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Personalised treatment of arthritis in the next eRA

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Rheumatoid arthritis (RA) is the most predominant chronic inflammatory joint disease affecting approximately 1% of the population. Over the last 20 years great progress has been made in the treatment of this immune-mediated inflammatory disease. Current practice consists of early goal-directed therapy with disease modifying antirheumatic drugