factors	0	21

BCS = Budd Chiari syndrome; PVT = portal vein thrombosis; PNH = paroxysmal nocturnal haemoglobinuria.

AETIOLOGY

Local risk factors for the development of BCS include solid malignancies, cysts or abscesses that compress or invade the venous tract.[2] This only accounts for the minority of BCS in the Western world. PVT, on the other hand, is most often seen as a complication of liver cirrhosis or hepatobiliary malignancies. Other frequent local risk factors are surgical trauma to the portal vein and infections or inflammation in the abdomen, which are often accompanied by an additional prothrombotic condition.

The aetiology of primary BCS and non-malignant, non-cirrhotic PVT often involves systemic, prothrombotic conditions. It should be kept in mind that most studies on aetiological factors for abdominal vein thrombosis included selected patient groups and only have a limited sample size. In a large multicentre European study (En-Vie study) 163 consecutive patients with primary BCS and 105 patients with non-malignant, non-cirrhotic PVT with a near-complete work-up for aetiological factors, reported prothrombotic factors in up to 84% and 42%, respectively.[5,7] In *table 1* the most common aetiological factors derived from these two large cohorts of patients with BCS and PVT are depicted. Several of these risk factors are also known risk factors for more common venous thrombosis, such as deep venous thrombosis and pulmonary embolism. However, some risk factors are more specific for abdominal vein thrombosis.

Inherited and acquired thrombophilia

Thrombophilia defines conditions that are associated with an increased risk of venous thromboembolism (VTE), and is characterised by a hypercoagulable state. Common clinical features of thrombophilia are thrombosis at a young age, recurrent venous thrombosis, a positive family history of VTE, and thrombosis located at unusual venous sites. The role of thrombophilia in the aetiology of abdominal vein thrombosis has recently been established. The exact prevalence of inherited deficiencies of the natural anticoagulants antithrombin, protein C and protein S is difficult to determine in BCS and PVT patients, because low levels of these factors may also be caused by reduced liver synthesis function, which frequently occurs in these patients. In addition, most of these patients are treated with long-term anticoagulant treatment with vitamin K antagonists, which hampers the diagnosis of protein C and protein S deficiency. In some studies, an inherited deficiency was diagnosed if protein C or S levels were lower than other vitamin K dependent coagulation factors synthesised by the liver or when a clear isolated deficiency was found.[8] In these studies the prevalence of antithrombin deficiency ranges from between 0-5% in both BCS and PVT, for protein C deficiency this is between 4-20% in BCS and 0-7% in PVT, and for protein S deficiency between 0-7% in BCS and 0-30% in PVT.[5,7-10] In one study protein C deficiency was significantly associated with both BCS and PVT,[8] whereas another large study did not find a significant association between these factors and PVT.[11] Although the data are not entirely consistent,

primary deficiencies of these coagulation inhibitors are likely to contribute to the pathogenesis of BCS and PVT, and should be included in diagnostic work-up.

In BCS patients the prevalence of the Factor V (FV) Leiden mutation ranges between 7% and 32%, which resembles the percentage in patients with DVT. The prevalence of the FV Leiden mutation in patients with PVT is lower, ranging between 3% and 9% (reviewed in Smalberg *et al.*[12]). Case-control studies have confirmed that the FV Leiden mutation is more strongly associated with BCS than with PVT. FV Leiden carriers have a four- to 11-fold increased risk of BCS, whereas a recent meta-analysis reported only a twofold risk of PVT in FV Leiden carriers.[13] On the contrary, the prothrombin G20210A gene variant is more common in PVT than in BCS with a prevalence ranging from 3%-8% in BCS.[12] A recent meta-analysis reported a four- to five-fold increase in risk of PVT in carriers of the prothrombin gene variant,[13] whereas the risk of BCS is approximately twofold increased.[8] So far, the mechanism behind the difference in prevalence of the FV Leiden mutation and the prothrombin gene variant in BCS and PVT remains unresolved. Despite the increased relative risk, the absolute life-time risk for abdominal vein thrombosis in carriers of FV Leiden mutation or prothrombin gene variant is very low and mainly dependent upon other risk factors mentioned below.

Although antiphospholipid antibodies (APA) are considered a risk factor for BCS and PVT, they have hardly been studied in case-control studies. The prevalence of APA in BCS and PVT has been estimated to be around 5-15%.[5,7,9] The importance as a risk factor is difficult to assess because anticardiolipin antibodies are also found in patients with chronic liver disease without thrombosis.[14] However, large studies confirming the relationship between APA and BCS and PVT are still lacking. In most studies only one single measurement was carried out, whereas for the correct diagnosis of the antiphospholipid syndrome, these should be measured at two different occasions 12 weeks apart.[15]

More recent studies have investigated whether increased levels of coagulation factors are increasing the risk of abdominal vein thrombosis. Recently, Martinelli *et al.* described significantly elevated factor VIII levels in patients with primary PVT, both with and without underlying cirrhosis, even adjusted for the acute-phase reaction. However, factor VIII is known to be strongly increased in patients with liver dysfunction, which is frequently seen in BCS and PVT patients.[16] These results were confirmed by Raffa *et al.* who studied several plasma coagulation factors in patients with non-cirrhotic PVT. They observed a significant increase of endogenous thrombin potential irrespective of the underlying prothrombotic or thrombophilic disorder.[17]

Emerging new thrombophilic risk factors

Only few studies have assessed the role of the fibrinolytic system in the pathogenesis of BCS and PVT. We recently observed an association between abdominal vein thrombosis (SVT) and genetic variation in the thrombin activatable fibrinolysis inhibitor (TAFI) gene.[18] A decreased risk of SVT in 147Thr/Thr homozygotes and a slightly, but not significantly, increased risk in carriers of the 325Ile variant was observed, suggesting a role for TAFI in the pathogenesis of SVT. The genotypes associated with an increased risk of SVT are associated with decreased TAFI levels.

Hoekstra *et al.* extensively investigated components of the fibrinolytic system in 101 BCS patients included in the En-Vie study.[19] This study showed significantly higher PAI-1 levels in BCS patients compared with controls, whereas TAFI and α2-antiplasmin levels were significantly lower. It was additionally shown that hypofibrinolysis, as determined using clot lysis times (CLT), was associated with an increased risk of BCS. A CLT above the 90th or 95th percentile of controls was associated with a 2.4-fold and 3.4-fold increase in risk of BCS, respectively. This study suggests that impaired fibrinolysis may also play a role in the pathogenesis of abdominal vein thrombosis.

Recently, a potential new factor in the pathogenesis of BCS was identified. Using a proteomic approach assessing fibrin binding proteins in plasma obtained from patients with BCS compared with healthy controls, Talens *et al.* initially showed that apolipoprotein A1 (ApoA1) was decreased in BCS patients. This observation was validated in a large cohort of BCS patients, in which ApoA1 levels were also significantly lower compared with controls.[20] ApoA1 is the principal component of high-density lipoprotein cholesterol, which has been shown to be inversely associated with other forms of VTE.[21] Low ApoA1 levels have also been associated with an increased risk of recurrence of common VTE.[22]

Other risk factors

It has been known for several decades that myeloproliferative neoplasms (MPNs) are a common underlying cause of abdominal vein thrombosis. MPNs are chronic clonal haematopoietic stem cell disorders characterised by an overproduction of mature and functional granulocytes, red blood cells and/or platelets.[23] It is estimated that MPNs are observed in 30-40% of patients with BCS or PVT, whereas this is rarely the cause of other types of VTE.[5,7,10,24,25] Portal hypertension, resulting from pre- or post-hepatic venous obstruction, can lead to hypersplenism and haemodilution. This may mask the characteristic peripheral blood cell changes (i.e. high haemoglobin levels and thrombocytosis) and make diagnosis of MPN difficult. Previously, diagnosis of MPNs in these patients relied on bone marrow (BM) biopsy findings and growth of erythroid

colonies in the absence of exogenous erythropoietin, referred to as spontaneous endogenous erythroid colonies (EEC). Patients were diagnosed with occult MPN in case of typical bone marrow changes, especially dysmegakaryopoiesis, or spontaneous EEC growth, but in whom blood cell counts were normal.[24] The discovery of the *JAK2V617F* mutation, a common gain of function mutation leading to development of MPN, has changed the diagnostic strategy of MPN. This mutation is present in nearly all patients with polycythemia vera and in about 50% of patients with essential thrombocythemia and primary myelofibrosis. The *JAK2V617F* mutation has been described in a large number of unselected BCS and PVT patients. In a recent meta-analysis we determined the prevalence of MPNs and their subtypes as well as *JAK2V617F* and its diagnostic role in these uncommon disorders.[26] In BCS, mean prevalence of MPNs and *JAK2V617F* was 28.4% and 41.1%, respectively. In PVT, mean prevalence of MPNs and *JAK2V617F* was 19.5% and 27.7%, respectively. MPN and *JAK2V617F* were more frequently found in BCS compared with PVT. Polycythemia vera was more prevalent in BCS than in PVT. *JAK2V617F* screening in SVT patients without typical haematological MPN features identified MPN in 17.1% and 15.4% of screened BCS and PVT patients, respectively.[26] It can be concluded that besides bone marrow histology, screening for *JAK2V617F* is an important diagnostic tool to detect MPN in these patients and should be performed in all patients with abdominal vein thrombosis as part of the standard diagnostic work-up.[27] The exact pathogenetic mechanism of thrombosis in MPNs still remains to be resolved, but besides characteristic erythrocytosis and thrombocytosis, platelet and leucocyte functional abnormalities seem to have a pathogenetic role.[28]

Paroxysmal nocturnal haemoglobinuria (PNH) is a rare, acquired haematological disorder of haematopoietic stem cells, which is associated with thrombosis at unusual sites. Remarkably, thrombosis of the abdominal veins is a frequent complication and accounts for the majority of deaths in this disorder.[29] PNH has been reported in 9-19% of tested BCS patients,[10,30] whereas a prevalence of 0-2% has been reported in PVT.[7] Several mechanisms, including intravascular haemolysis, increased platelet activation and aggregation, procoagulant microparticles resulting from complement-mediated platelet damage, hypofibrinolysis and increased tissue factor expression may contribute to the pathogenesis of venous thrombosis in PNH. Patients with a PNH cell population above 60% of the granulocytes appear to be at greatest risk for thrombosis.[31] Testing for PNH should be routinely performed in all BCS and PVT patients, especially since treatment with eculizumab may be indicated in these individuals.[32]

A number of systemic, autoimmune-mediated diseases have been implicated in the pathogenesis of both BCS and PVT, including Behcet's disease, inflammatory bowel disease, vasculitis, sarcoidosis and connective tissue disease. In the previously mentioned EnVie study these disorders occurred rarely.[6]

Oral contraceptive use and pregnancy are well-known risk factors for VTEs. Since abdominal vein thrombosis occurs frequently in young women, hormonal factors may be of importance in the pathogenesis. In the recent En-Vie study, 50% of patients with BCS and 25% of the patients with PVT were women aged 15-45 years.[5,7] Oral contraceptives have been shown to be associated with at least a twofold risk for BCS.[8,33] For PVT the risk may be slightly increased, but this has not yet been well established.[8]

Multifactorial aetiology

As is well known for other more common forms of venous thromboembolism (VTE) the aetiology of primary BCS and PVT is often multifactorial. In the En-Vie study a combination of two or more genetic or acquired prothrombotic factors was found in 46% of BCS and 48% of PVT patients.[5,7] In BCS patients, 18% of the patients even displayed three risk factors. In over 60% of patients presenting with an inherited thrombophilia factor an additional risk factor was found. In a paediatric population of 31 patients with PVT it was shown that despite the fact that almost all children had a local underlying aetiological factor, deficiencies of the natural anticoagulants and prothrombotic mutations were observed in over 30% of the patients.[34]

Based on these findings, a complete haematological work-up, including diagnosis of inherited thrombophilia, APA, MPN and PNH, should always be performed in BCS and PVT patients, irrespective of whether one prothrombotic factor has already been identified. This is in particular relevant for identifying MPN, which are also often accompanied by other prothrombotic factors, and may require additional treatment, such as aspirin and antiproliferative treatment, such as interferon or hydroxyurea.

ANTICOAGULANT TREATMENT OF ABDOMINAL VEIN THROMBOSIS

The mainstay of therapy is the immediate institution of anticoagulant therapy with low-molecular-weight heparin followed by long-term use of vitamin K antagonists. In BCS, life-long therapy is warranted considering the severity of the disorders. In individuals with acute portal vein thrombosis, anticoagulant therapy is given for three to six months, but depending upon the underlying disorder is sometimes given indefinitely. The duration of anticoagulant therapy is strongly dependent upon the risk

of recurrent thrombosis. So far, only a few studies have focused on the risk of recurrence in PVT. Condat *et al.* assessed the outcome of PVT in relation to prothrombotic conditions in a cohort of 136 patients of whom 84 received anticoagulant therapy.[35] In this study, an incidence rate of 5.5 per 100 person-years for all types of thrombotic events was reported and an underlying prothrombotic state was shown to be an independent predictor of recurrent thrombosis. In another study with a median follow-up of 41 months in 121 patients with abdominal vein thrombosis, the recurrence rate of thrombosis was 10.5%.[36] Nearly 75% of these recurrent events were abdominal vein thrombosis. Most of these patients had an MPN and none were on anticoagulant treatment. In a recent analysis of 120 PVT patients treated in the ErasmusMC we observed recurrent thrombosis in 4%, 8% and 27% of the patients after a follow-up of one, five and ten years, respectively. This was strongly dependent upon an underlying prothrombotic disorder.[37]

As mentioned above, patients with abdominal vein thrombosis have a high risk of recurrence; however, they are also at a higher risk of bleeding. Because patients with acute PVT frequently present with variceal bleeding, local measures should be taken to stop bleeding and prevent rebleeding. In two previously mentioned studies the rates of bleeding after follow-up of 41 months and five years were 15% and 46% respectively.[36,37] In the latter study anticoagulant therapy was a significant predictor of bleeding. Therefore it is very difficult to give strict recommendations on the duration of anticoagulant treatment in patients with PVT. It has been suggested to give long-term anticoagulant therapy only to those individuals with major underlying thrombophilic risk factors, such as homozygous FV Leiden mutation and prothrombin gene variant.[38] Follow-up studies are needed in order to establish the duration of anticoagulant treatment, especially those with no or mild thrombophilic disorders.

In case of an underlying MPN, anticoagulant treatment should also be given indefinitely. It is still being debated whether aspirin should be added to the anticoagulant treatment with vitamin K antagonists in patients with MPN and abdominal vein thrombosis. In a recent retrospective study we showed a potential benefit of aspirin in patients with PVT and MPN with lower recurrent thrombosis rates in individuals also treated with oral anticoagulant therapy.[39] This has to be confirmed in prospective studies. These patients should also be treated with antiproliferative therapy, such as alpha interferon or hydroxyurea, in order to normalise peripheral blood cell counts. Additional management of abdominal vein thrombosis with invasive procedures, stents or surgery is outside the scope of this article and has been reviewed before.[6]

CONCLUSIONS

Abdominal vein thrombosis is a rare, but potentially life-threatening thrombotic event. The aetiology is multi-factorial, in which both genetic and acquired prothrombotic factors are frequently encountered simultaneously. Indefinite anticoagulant treatment is advised for patients with BCS and for patients with PVT with a major underlying prothrombotic risk factor; however, this must be balanced against an increased risk of bleeding. Long-term treatment studies are urgently needed to establish the optimal duration of anticoagulant treatment in these thrombotic disorders.

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ORIGINAL ARTICLE

Protocolised inpatient care of diabetes mellitus

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ABSTRACT

Background: The prevalence of hyperglycaemia in patients with diabetes mellitus at admission is high. Prevention and treatment is important to prevent further clinical complications. We have conducted a study evaluating implementation of a new protocol to standardise inpatient care of patients with diabetes mellitus.

Methods: A retrospective study including all glucose measurements of adult patients with diabetes mellitus type 1 or 2, admitted to a surgery department, was performed before and after implementation of the new protocol. This protocol included direct consultation of an internist and diabetes specialist nurse at admission, who initiated a daily treatment program and adjustment scheme based on glucose measurements four times a day by the HemoCue201DM glucose point of care device. We compared the prevalence of hyperglycaemia and hypoglycaemia before and after implementation with logistic regression analyses adjusted for age and gender.

Results: Overall, 360 patients with diabetes mellitus type 1 or 2 with 5322 glucose measurements were included. The risk of developing hyperglycaemia was significantly reduced after implementation of the protocol (22 patients with 65 hyperglycaemias) compared with before the intervention (70 patients with 417 hyperglycaemias) (RR adjusted 0.24 (95% confidence interval 0.19; 0.32)). Overall, 45 patients experienced 95 episodes of hypoglycaemia, which did not differ significantly between the two groups.

Conclusion: After implementation of a new protocol to standardise inpatient care of diabetes mellitus we established a decrease in the risk to develop hyperglycaemia of 76% without an increased risk of developing hypoglycaemia. Implementation of this protocol required frequent glucose measurements which are facilitated by point of care glucose measurements.

KEYWORDS

Diabetes mellitus, hyperglycaemia, hypoglycaemia, inpatient care

INTRODUCTION

Diabetes mellitus is a major public health issue with a total prevalence of 14% in 2010 rising to an expected 21% of the USA adult population by 2050.[1] Approximately 10% of all patients who are admitted in an acute setting have diabetes mellitus.[2] The regulation of diabetes mellitus is often disturbed in hospital due to, for instance, the use of several drugs, infection, altered eating patterns or decreased mobilisation.

Hyperglycaemia due to decompensated diabetes mellitus, unrecognised diabetes mellitus or hospital-related hyperglycaemia leads to increased morbidity and mortality, mainly due to an increase in infections due to immunosuppression, cardiovascular events, venous thromboembolic events, inflammation, endothelial cell dysfunction and cerebral ischaemia.[3] Therefore, it is important to treat and prevent hyperglycaemia.

Recently, we implemented a new protocol to standardise inpatient care of patients with diabetes mellitus, which included a direct consultation of an internist and diabetes specialist nurse at the admission of patients with diabetes mellitus type 1 or 2, irrespective of the reason for admission. In addition to a daily treatment program, the attending nurse could adjust the insulin dose using a standardised adjustment scheme based on point of care glucose measurements available on each department. Implementation of intensive diabetes mellitus therapy requires frequent and accurate blood glucose data. Glucose monitoring using capillary blood has an advantage over

laboratory venous glucose testing since the results can be obtained rapidly at the 'point of care'.[3] Glucose results were monitored by a point of care testing (POCT) device, whose performance appeared to be in accordance with the guidelines for decentralised monitoring of glucose.[4]

We have conducted a retrospective study in the Maasstad Hospital in Rotterdam comparing the prevalence of hyperglycaemia and hypoglycaemia before and after implementation of this new protocol.

METHODS

Setting and study design

The study population comprised all patients of 18 years and older with diabetes mellitus type 1 or 2 using oral antidiabetics and/ or insulin therapy admitted to one of the surgery departments of the Maasstad Hospital in Rotterdam.

A retrospective study was conducted comparing all glucose measurements after implementation of the inpatient care of diabetes mellitus protocol (September and October 2010) with all glucose measurements before implementation (March, April and May 2010). All glucose measurements during admission were included. Overall, there were 409 patients with 5466 glucose measurements. We excluded patients (n=49; 144 glucose measurements) who were not referred to the internist or diabetes specialist nurse by the attending nurse of the department where the patient was admitted.

Age on the day of the glucose measurement, gender and department were retrieved from the computer-based healthcare information system.

Glucose measurements

At admission, glucose concentration was measured by the laboratory reference method. The protocol included direct consultation of an internist and diabetic specialist nurse at admission, who initiated a daily treatment program. Glucose measurements were monitored four times per day with POCT devices available on each department. The values are present within seconds and are automatically registered in the computer-based healthcare information system. In the Maasstad Hospital, the HemoCue Glucose 201DM device (201DM) was used, which has recently been compared with a new generation device (201DMRT).[4] This comparison showed a high correlation coefficient of 0.998 and acceptable total measurement error (total error <10% in the concentration range 4-20 mmol/l). Moreover, the method correlated well with the reference laboratory method. It is generally accepted that the hexokinase glucose method is the reference method for glucose measurement. Recently, an excellent agreement between the HemoCue Glucose 201DMRT device and the Vista hexokinase reference method within the measured range was found (2-30 mmol/l). The results were not influenced by changes in partial oxygen pressure, although they were influenced by changes in haematocrit in a predictable fashion.[4]

Glucose measurements below 4 mmol/l were defined as hypoglycaemia and measurements more than 15 mmol/l were defined as hyperglycaemia.

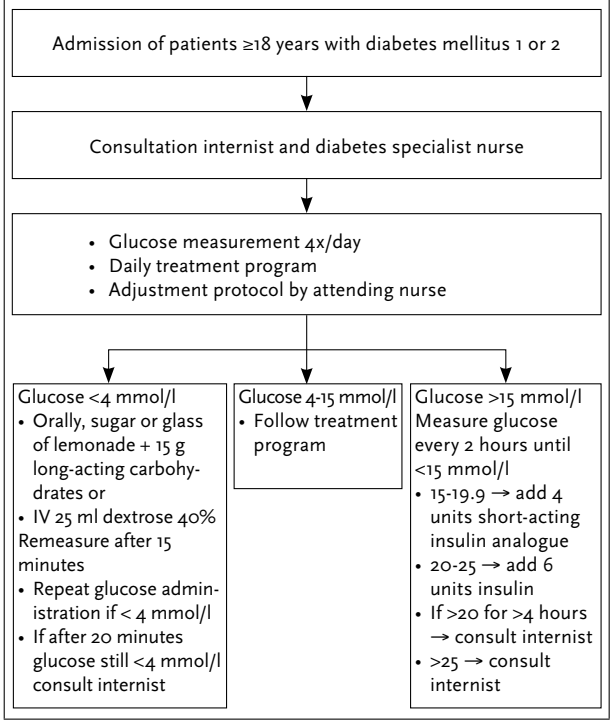
Intervention

In September 2010, a new protocol was implemented to standardise the treatment of patients with diabetes mellitus in the inpatient clinic. The implementation was started in the surgery departments and later on extended to all departments. Before implementation all nurses from the surgery departments were trained.

Before implementation of the protocol, patients used their own dosage of oral antidiabetic drugs or insulin. The primary caregiver could consult the internist when patients experienced hypo- or hyperglycaemia or when the patient's intake had to be altered (e.g. fasting, fluid diet).

After implementation of the protocol (*figure 1*), the attending nurse of the department set up a direct consultation with the internist and diabetes specialist nurse. The diabetes specialist nurse and the internist agreed on a daily treatment program based on the previous treatment dosages, reason for admission, intake and mobilisation and current glucose measurements,

Figure 1. *Inpatient care of diabetes mellitus*



pursuing a glucose target value of 4-10 mmol/l. Glucose was measured four times a day (preprandial and before bedtime). In addition, the insulin dosage could be adjusted by the attending nurse according to the glucose measurement using the protocol when glucose measurements were <4 or >15 mmol/l (*figure 1*). The diabetes nurse contacted the attending nurse on a daily basis and changed the treatment program if necessary, under supervision of the internist.

Statistical analysis

The baseline characteristics before and after the intervention were compared using the t-test as well as the number of episodes of hyperglycaemia and hypoglycaemia overall and within one patient.

Linear regression was used to calculate the difference in glucose level after the intervention compared with before the intervention. The relative risk with 95% confidence interval of hyperglycaemia or hypoglycaemia in the group of patients after the intervention was implemented was compared with the group of patients before implementation of the intervention. This was estimated by calculation of the unadjusted and adjusted (for age and gender) odds ratios using univariate and multivariate logistic regression analyses. In addition, in a sensitivity analysis, patients with >20 episodes of hyperglycaemia and hypoglycaemia were excluded to eliminate the possibility that a few patients with a lot of episodes of hyperglycaemia altered the risk estimate. All analyses were performed using SPSS for Windows version 18.0.

RESULTS

Patient characteristics

Overall, 360 patients with diabetes mellitus type 1 or 2 with 5322 glucose measurements were included during the study period (*tables 1* and *2*). Of all patients, 47.8% were male, which did not differ significantly between the two groups. The group of patients included after the intervention was slightly younger, although not significantly different from the group before the intervention.

Complications

Overall, 45 patients experienced 95 hypoglycaemia episodes. There was no significant difference in the prevalence of hypoglycaemia between the two groups (*table 2*). In contrast, significantly less patients experienced hyperglycaemia after implementation of the intervention (22 patients with 65 hyperglycaemias) compared with before the intervention (70 patients with 417 hyperglycaemias). After adjustment for age and gender, there was a 76% (relative risk (RR) 0.24 (95% CI 0.19; 0.32)) risk reduction to develop hyperglycaemia (*table 3*). This risk was still significantly lower after excluding two patients with >20 hyperglycaemias in the group of patients before the intervention (RR 0.30 (95%CI 0.23; 0.40)). Both patients experienced 46 episodes of hyperglycaemias and hypoglycaemias in total. One patient was admitted due

Table 1. *Baseline characteristics*

	Before intervention (March – May 2010) n (%)	After intervention (September – October 2010) n (%)
Number of patients	249	111
Gender (male)	123 (49.4%)	49 (44.1%)
Age (mean (SD) / range in years)	74.8 (16.1)/ 24.8 – 99.6	68.9 (16.2)/ 25.0 – 93.9
Department:		
• Abdominal surgery (1)	78 (31.3%)	32 (28.8%)
• Abdominal surgery (2)	55 (22.1%)	28 (25.2%)
• Trauma surgery	52 (20.9%)	22 (19.8%)
• Vascular surgery	64 (25.7%)	29 (26.1%)

Table 2. *Number of episodes of hyperglycaemia and hypoglycaemia*

	Before intervention (March to May 2010) n (%)	After intervention (September to October 2010) n (%)
Number of patients	249	111
Number of glucose measurements	3373	1949
Number of hyper- and hypoglycaemias	472 (14.0%)*	105 (5.4%)*
- Hypoglycaemia(<4 mmol/l)	55 (1.6%)	40 (2.1%)
- Hyperglycaemia(>15 mmol/l)	417 (12.4%)*	65 (3.3%)*
Number of patients with hyper- and hypoglycaemias		
- Hypoglycaemia(< 4 mmol/l)	29 (11.6%)	16 (14.4%)
- Hyperglycaemia(>15 mmol/l)	70 (28.1%)*	22 (19.8%)*
Number of hyper- and hypoglycaemias within one patient		
- 1	45 (1.3%)	20 (1.0%)
- 2-4	25 (0.7%)	10 (0.5%)
- 5-9	13 (0.4%)	6 (0.3%)
- 10-19	14 (0.4%)	2 (0.1%)
- ≥20	2 (0.1%)	0
Glucose (median/ 25-75 interquartile range in mmol/l)	9.4/7.4-12.4*	8.5 / 7.0-10.5*
Glucose (in mmol/l) after intervention compared with before intervention	Reference	-1.3 (-1.5; -1.1)*

***P<0.0001.**

Table 3. *Risk of hyperglycaemia and hypoglycaemia*

	Before intervention	After intervention	Risk of hyper- and hypoglycaemias (95% CI)	Risk of hyper- and hypoglycaemias adjusted for age and gender (95% CI)
Number of glucose measurements	3373	1949		
- Normoglycaemia	2901	1844	Reference	Reference
- Hypoglycaemia	55	40	1.14 (0.76; 1.73)	1.24 (0.82; 1.88)
- Hyperglycaemia	417	65	0.25 (0.19; 0.32)*	0.24 (0.19; 0.32)*
Sensitivity analysis				
Number of glucose measurements	3080	1860		
- Normoglycaemia	2700	1755	Reference	Reference
- Hypoglycaemia	51	40	1.21 (0.79; 1.83)	1.36 (0.89; 2.07)
- Hyperglycaemia	329	65	0.30 (0.23; 0.40)*	0.30 (0.23; 0.40)*

Sensitivity analysis: exclusion of two patients with >20 hyper- and hypoglycaemias each; *P<0.0001; CI = confidence interval.

to a necrotectomy of the right hallux. The other patient was admitted for gastric perforation. This patient chose to refrain from further treatment due to his comorbidities.

DISCUSSION

In this retrospective study concerning inpatient care of diabetes mellitus, we found a significant greatly decreased risk of developing hyperglycaemia during admission without an increased risk of developing hypoglycaemia after implementation of a new protocol. This protocol included direct consultation of an internist and diabetes specialist nurse at admission, who initiated a daily treatment program. In addition, the attending nurse could adjust the insulin dose using an adjustment scheme based on point of care glucose measurements. The insulin intervention and dose adjustment was performed based on decentralised glucose measurement by a POCT device. Compared with our study, in Atlanta, USA, a higher prevalence of hyperglycaemia was present in 38% of patients at or during admission.[5] In our study approximately 28% of patients with hyperglycaemia before implementation of the intervention versus approximately 20% after implementation was found. This is probably due to the fact that other definitions of hyperglycaemia were used and possibly due to another patient population. In Atlanta, a fasting glucose >7 mmol/l or a non-fasting glucose >11.1 mmol/l were used to determine hyperglycaemia. The group of patients with new hyperglycaemia had a longer length of hospital stay and an 18-fold increased mortality rate compared with the normoglycaemia group.[5]

A number of studies have shown that inpatient care by a multidisciplinary team including a diabetes specialist nurse can reduce the length of stay in hospital without increased readmission rates.[6-9] Consultation of a diabetes team instead of an endocrinologist or an internist alone resulted in 35% and 56% decreased lengths of stay, respectively. Delayed consultation was associated with an increased length of stay.[7] A randomised study comparing consulting a diabetes team and usual care showed a reduction in length of stay from 7.5 to 5.5 days for patients who were admitted because of diabetes mellitus as a primary diagnosis. In patients with another reason for admission with diabetes mellitus as comorbidity, there was no reduction in length of stay; however, a decrease of readmissions in the following three months of 55% was shown.[6] Another randomised study with or without the intervention of a diabetes specialist nurse showed a reduced length of stay (11 versus 8 days). The majority of patients were admitted for reasons unrelated to their diabetes mellitus. Readmission rates were similar in both groups.[9]

The decrease in prevalence of hyperglycaemias in our study did not lead to an increase of hypoglycaemias. Hypoglycaemias have been associated with increased mortality.[10,11] However in a large study it was shown that hypoglycaemia was not an independent predictor for mortality, implying that it is only a marker of poor health.[12]

The strengths of this study are the fact that there is no selection bias, since we included all consecutive patients from the same departments before and after the intervention. We assume a random distribution of the reasons for admission in both groups. We selected a period directly after implementation of the protocol and compared it with a period before the summer in the same year. We excluded a small group of patients who were mistakenly not signed up by the diabetes specialist nurse and internist since this group did not receive the intervention. The relative risk is, however, still significant if we include these patients (p<0.0001, data not shown). In addition, there is no information bias, since all measurements during admission were included; they were retrieved from the computer-based healthcare information system.

Furthermore, one of the strengths of this study was the use of a POCT device, which fulfils the criteria needed for safe point of care testing of glucose. Most of the studies validating performance of POCT devices do not address all issues important for safe measurement of glucose or were not performed according to strict criteria on total error. POCT glucose devices were primarily not

designed to be used for the purpose of monitoring and adjustment of insulin dosing schedules as the quality of their performance was far behind the laboratory reference method. The POCT device used in this study was extensively validated according to strict international criteria. The device used showed a very good correlation with the reference method and with the newest generation of device. Moreover, the total error was acceptable for the measuring range acquired. This made it possible to obtain glucose measurements at the 'point of care' and makes the results of this study very valuable. This device showed that the results of the glucose measurements are not influenced by changes in partial oxygen pressure, although they are influenced by changes in haematocrit in a predictable fashion.[4] This implicates a limitation of this study because we did not correct the glucose measurements for haematocrit, since this is difficult to implement in daily practice. However, we assume this did not influence the outcome of this study since we expect random changes in haematocrit in the patients before and after implementation of the protocol.

Another limitation of the study is the retrospective design of the study. Ideally, a randomised study would have been performed. We assume, however, a random distribution of comorbidities and drug use. We adjusted for age and gender. Since information about the reason for admission, comorbidities, drug use and duration of admission was not available, we could not study the effect of these parameters as possible confounders. In addition, we could not study the effect of implementation of the intervention on the duration of admission and number of readmissions. Unfortunately, information about the time of glucose measurement was not available either. Therefore we could not examine the separate effect on fasting, pre- or postprandial glucose measurements.

In conclusion, after implementation of a new protocol to standardise inpatient care of diabetes mellitus by an internist and diabetes specialist nurse, we established a decreased risk of hyperglycaemia of 76% without an increased risk of developing hypoglycaemia. Implementation of this protocol required frequent glucose measurements which is possible due to point of care glucose measurements. However, the point of care measurement is only possible if the performance of the glucose device fulfils strict criteria on total error and other references. Before implementing adjustment of insulin dosage based on point-of-care measurements, the quality of glucose POCT devices should always be checked with the central hospital laboratory. In the future, we would like to study the influence on morbidity and mortality and the duration of admission and prevalence of readmissions.

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Netherlands
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CASE REPORT

Waldenström's macroglobulinaemia presenting with nephrotic syndrome

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KEYWORDS

Lymphoplasmacytic lymphoma, nephrotic syndrome, minimal change nephropathy, Waldenström's macroglobulinaemia

What was known about this topic?

Nephrotic syndrome in the presence of lymphoplasmacytic lymphoma with IgM monoclonal gammopathy (Waldenström's macroglobulinaemia) can have several causes of which amyloidosis is probably the most thought of. The occurrence of nephrotic syndrome caused by minimal change nephropathy occurring as a paraneoplastic manifestation of Waldenström's macroglobulinaemia is a rare phenomenon, with only two cases previously reported in the literature.

What does this add?

With this case we hope to raise awareness that minimal change nephropathy should be considered as a rare but possible cause of nephrotic syndrome in Waldenström's macroglobulinaemia. Curing Waldenström's macroglobulinaemia can result in complete remission of the minimal change nephropathy.

INTRODUCTION

Minimal change nephropathy (MCN) is a major cause of nephrotic syndrome in both children and adults. It is characterised by normal appearing glomeruli by light microscopy and the absence of immunoglobulin deposits by immunoflorescence microscopy. On electron microscopy there is diffuse fusion of the epithelial foot processes. MCN is suggested to be caused by a T-cell or B-cell dysfunction and/or a yet undefined circulating glomerular permeability factor. Most cases of MCN are idiopathic, but sometimes MCN seems to be associated with an underlying cause such as drugs, neoplasms, atopy, other glomerular diseases or infection.

Here we describe a case of a 55-year-old male who was diagnosed with a nephrotic syndrome caused by MCN, which seems to be associated with Waldenström's macroglobulinaemia. Treatment not only resulted in complete remission of the Waldenström's macroglobulinaemia but also in complete remission of the nephrotic syndrome.

CASE

A previously healthy 55-year-old male was referred to the cardiology outpatient clinic because of oedema of both legs as well as an irregular pulse. He was diagnosed with mitral valve insufficiency grade 4/4, caused by mitral valve prolapse (Barlow's syndrome) as well as atrial fibrillation for which cardioversion was performed. He showed signs of (mostly right-sided) cardiac failure. At the same time, a normocytic anaemia and hypoalbuminaemia were found for which the patient was referred to the department of internal medicine. Physical examination revealed a systolic murmur II/VI on the apex of the heart and pitting oedema of both legs up to the inguinal area. Further physical examination was unremarkable.

Laboratory testing showed an erythrocyte sedimentation rate of 132 mm/hour, haemoglobin (Hb) 7.5 mmol/l,

MCV 92 fl, reticulocytes 27 x 10^9/l, thrombocytes 175 x 10^9/l, leucocytes 5.6 x 10^9/l with normal differentiation, creatinine 55 µmol/l, urea 4.9 mmol/l, normal liver function tests, normal electrolytes, albumin 16.1 g/l, total serum protein 57.0 g/l, monoclonal protein (M-protein) IgM kappa 10 g/l, IgG 7.0 g/l, IgA 1.54 g/l, IgM 9.8 g/l, and β_2-microglobulin 3.7 mg/l. Immunophenotyping showed monoclonal B-cells IgM (IgD) type kappa: 0.03 x 10^9/l in the peripheral blood and 7% in bone marrow aspirate (probably underestimated because of blood admixture). Bone marrow biopsy showed a large part of the bone marrow cavities infiltrated by lymphoplasmacytoid cells.

Serology testing for autoimmune diseases showed absence of antinuclear factor and antineutrophilic cytoplasmatic antibodies, antiglomerular basement membrane antibodies of 0.6 kU/l, and normal C3 and C4 complement. Urine analysis revealed proteinuria of 5.6 g/24 hours and Bence Jones protein type kappa (<0.1 g/l) as well as M protein IgM type kappa (<0.1 g/l).

Renal biopsy was performed which showed nine normal glomeruli on light microscopy. Immunofluorescence showed only minor granular deposits of kappa light chains in the mesangium and electron microscopy showed diffuse flattening of the podocytes as well as an intact but thin glomerular basement membrane and no signs of amyloid deposition or lymphoma localisation.

A computed tomography scan of the chest and abdomen revealed no abnormalities, especially no pathologically enlarged lymph nodes or spleen.

The patient was therefore diagnosed with a nephrotic syndrome caused by MCN, as well as an indolent non-Hodgkin's lymphoma: lymphoplasmacytic lymphoma with bone marrow involvement and IgM monoclonal gammopathy, also known as Waldenström's macroglobulinaemia.

Treatment was instituted with prednisone 60 mg daily but the nephrotic syndrome showed only a partial response: the oedema diminished but did not disappear, the proteinuria dropped to 1.9 g/24 h, while the serum albumin stabilised at 20 g/l in six weeks time. During prednisone therapy, the patient developed a sensomotor polyneuropathy and his anaemia worsened (Hb 6.5 mmol/l), while the M protein rose to 25 g/l.

Because the response of the nephrotic syndrome was only partial and other Waldenström's macroglobulinaemia-associated symptoms developed at the same time (polyneuropathy, worsening anaemia and rising M protein), the treatment with prednisone was discontinued and a combination of rituximab (375 mg/m^2 = 750 mg iv) and chlorambucil (6 mg/m^2 = 12 mg orally on day 1-14) every four weeks was started. He received a total of six courses after which his blood count and albumin normalised and the M protein was absent. Both the proteinuria and the polyneuropathy had fully resolved. Bone marrow examination showed complete remission of the lymphoplasmacytic lymphoma.

At the present time, almost five years after completion of therapy, the patient is still in complete remission of both Waldenström's macroglobulinaemia and nephrotic syndrome. He has undergone successful mitral valve surgery and is now functioning normally.

DISCUSSION

Waldenström's macroglobulinaemia is defined as lymphoplasmacytic lymphoma with bone marrow involvement and an IgM monoclonal gammopathy of any concentration.[1] The overall incidence is 3.8 per million persons per year, with the incidence increasing with age. The need for treatment is determined by symptoms caused by the tumour mass or M protein.[2]

With renal disease in the presence of Waldenström's macroglobulinaemia, amyloidosis might be the first thing that comes to mind. In this case, however, we have a patient who presented with MCN as well as Waldenström's macroglobulinaemia. Kidney needle biopsy showed the characteristic signs of MCN with the absence of immune-complex deposits or localisation of the lymphoplasmacytic lymphoma itself.

In the Netherlands, primary MCN is the cause of nephrotic syndrome in adults in about 21% of cases.[3] Initial treatment consists of prednisone, with which only 45-60% of patients reach complete remission at six weeks, to 75-90% reaching complete remission at 16 weeks.[4] In patients refractory to prednisone therapy or with frequent relapses of MCN, other immunosuppressive agents such as cyclophosphamide, chlorambucil, cyclosporine A and rituximab are also used.[4,5]

In our patient, the time relationship between the occurrence of MCN and Waldenström's macroglobulinaemia is suggestive of MCN occurring as a paraneoplastic manifestation of Waldenström's macroglobulinaemia. Several glomerulopathies are known to occur as paraneoplastic manifestations of malignant disease.[6] For instance, the occurrence of MCN as a paraneoplastic manifestation of classical Hodgkin's lymphoma is well established.[7]

Glomerulopathies are also described as a paraneoplastic manifestation in non-Hodgkin's lymphoma with MCN occurring most frequently.[8-10] MCN associated with Waldenström's macroglobulinaemia, however, seems to be a rarely mentioned phenomenon with only two cases reported previously in literature.[11,12]

Little is known about steroid responsiveness of MCN occurring as a paraneoplastic phenomenon. In MCN

occurring prior to classical Hodgkin's lymphoma, remission is accomplished in about 62.5% of patients treated with steroids alone; the prognosis of MCN seems to be directly related to the prognosis of the classical Hodgkin's lymphoma, irrespective of the therapy regimen used to treat the Hodgkin's lymphoma and whether or not this regimen contained steroids.[7]

In our patient a partial response of the nephrotic syndrome was achieved in six weeks of prednisone treatment after which it was discontinued because the Waldenström's macroglobulinaemia required treatment. In our patient as well as in the two previously reported cases of MCN and Waldenström's macroglobulinaemia occurring simultaneously, treatment of Waldenström's macroglobulinaemia resulted in the disappearance of MCN. However, it must be noted that in all three cases the medication used to treat the Waldenström's macroglobulinaemia could also be effective in the treatment of (primary) MCN.[4,5]

Although the time relationship between Waldenström's macroglobulinaemia and MCN is suggestive of causality between these two conditions, definite proof will be furnished by reappearance of the nephritic syndrome at the relapse of the Waldenström's macroglobulinaemia. The pathogenesis of MCN as a paraneoplastic manifestation in haematological malignancies is largely unknown. In classical Hodgkin's lymphoma, there seems to be a molecular link between the two entities with induction of c-mip in both Hodgkin-Reed Sternberg cells and podocytes.[13]

We conclude that in case of nephrotic syndrome occurring in the setting of Waldenström's macroglobulinaemia, not only amyloidosis, but also MCN should be considered as a cause. Treating Waldenström's macroglobulinaemia can also cure nephrotic syndrome.

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Netherlands
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PHOTO QUIZ

A case of painless jaundice

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CASE REPORT

A 64-year-old geologist with Crohn's disease presented with fatigue, anorexia, nausea and painless jaundice. His serum carcinoembryonic antigen was 7.2 ng/ml (normal 0-3 ng/ml) and the calcium 19-9 was 965 U/ml (normal <55 U/ml). Ultrasound and computed tomography (CT) scan of the abdomen revealed diffusely dilated bile ducts, with a 3 cm filling defect distending the distal common bile duct (CBD) (*figure 1*). Endoscopic retrograde cholangiopancreatography (ERCP) showed a large filling defect in the distal CBD (*figure 2*). Biliary brushings and biopsies revealed large oval to polygonally shaped nuclei with very prominent nucleoli, surrounded by attenuated cytoplasm.

Figure 1. *Imaging studies including right upper quadrant ultrasound, CT of the abdomen and MRI of the abdomen showing a mass within the common bile duct*

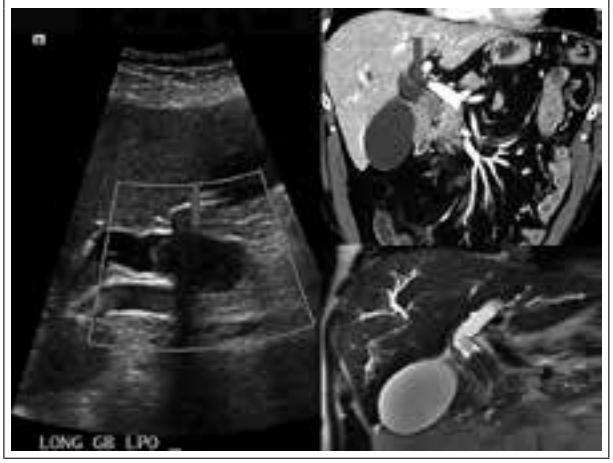
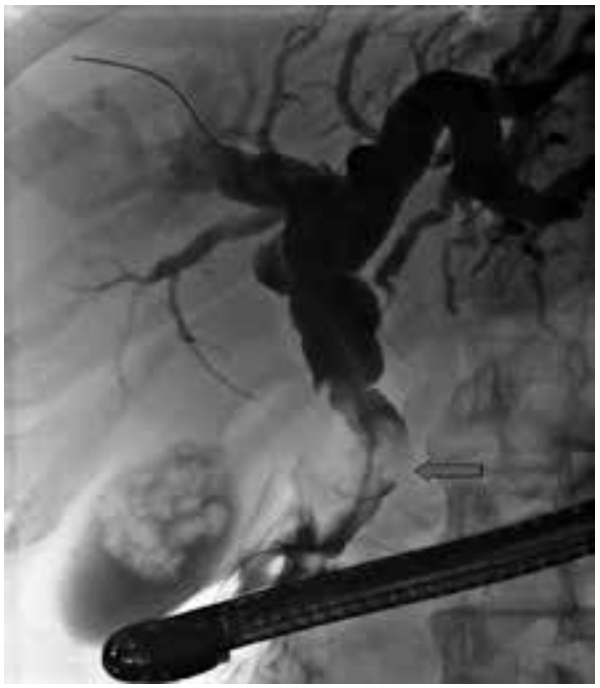


Figure 2. *Cholangiogram showing a filling defect within the common bile duct*



WHAT IS YOUR DIAGNOSIS?

See page 418 for the answer to this photo quiz.

Netherlands
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PHOTO QUIZ

Rapid widening of the mediastinum after coronary angiography

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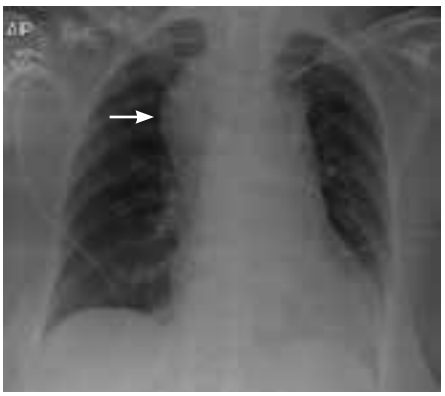
An 86-year-old woman was evaluated for increasing anginal symptoms for which antiplatelet drugs were started besides her coumarin derivative, which she took for paroxysmal atrial fibrillation. A coronary angiogram showed a left main pinpoint stenosis.

In the hour following her coronary angiogram, her neck circumference increased rapidly. We saw a pale woman with cold extremities in respiratory distress sitting upright with an inspiratory stridor for which she was intubated. The initial diagnosis of a contrast media reaction was clinically deemed unlikely but refuted more definitely on the chest X-ray (*figure 1*) taken after intubation.

Figure 1. *Widening of the mediastinum*



WHAT IS YOUR DIAGNOSIS?

See page 419 for the answer to this photo quiz.

Netherlands
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PHOTO QUIZ

A longstanding non-painful tumour of the back

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CASE REPORT

A 46-year-old Somalian man was referred to our outpatient clinic because of a longstanding non-painful tumour on his back. The patient reported gradual weight loss of 6 kg and night sweats. The patient was taking lamotrigine and valproic acid because of epilepsy.

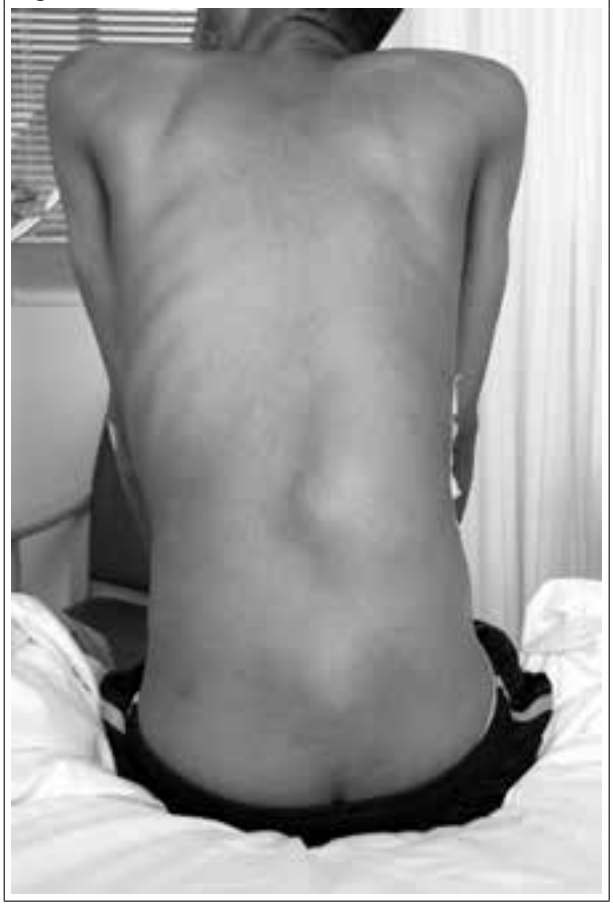
On physical examination the patient had a temperature of 36.4 °C. At the lower back he had two subcutaneous swellings, with a diameter of 10 cm and 5 cm (*figure 1*). There was no loss of neurological functions.

Laboratory findings showed an erythrocyte sedimentation rate of 51 mm/hour, a mild leucocytosis (11.1 x 10^9/l) and a C-reactive protein of 124 mg/l. HIV serology was negative.

WHAT IS YOUR DIAGNOSIS?

See page 420 for the answer to this photo quiz

Figure 1. *Subcutaneous masses on the lower back*



Netherlands
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PHOTO QUIZ

Floppy ears and tracheal wall narrowing

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CASE REPORT

A 64-year-old woman presented at our instution with a one-month history of polyarthritis, along with left hearing impairment, blurred vision, progressive dyspnoea, and dry cough. The symptoms had started 18 years previously, when she presented with a sudden episode of pain and swelling in both ears that spared the lobules. At that time, she was diagnosed with infectious perichondritis and treated with antibiotics, without response. She also developed recurrent episodes of intermittent polyarthritis, as well as repeated episodes of pain, swelling, and heat in the nose. Serological findings were negative. The patient also reported an episode of subglottic stenosis five years after the onset of symptoms.

On physical examination, a saddle nose deformity and floppy, deformed ears were noted.

Figure 1. *Floppy, deformed ears were noted*

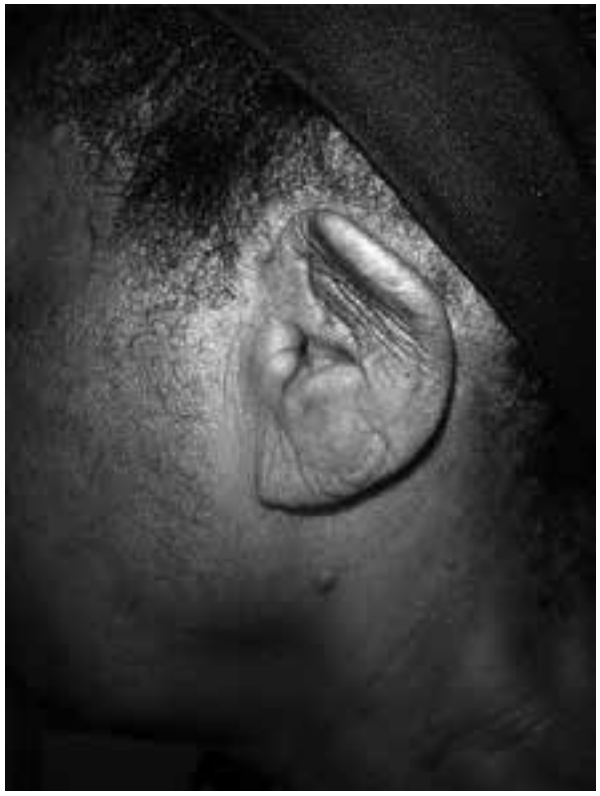
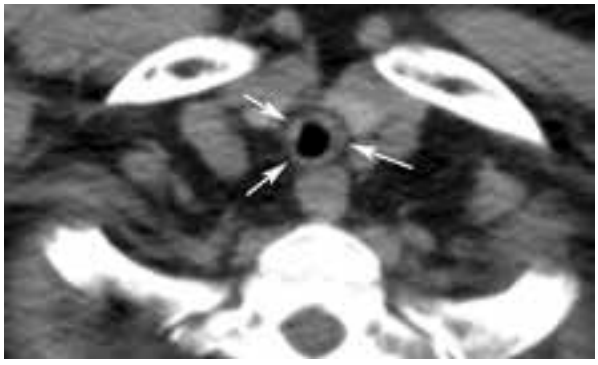


Figure 2. *Chest computed tomography revealed smooth tracheal and main bronchial wall thickening that spared the posterior membranous wall, with narrowing of its lumen. No significant parenchymal findings were observed*



WHAT IS YOUR DIAGNOSIS?

See page 421 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 414)

A CASE OF PAINLESS JAUNDICE

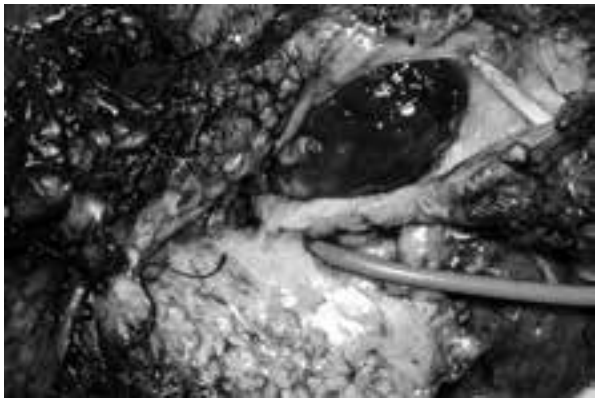
DIAGNOSIS

Primary malignant melanoma was diagnosed by cells staining positive for S100 and HMB45, and negative for pancytokeratin, hepatocyte-specific antigen, CK7, and CD68 (*figure 3*). BRAF mutation was detected with V600E mutation.

Further evaluation including magnetic resonance imaging of the abdomen did not detect melanoma distinct from the common bill duct (CBD) mass (*figure 1*). Dermatological and ophthalmological evaluation showed no evidence of cutaneous or ocular melanoma. MRI of the brain and abdomen, CT of the chest, abdomen and pelvis and CT PET scan showed no other lesions.

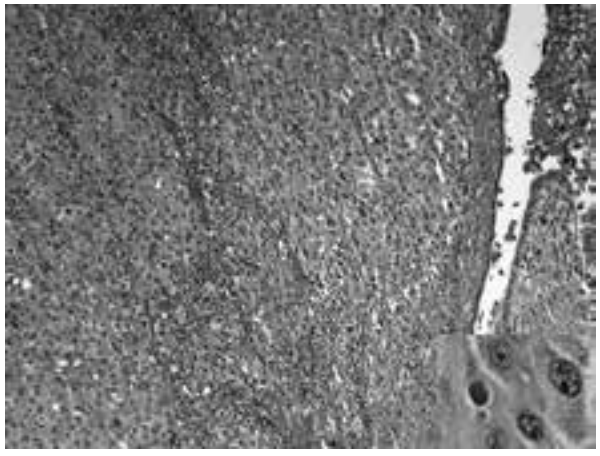
The patient underwent Whipple surgery in which the melanoma was seen to be confined to the common biliary duct (*figure 4*). The patient recovered uneventfully.

Figure 4. *Gross pathology of the melanoma within the common bile duct*



The majority of melanomas originate from the skin. However, a minority arise from other noncutaneous organs including uvea and the retina.[1] Whenever melanoma is encountered in the bile duct, it usually presents as secondary melanoma.[1,2] Nonetheless, ten cases of primary melanoma of the bile duct have been reported.[1-4] Primary melanoma of the bile duct originates from the melanocytes that exist in the biliary system. This was shown by Ricci *et al.* who reported a case of primary melanoma of the gallbladder. In this case, they found ultrastructural evidence of two distinct benign and malignant melanocyte populations.[4] The majority of the ten described cases of primary melanoma of the bile duct have been reported in males with an 8:2 male to female ratio. The ages of the patients range from 30 to 58 years with the majority in their fourth or fifth decades. Most of the cases present as a solitary polypoid lesion. Outcomes of primary melanoma of the bile ducts are variable. Half of reported cases of primary biliary melanoma underwent Whipple surgery. Three of these patients were disease-free after ten months, including one who was disease-free six years after Whipple surgery.[1-4]

Figure 3. *Haematoxylin and eosin showing melanoma, ductal epithelium and pigment*



Magnification is x 200 in main picture and x 600 in inset.

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ANSWER TO PHOTO QUIZ (PAGE 415)

RAPID WIDENING OF THE MEDIASTINUM AFTER CORONARY ANGIOGRAPHY

DIAGNOSIS

A computed tomography scan showed a blush in the right inferior thyroid artery (arrow *figure 2*) most probably caused by the guide wire (notably with a soft curled end) harming the vessel when entering from the right radial artery used for the coronary angiogram.

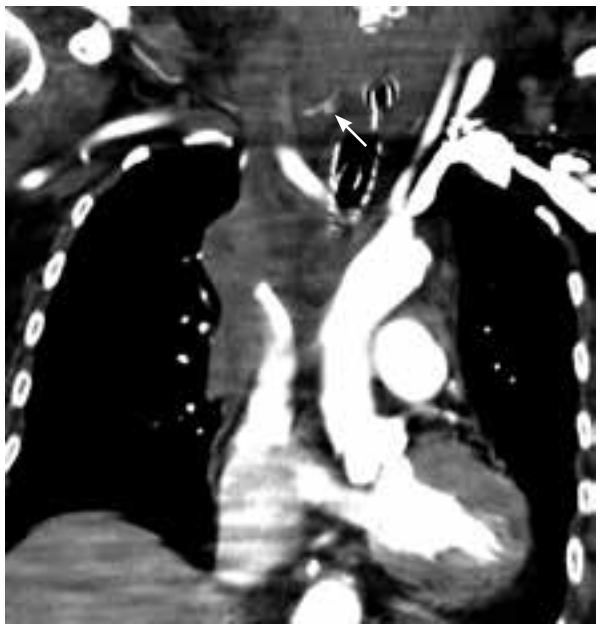
The patient's right inferior thyroid artery stems from the thyrocervical trunk, also known as the innominate artery, originating most cranially from the right subclavian artery as is most common. Yet, several different origins have been delineated.[1,2] Park *et al*. were the only ones to report this specific complication.[3] Furthermore, with five cases described in the literature, mediastinal haematoma as a result of radial cardiac catheterisation appears to be a rare complication.[3-5] This is the first case describing concomitant respiratory failure. In a cohort of 3369 cardiac catheterisations via the radial approach, Sanmartin *et al*. described several bleeding complications but none were located more proximally than the brachial artery.[6] Other vascular complications include bleeding anywhere from the access site (most frequently) to the branches of the aorta along the route of the vessel, pseudoaneurysm, arterovenous fistula and thromboembolism.[7-9]

The evolving haematoma in our patient led to compression of the upper airways and consequently caused respiratory distress requiring intubation. Four days later she was extubated with no respiratory sequelae.

Although, in the past, not everyone needing a diagnostic catheterisation was on anticoagulation, Fransson *et al*. demonstrated an increase in vascular complications when they were.[9] Nowadays, when using the radial artery, there is a trend towards adding platelet inhibitors to coumarin derivatives, which will have contributed to the haemorrhagic diathesis in our patient.

Anticoagulation was reversed and the idea of coiling the bleeding artery was considered but abandoned because of clinical improvement. A week later she underwent successful coronary stenting and her anticoagulation therapy was resumed uneventfully.

Figure 2. *Computed tomography of the chest with contrast shows an active bleeding focus in the right inferior thyroid artery*



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ANSWER TO PHOTO QUIZ (PAGE 416)

A LONGSTANDING NON-PAINFUL TUMOUR OF THE BACK

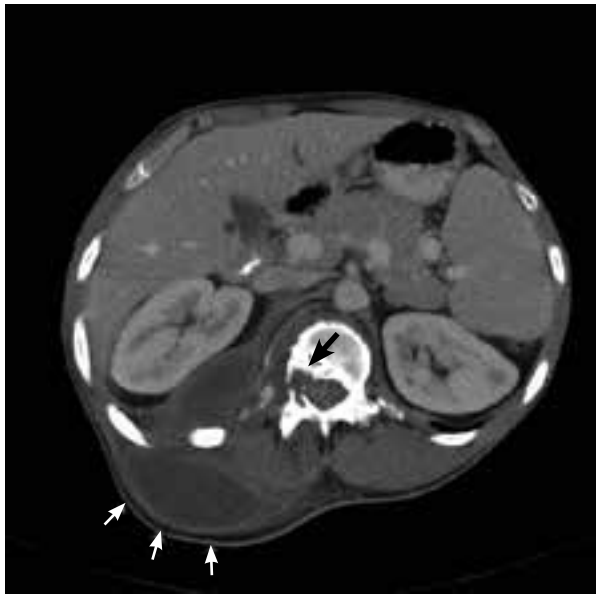
DIAGNOSIS

Skeletal tuberculosis (Pott's disease)

In this case of a Somalian man with a non-progressive disease history, the radiological findings were very suggestive for skeletal tuberculosis. Auramine of the aspirate was negative, but polymerase chain reaction for mycobacterium tuberculosis was positive, later confirmed by a positive culture. Antituberculous treatment was given for six months, after which he recovered fully.

In the Netherlands, around 1100 patients were diagnosed with tuberculosis in 2010. Of these cases, 43% were caused by extrapulmonary tuberculosis of which 11% involved bones and joints.[1] It is more common in children.[2]

Figure 2. *Axial CT image showing bony destruction of L1 (large arrow). Also abundant fluid collections in close contact to the spine as well as extending along the long spinal muscles (smaller arrows) creating a clear swelling*



When extrapulmonary tuberculosis manifests with vertebral osteomyelitis it is called Pott's disease. In contrast with pyogenic spondylitis, systemic symptoms are often absent.[2] In adults, the most frequently involved part of the spine is the lower thoracic spine, followed by the lumbar vertebrae.[2] Pathogenesis is related to reactivation of haematogenous and lymphogenous foci. Destruction of the intervertebral disc space and vertebral bodies causes the clinical symptoms.[3] Early symptoms are back pain and stiffness. Weight loss, fever and drenching night sweats are less frequent signs. Neurological symptoms may occur, such as muscle weakness of the legs or sensory loss. In many cases a delay in diagnosis is common. Paraspinal 'cold' abscesses around the affected area occur in 50% of the cases, which means that there is little inflammation.[2] Radiologically, Pott's disease presents with characteristic signs of spondylitis with or without abscess formation.

Definitive diagnosis is established by culture of *Mycobacterium tuberculosis* through aspiration or biopsy of the affected vertebra or abscess. The differential diagnosis includes spondylitis by other micro-organisms (pyogenic or fungal) and neoplasma. Treatment consists of antituberculous drugs for at least six months. Surgical involvement is necessary in refractory disease or unstable spine.[2,4]

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ANSWER TO PHOTO QUIZ (PAGE 417)

FLOPPY EARS AND TRACHEAL WALL NARROWING

DIAGNOSIS

The diagnosis of relapsing polychondritis was established on a clinical basis, according to the criteria of nasal, auricular, and laryngotracheal chondritis and seronegative inflammatory arthritis.[1] A biopsy was not necessary for the diagnosis.[2]

Relapsing polychondritis is characterised by recurrent, potentially severe, episodes of inflammation in cartilaginous tissues, including the elastic cartilage of the ears and nose, the hyaline cartilage of peripheral joints, the fibrocartilage at axial sites, and the cartilage in the tracheobronchial tree. It may also affect other proteoglycan-rich structures, such as the eye, heart, blood vessels, and inner ear.[3]

External ear pain is the most common symptom of relapsing polychondritis, and is usually an isolated presenting symptom. Almost invariably, this condition is misdiagnosed as infectious perichondritis of the ear; however, relapsing polychondritis typically spares the lobule, in contrast to infectious processes. With repeated attacks, the ear can become nodular and, in severe cases, floppy and deformed as the cartilaginous support is lost.[3] Nasal chondritis is painful, affects the distal part of the nasal septum, and through recurrent episodes leads to a saddle nose deformity.[2]

Arthritis is the second most common symptom of relapsing polychondritis. Intermittent, migratory, asymmetric, seronegative, and usually nonerosive poly- or oligoarthritis, lasting weeks to months, is the most frequently encountered pattern. Ocular symptoms occur in approximately 60% of patients.[3] Airway involvement is common and sometimes severe in patients with relapsing polychondritis. Airway symptoms include progressive dyspnoea, cough, stridor, hoarseness, and chest discomfort,[4] which can be explained by destruction and fibrosis of the laryngeal and tracheal cartilaginous rings, creating luminal collapse, and also by airway narrowing due to inflammation and cicatricial fibrosis.[3,4] Cardiovascular involvement is the second most frequent cause of death in these patients.[1] Clinicians should be aware of the existence of relapsing polychondritis, as the disease can be managed with medication to reduce the frequency, duration, and severity of flare-ups.

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SPECIAL ARTICLE

Life-threatening complications of ibogaine: three case reports

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ABSTRACT

Ibogaine is a naturally occurring psychoactive alkaloid extracted from the roots of the *Tabernanthe iboga* plant, which in alternative medicine is used to treat drug dependency. However, this upcoming, online advocated therapy can be dangerous due to its potentially lethal adverse effects. We present three cases in which toxic side effects were noted. We used the Naranjo scale to estimate the probability of a causal relationship between these effects and ibogaine. Findings in these three cases are suggestive of a causal relationship between the use of ibogaine and serious respiratory and cardiac problems (including lengthening of the QT interval). In our opinion it is of great importance that clinicians are aware of these potentially serious side effects and realise that widespread online marketing practices will give many more people access to ibogaine.

KEYWORDS

Iboga, ibogaine, long QT interval, tachyarrhythmia

What was known about this topic?
Ibogaine is a naturally occurring psychoactive alkaloid which in the last decades drew the attention of the Western world for its potential to inhibit withdrawal symptoms associated with weaning from drugs.[1,2] Even though small open-label studies investigating the effects of ibogaine have been promising,[2,3] controlled clinical trials were never carried out because research showed possible serious side effects and one fatality in Alper's open-label study.[3,4]

What do these cases add?
The non-medical use of ibogaine in the treatment of opioid addiction is increasing; it is important for health professionals to be aware of the risks and take a clear stand on this practice. The limited available evidence will be discussed here and complemented with new cases of probable ibogaine intoxications.

INTRODUCTION

Ibogaine is a naturally occurring psychoactive alkaloid which is extracted from the roots of the *Tabernanthe iboga* plant. The plant is mainly found in West and Central Africa and has long been used in rituals and to fight fatigue, hunger and thirst. In the last decades it drew the attention of the Western world for its potential to inhibit withdrawal symptoms associated with weaning from drugs.[1,2]
Anecdotal evidence and results of small open-label studies investigating the effects of ibogaine have been promising.[2,3] However, controlled clinical trials were never carried out because both laboratory studies pointed to the risk of serious side effects and one fatality in Alper's open-label study.[3,4] Nevertheless, the non-medical use of ibogaine in the treatment of opioid addiction is increasing; it is important for health professionals to be aware of the risks and take a clear stand on this practice.
The limited available evidence will be discussed here and complemented with new cases of probable ibogaine intoxications. One case was previously published by Hoelen *et al.* in 2009.[5] We applied the Naranjo scale to estimate the probability of a causal relationship.[6]

CASE 1

A 49-year-old Scottish male with a history of heroin addiction presented to the emergency department with collapse. He had received his first anti-addictive dose of ibogaine one or two days earlier. History taking was complicated due to intermittent unresponsiveness, but the patient stated he had no specific complaints. Apart from hypothyroidism and asthmatic symptoms, his medical history was unremarkable.

Electrocardiography (ECG) of this patient showed intermittent ventricular tachyarrhythmias, known as torsade de pointes, with underlying sinus rhythm and a QT interval of >700 ms. Laboratory testing showed mild hypophosphataemia (0.76 mmol/l [reference 0.8-1.50]), and mild hypokalaemia (3.5 mmol/l [reference 3.5-5.0]) but no other deviations (calcium 2.23 mmol/l [reference 2.1-2.6]). A computed tomography (CT) scan of the brain showed no abnormalities. Urine screening showed traces of opioids.

The patient was admitted to the intensive care unit (ICU), where he was defibrillated twice for tachyarrhythmias. Over the following days he recovered quickly; his QT interval, however, remained prolonged during the entire stay in our hospital. He was discharged after being free of ventricular tachyarrhythmias for ten days, with a QT interval of 475 ms.

CASE 2

A 31-year-old American woman with a history of persistent alcohol addiction presented to the emergency room with a seizure-like attack after taking a first dose of 3.5 g of ibogaine.[5] She had started on ibogaine as an alternative therapy for her treatment-resistant alcohol dependence. Apart from this single dose of 3.5 g ibogaine 15% (usual dose, 2 to 6 g) she had not taken any other medication or drugs, and her family history was unremarkable. She only complained of nausea.

ECG revealed a strikingly prolonged QT interval (corrected 616 ms) and torsade de pointes. Laboratory testing showed mild electrolyte deviations (magnesium 0.49 mmol/l [reference 0.65-1.05], potassium 3.2 mmol/l [reference 3.5-5.0]), which were rapidly corrected, however without any effect on the QT interval.

The patient was admitted to the ICU, where after 42 hours of monitoring her QT interval normalised. During her stay in the ICU no new seizures occurred and no further intervention was needed. She was discharged home in good condition.

CASE 3

A 43-year-old Italian woman, also being treated with ibogaine for heroin and benzodiazepines addiction, was admitted to the emergency room in an unresponsive state, which had lasted longer since she had been found earlier that morning. She had vomited and possibly had shown some contractions around her mouth.

Physical examination showed a non-responsive, tachypnoeic and subfebrile woman, who moved her arms and legs symmetrically. Blood testing showed only leukocytosis (12.7 [reference 4.0-10.0]), slightly elevated erythrocyte sedimentation rate of 38 mm/h [reference 2-12] and mild hypokalaemia (3.1 mmol/l). A CT scan of the brain, chest X-ray and ECG (QTc around 480 ms) were performed but showed no abnormalities. Because urine screening tested positive for opioids, she was injected with flumazenil (Anexate) and naloxone upon which she developed mild withdrawal symptoms, but no improvement in consciousness. Electroencephalography showed encephalopathy of unknown origin, possibly related to earlier hypoxia. No epileptic activity was seen.

She was admitted to the ICU, where she was intubated on day 2, due to respiratory insufficiency. She remained unstable for several days, but was extubated after 24 hours. No cardiac arrhythmias were observed, but she did develop urine retention and aspiration pneumonia during the ICU stay. Later, blood samples showed potentially lethal ibogaine levels (0.37 mg/ml).[7] The patient was discharged home in good condition after seven days.

DISCUSSION

The three cases presented here concerned patients who used ibogaine shortly before experiencing cardiac or respiratory instability. Unexplained death or adverse events after the use of ibogaine have been described before incidentally; they have rarely been related with ibogaine blood levels.[8]

First, we would like to discuss the likelihood of whether the adverse reactions in these cases were actually due to ibogaine rather than the result of other factors. We applied the Naranjo scale to assess the probability of a causal relationship. Probability is assigned via a score termed definite, probable, possible or doubtful (*table 1*).[6] In cases 1 and 3 traces of opioids were found in serum and/or urine. The patient in case 1 claimed not to have used drugs recently before presentation, but in case 3 the patient was on a methadone regime. Thus, in case 3, a combination of ibogaine and opioid intoxication could be considered, especially in view of the dominant respiratory problems. In this context, it is of interest that ibogaine is thought to potentiate opioids and their toxic effects.[8] Applying the score to patient 1 and 3, they score 6 to 8 points, thus a causal relationship is probable. In case 2 there appeared to be no other explanation for the symptoms, adding up to 8 points and a probable causal relationship. Electrolyte deviations in all three patients were too minimal to expect to produce the symptoms as presented here.

Table 1. *Scale of Naranjo. Causal relationship is certain (≥9), probable (5-8), possible (1-4), doubtful (0)*[6]

	Yes	No	Unknown
Previous reports of adverse event of X	1	0	0
Adverse event after intake of X	2	-1	0
Improvement after discontinuation of X	1	0	0
Relapse of symptoms after readministration of X	2	-1	0
Alternative causes of adverse event	-1	2	0
Adverse event occurs when placebo is administered	-1	1	0
Toxic concentration in body fluid detected	1	0	0
Severity of symptoms is dose related	1	0	0
Patient had similar adverse event before when using X	1	0	0
Adverse event is confirmed by objective evidence	1	0	0

Apart from the presented cases, there is only one previous report in the literature describing ventricular tachyarrhythmia and long QT interval in relation to recent ibogaine ingestion.[9] The similarity of this case report with cases 1 and 2 described here is striking and contributes to our view that ibogaine can cause serious cardiac problems. In conclusion, findings in these three cases are suggestive of a causal relationship between the use of ibogaine and serious respiratory and cardiac problems (including prolonged QT interval). One other publication supports these findings.[9] Considering more and more people have access to ibogaine through widespread online availability, we would suggest professionals to get in line and explicitly issue a clear negative advice against the use of ibogaine. Furthermore, in our opinion ibogaine should be added to the list of drugs causing long QT interval.

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SPECIAL ARTICLE

Approach to hypophosphataemia in intensive care units – a nationwide survey

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ABSTRACT

Background: Evidence-based guidelines for monitoring of serum phosphate levels and for the treatment of hypophosphataemia in critically ill patients are lacking. The aim of this survey was to evaluate current practice with respect to diagnosis and treatment of hypophosphataemia in critically ill patients among intensive care unit (ICU) physicians in the Netherlands.

Methods: A survey was conducted among all hospitals with an ICU in the Netherlands. Paediatric ICUs were excluded from participation. A questionnaire was sent, with questions on practice regarding serum phosphate monitoring and treatment of hypophosphataemia. Respondents returned the questionnaire either by mail or through a web-based survey.

Results: A response was received from 67/89 ICUs (75%). Respondents mentioned renal replacement therapy, sepsis and malnutrition, as well as surgery involving cardiopulmonary bypass as the most important causes of hypophosphataemia in intensive care unit patients. Of all respondents, 46% reported to measure serum phosphate levels on a daily basis, whereas in 12% serum phosphate levels were measured only on clinical indication. Less than half of the respondents had some sort of guideline for correction of hypophosphataemia. In a vast majority (79%), correction of hypophosphataemia was reported to start with serum phosphate levels <0.60 mmol/l. Intravenous administration of phosphate was the preferred method of correction, with widely variable dosages and speeds of infusion. Complications of intravenous phosphate were reported to occur infrequently.

Conclusion: There is large variability in the way serum phosphate is monitored and hypophosphataemia is treated in critically ill patients in the Netherlands.

KEYWORDS

Hypophosphataemia, intensive care, monitoring, treatment, survey

INTRODUCTION

Intensive care unit (ICU) patients are at increased risk for developing hypophosphataemia due to the presence of multiple causal factors including – but not restricted to – volume expansion, diuretics, metabolic acidosis, respiratory alkalosis and the refeeding syndrome.[1] Reported incidences of hypophosphataemia, most frequently defined as a serum phosphate level <0.80 mmol/l, vary widely,[2,3] with highest incidences in patients with sepsis[4] and after hepatic[5] or cardiothoracic surgery.[6]

Hypophosphataemia may have serious consequences, such as respiratory failure and myocardial dysfunction. However, it is not known whether correction of hypophosphataemia affects the outcome of critically ill patients.[1] Notably, correction of hypophosphataemia by means of intravenous administration of phosphate concentrates may cause abnormalities of other electrolytes, Hyperkalaemia is of particular concern when sodium-potassium-phosphate solutions are used for correction, especially when administered at high speeds. Currently, no evidence-based guidelines exist for the monitoring of serum phosphate levels and treatment of hypophosphataemia in ICU patients. Consequently, suggested treatment regimens described in the literature are inconsistent.[7-12]

We hypothesised that the approach to hypophosphataemia in ICUs in the Netherlands would vary widely. The aim of this study was to evaluate current practice of monitoring

serum phosphate levels and treatment of hypophosphataemia in critically ill patients in the Netherlands. For this purpose, we sent a questionnaire to ICUs in the Netherlands.

METHODS

Design

We conducted a survey using a postal questionnaire among all hospitals with an ICU in the Netherlands. Paediatric ICUs were excluded from participation. The items in the questionnaire were selected on the basis of the current literature and professional experience. We chose to use only closed-ended questions as these enable comparison across respondents, require less time to complete than open-ended questions and are easy to code and process.[13] Approval by the Institutional Review Board was not deemed necessary since participation involved neither patients and experimental subjects nor patient data. Respondents were assured that confidentiality of individual and institutional response was protected. Completion and return of the questionnaire was considered equivalent to consent to participate in the study.

Questionnaire

The questionnaire consisted of 29 questions regarding causes of hypophosphataemia, frequency of serum phosphate measurements, triggers for correction and methods for correction of hypophosphataemia (the complete questionnaire is available online at http://home.kpn.nl/weae19qy/questionnaire.htm). To ensure clarity and consistency members of our local research group assessed the questionnaire for face and content validity before the final version was compounded and sent.

In November 2011, the questionnaire was sent by mail to all 89 ICUs. Respondents could either return the questionnaire by mail or complete the web-based version of the survey. Six weeks after sending the questionnaire, a reminder letter was sent. In addition, ICUs that did not respond were contacted by telephone one month after sending the reminder letter. Two months thereafter, the results were analysed.

For the purpose of the questionnaire, hypophosphataemia was defined as moderate or severe when the serum phosphate level was 0.32-0.65 mmol/l (1.0-2.0 mg/dl) or <0.32 mmol/l (<1.0 mg/dl), respectively, consistent with definitions in the international literature. The trigger for treatment of hypophosphataemia was expressed in decimals of the serum phosphate concentration in mmol/l (e.g. 0.30, 0.40 and 0.50 mmol/l), the preferred SI units used to report serum phosphate in hospitals in the Netherlands.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 19 (Chicago, IL, USA). Descriptive statistics of categorical variables are reported as total numbers and percentages. Continuous variables are reported as median and interquartile range. Pearson's chi-squared tests were used to evaluate the relationship between different categorical variables.

RESULTS

Survey response

Of the 89 questionnaires sent, 67 were returned (75%); 51 questionnaires by mail, 16 through the online survey; 39 (58%) respondents were internist-intensivists, and 22 (33%) were anaesthesiologist-intensivists. Characteristics of the responding ICUs are shown in *table 1*.

Table 1. *ICU characteristics*

		No. ICUs	
Type of hospital	Academic	8	(12%)
	Non-academic teaching hospital	37	(55%)
	Non-teaching hospital	21	(31%)
	Other	1	(2%)
Hospital size (beds)	<200	3	(4%)
	200-500	36	(54%)
	500-800	20	(30%)
	>800	8	(12%)
ICU size (ventilation beds)	<5	9	(13%)
	5-9	24	(36%)
	10-14	16	(24%)
	15-19	4	(6%)
	>20	14	(21%)
Number of ICU admissions per year	<500	10	(15%)
	500-1000	29	(45%)
	1000-1500	10	(15%)
	1500-2000	9	(14%)
	>2000	6	(11%)
Patient categories	Medical	67	(100%)
	General surgery	67	(100%)
	Major surgery*	45	(67%)
	Cardiothoracic surgery	10	(15%)
	Neurosurgery	15	(22%)
Number of full-time intensivists (median, IQR)		4.8	(3-7)
Number of full-time ICU nurses (median, IQR)		40	(26-75)

***Major surgery: major trauma, vascular, gastrointestinal and orthopaedic surgery; IQR = interquartile range.**

Causes of hypophosphataemia

Responses from academic, non-academic teaching and non-teaching hospitals were consistent regarding causes of hypophosphataemia. Particularly, renal replacement therapy was considered a risk factor for the development of hypophosphataemia (84%), as well as sepsis (84%) and malnutrition (79%). Respondents from ICUs where cardiac surgery patients were admitted, more frequently considered cardiopulmonary bypass during surgery to be an important cause of hypophosphataemia than respondents from other ICUs. Other assumed important causes of hypophosphataemia are displayed in *table 2*.

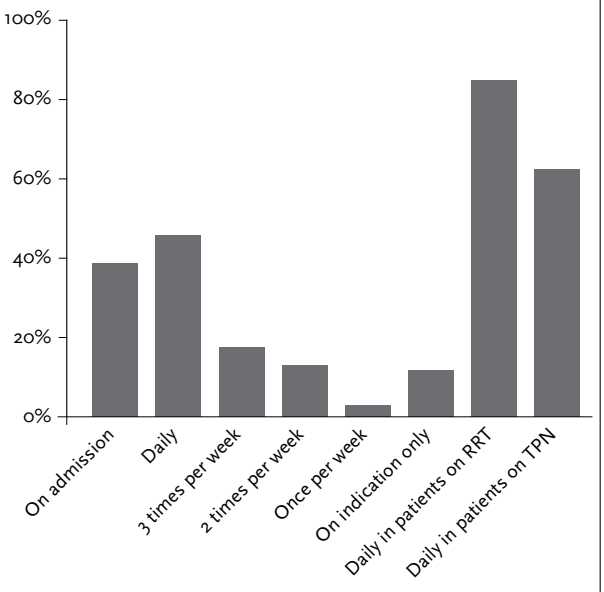
Monitoring of serum phosphate levels

Responses from academic, non-academic teaching and non-teaching hospitals were consistent, but varied more widely regarding policies of monitoring of serum phosphate levels. Of all respondents, 46% reported to measure serum phosphate levels every day in every patient; 12% measured serum phosphate levels only on indication (*figure 1*). Serum phosphate levels were routinely measured on admission to the ICU by 39% of the respondents. In patients receiving renal replacement therapy, serum phosphate levels were reported to be measured every day by 85%.

Incidence of hypophosphataemia

The estimated incidences of moderate and severe hypophosphataemia in the ICU are shown in *table 3*. Respondents from ICUs measuring serum phosphate levels every day in all patients estimated the frequency of moderate hypophosphataemia higher than those who performed phosphate measurements less frequently (median estimated incidence of moderate hypophosphataemia 25-40% versus 15-25%, p<0.05). The estimated incidence of severe hypophosphataemia was not different between respondents from ICUs where serum phosphate levels are measured every day in all patients and ICUs that perform phosphate measurements only on indication.

Figure 1. *Monitoring frequency of serum phosphate levels*



RRT = renal replacement therapy; TPN = total parenteral nutrition.

Table 3. *Estimated incidences of hypophosphataemia*

	Estimated incidence	% of respondents
Moderate hypophosphataemia (serum phosphate <0.65 mmol/l)	5-15%	27
	15-25%	33
	25-40%	25
	>40%	15
Severe hypophosphataemia (serum phosphate <0.32 mmol/l)	0-5%	71
	5–15%	19
	15–25%	7
	25–40%	3

Table 2. *Causes of hypophosphataemia*

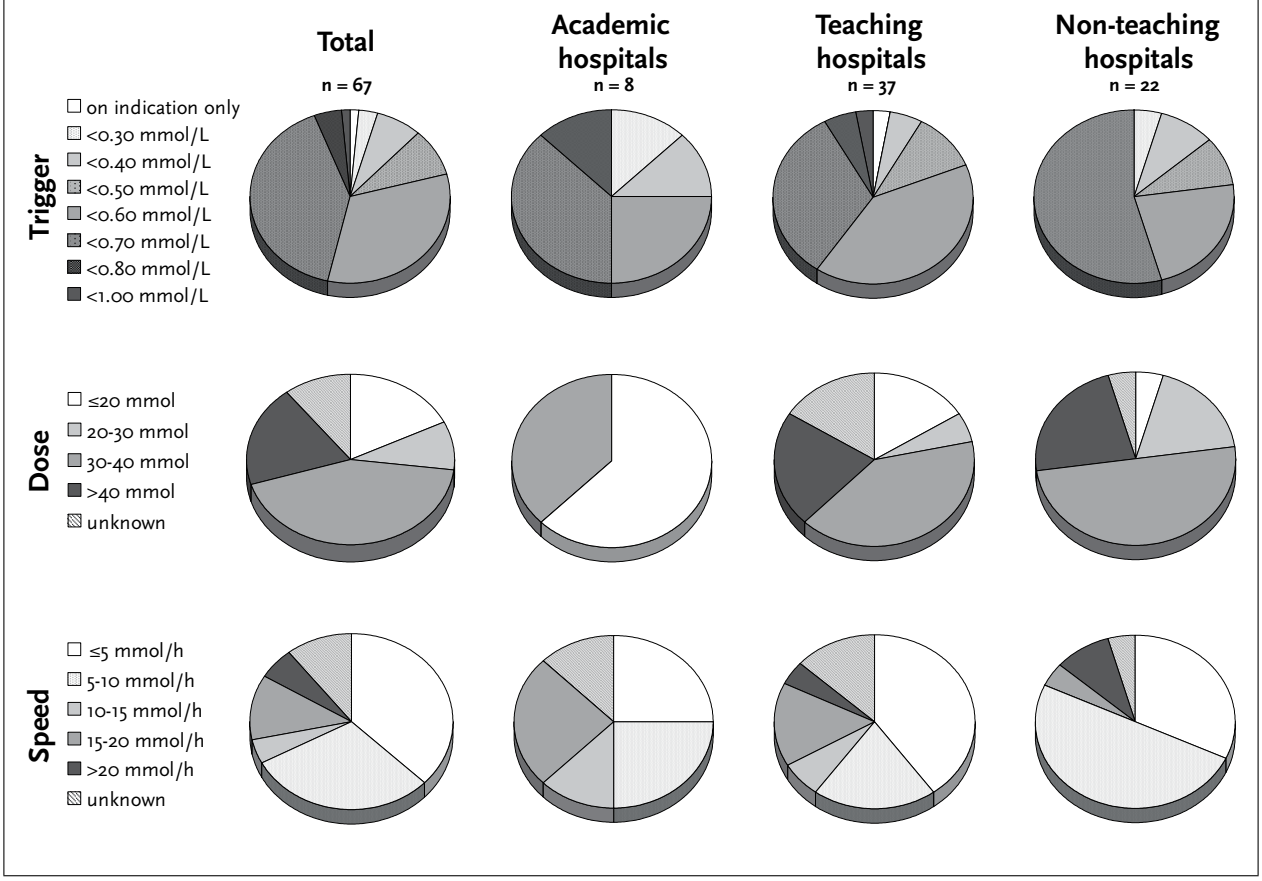
Risk factor	% of respondents
Renal replacement therapy	84
Sepsis	84
Malnutrition	79
Acid-base disorders	67
Diabetic ketoacidosis	61
Diuretic therapy	60
Major surgery	60
Diarrhoea	54
Increased risk for all ICU patients	46
Volume therapy	43
Inotropic/vasopressor therapy	21
Cardiopulmonary bypass	21
Mechanical ventilation	12

For every risk factor, the percentage of respondents considering this to be a risk factor for development of hypophosphataemia is reported.

Correction of hypophosphataemia

Fifty-three percent of respondents reported correction of hypophosphataemia to be guided by a local guideline. The trigger for correction of hypophosphataemia varied widely between responding ICUs, but was independent of the type of responding ICU. In the majority of ICUs (79%), intravenous infusion of phosphate is started if the serum phosphate is <0.60 mmol/l; in 13% hypophosphataemia is corrected only if the serum phosphate is <0.30 mmol (*figure 2*).

Figure 2. *Trigger, dose and speed of phosphate suppletion in different hospital categories*



Of all respondents, 87% reported phosphate to be exclusively administered intravenously. For intravenous correction, 69% reported using sodium-potassium-phosphate concentrates, 22% sodium-phosphate concentrates; 5% mentioned using both solutions; 5% mentioned using glycerophosphate. The maximum dose and rate of phosphate infusion varied widely, with phosphate dosages ranging from ≤20 mmol up to >40 mmol and speed of infusion ranging from ≤5 mmol up to >20 mmol per hour (*figure 2*); 43% reported using a dose of 30-40 mmol phosphate, and 37% reported using a speed of infusion of phosphate ≤5 mmol per hour. Differences between types of hospitals are shown in *figure 2*.

Complications of intravenous correction of hypophosphataemia

Of all respondents, 66% reported that complications of intravenous phosphate administration never occurred. Reporting of complications is neither dependent on the type of hospital nor on the reported phosphate infusion rates.

DISCUSSION

Dutch intensivists consider hypophosphataemia to be common in ICU patients. Consequently, serum phosphate levels are monitored frequently in those patients. To our knowledge, this is the first survey to investigate the approach to monitoring of serum phosphate levels and treatment of hypophosphataemia in critically ill patients. The results of this survey indicate that this approach varies considerably between hospitals.

In general, critically ill patients have multiple reasons for developing hypophosphataemia. Indeed, sepsis, trauma, major surgery, fluid therapy, acid-base disorders, refeeding and treatment with catecholamines or diuretics are all risk factors for the development of hypophosphataemia.[1] In addition, hypophosphataemia is considered to be not without consequences. Hypophosphataemia has been associated with respiratory muscle and myocardial dysfunction, cardiac arrhythmia, neuromuscular symptoms and leucocyte dysfunction,[1,14] and may therefore cause additional morbidity and maybe even mortality. Intravenous infusion of phosphate may not be without risk,

as it may induce hypocalcaemia, hyperphosphataemia and hyperkalaemia, depending on which type of solutions are used.[1,15] It would be appropriate to have a guideline for the frequency of monitoring of serum phosphate levels and for the correction of hypophosphataemia in the ICU setting, which may even be different for distinctive patient groups in the ICU.

Evidence from randomised controlled trials regarding correction of hypophosphataemia is lacking. Several case reports, though, show improvement of myocardial function after correction of severe hypophosphataemia.[16-18] Two small prospective studies report improvement of myocardial performance after correction of hypophosphataemia in patients with sepsis[8] and after cardiac surgery.[3]

The reported frequency of measuring serum phosphate levels and policies regarding correction of hypophosphataemia varied widely in this survey. Despite the high incidence of hypophosphataemia and its possible detrimental effects in critically ill patients, there seems to be no consensus on how frequently serum phosphate levels should be measured. The estimated incidence of hypophosphataemia by the respondents of this survey, however, is largely consistent with reported incidences in the literature.[1] Maybe not too surprisingly, the survey showed an association between awareness of a high incidence of hypophosphataemia and the frequency of monitoring of serum phosphate levels. Frequency of measurement ranged from daily measurements to those who measure phosphate on indication only. The literature lacks advice regarding the frequency with which serum phosphate should be measured in ICU patients. Because risk factors for hypophosphataemia are frequently present in almost all ICU patients, and also because hypophosphataemia may have therapeutic consequences, we consider it appropriate to measure serum phosphate levels frequently and routinely. It is unclear, though, how frequently serum phosphate levels are to be measured. Routine daily measurement of serum phosphate may be unnecessarily frequent, except for those at high risk for hypophosphataemia.

Patients with malnutrition in whom feeding is initiated represent one of those high-risk groups. The refeeding syndrome is almost universally associated with hypophosphataemia.[19] Patients who are at risk for this syndrome should be monitored frequently and should promptly receive phosphate-enriched feeding. Malnutrition was considered an important risk factor for the development of hypophosphataemia by the respondents. Early feeding in critically ill patients, either enterally through a nasogastric tube or parenterally, is common practice. Although the type of feeding administered to a patient may potentially influence the risk for the development of hypophosphataemia, this issue was not addressed in the current survey.

Our survey showed a large variability with regard to the trigger for correction of hypophosphataemia. Only half of the respondents reported to have a guideline for correction of hypophosphataemia. The literature advises to correct hypophosphataemia when it is symptomatic and/or when serum phosphate levels fall <0.32.[1,20-22] Whether also moderate hypophosphataemia should be corrected in critically ill patients is less clear, but correction is advised in patients on mechanical ventilation.[14] Improved myocardial function has been reported after treating patients with phosphate levels between 0.30 and 0.40 mmol/l.[3,8] It seems reasonable to correct hypophosphataemia in patients with serum phosphate <0.40 mmol/l. Over 95% of the respondents to our survey reported to use at least this trigger. More research is needed, however, to investigate whether correction of hypophosphataemia improves outcome.

Dose and speed of phosphate administration varies greatly among Dutch ICUs, which may not be too surprising as different treatment protocols are suggested in the literature. Advised doses of phosphate boluses in the literature range from ≤15 mmol[7,9] to 40 mmol.[10,23] Two recent studies report on body weight-dependant phosphate doses ranging from 0.4 to 1.0 mmol of phosphate per kilogram[11,12] with acceptable safety and efficacy. The most commonly reported dose of phosphate administration in our study was 30 to 40 mmol. Notably, the reported speed of phosphate administration was ≤5 mmol per hour in most respondents. This is lower than the administration speeds reported in the literature ranging from 5-7.5[7,9,12] to 20 mmol per hour,[8,23] with acceptable safety. Given the currently available evidence, administering a phosphate dose of 40 mmol or 0.5 mmol per kilogram seems a practical approach for patients with hypophosphataemia. Although there is no evidence for the superiority of a certain speed, it seems acceptable and more practical to use an administration rate between 10 and 20 mmol per hour. While intravenous administration of hypophosphataemia may lead to hyperphosphataemia, hyperkalaemia and hypocalcaemia, depending on the type of electrolyte concentrate used, respondents in this survey reported these to occur very seldom. The exact incidence of these complications is not reported in the literature. Frequent measurement of electrolytes and avoidance of potassium-containing formulas in the presence of hyperkalaemia is advised to prevent complications, in particular life-threatening arrhythmias.

There are some limitations to this survey. First, the questionnaire was only sent to ICUs in the Netherlands. Practice in the Netherlands may differ from that in other countries and the generalisability of the results

may be poor. In addition, only one physician filled in the questionnaire for each responding ICU. The answers given may not represent the opinion of the entire ICU staff. Although there was a good response rate, selection bias may have been introduced because the responding ICUs may be those where hypophosphataemia receives more attention than in the other ICUs. Finally, both web-based and surveys returned by regular mail were analysed as one group. The two questionnaires were exactly identical, but we cannot rule out a difference in way of responding to the questions in these different formats. However, results between both groups did not differ when analysed separately.

In conclusion, there is a large variability in the monitoring of serum phosphate and treatment of hypophosphataemia in critically ill patients in ICUs in the Netherlands. Pending studies necessary for evidence-based guidelines, we propose to monitor serum phosphate levels frequently in all critically ill patients, and to correct hypophosphataemia when serum phosphate levels are <0.40 mmol/l, administering a dose of 40 mmol in two to four hours.

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LETTER TO THE EDITOR

Hypertension and use of an intrauterine levonorgestrel-releasing device

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Dear Editor,

Hypertension is a common disorder in the general population. In 95% no underlying cause can be identified. In woman use of contraceptive drugs should be considered. Less known is that even a levonorgestrel-containing intrauterine device (LNG-IUD) can cause high blood pressure (BP). However, since 2007 this side effect is no longer mentioned in the official drug information in the Netherlands.

In our clinic we saw a 46-year-old woman with an increase in BP after insertion of an LNG-IUD. She had used oral contraception (Stediril '30' containing thinylestradiol 30 μg and levonorgestrel 150 μg) for ten years without problems; however, her BP had not been registered. She did not have any cardiovascular risk factors. Twelve years after she stopped oral contraception she was diagnosed with hypertension. No underlying cause could be identified. Five years later her BP was well regulated (114/73 mmHg) with metoprolol retard 50 mg and losartan 100 mg, both once daily. Three months later, her office BP had increased to 150/100 mmHg. Ambulatory 24-hour BP monitoring revealed an average value of 159/103 mmHg. One month earlier she had a levonorgestrel-containing IUD implanted. She was not experiencing any psychological or social stress at that moment, and she was still taking the same drugs as before. Since the relation in time was striking, the IUD was removed. Two weeks later her office BP decreased to 140/90mmHg. Four months later repeated self-measured home BP was well regulated again (130/80 mmHg). The medication was unchanged. After some months she underwent an uncomplicated sterilisation.

The LNG-IUD releases on average 14 μg levonorgestrel daily into the uterine cavity, with the highest values in the first year after insertion.[1] Although the plasma concentration of levonorgestrel is low as compared with oral contraceptive drugs, systemic side effects can still occur. Reported symptoms are headache, acne, hirsutism and mood disturbance.[2]

The occurrence of hypertension during use of an LNG-IUD has been studied several times, but none of the studies, with a follow-up of one to ten years, could find a relation.[3-5] However, no studies have addressed the short-term effect of an LNG-IUD on the BP.

The mechanism by which progestagens can cause hypertension is still not completely understood and study results are conflicting. Likely the renin-angiotensin-aldosterone system and genetic predisposition play an important role.

With this letter we would like to highlight that increase in blood pressure might be due to an LNG-IUD.

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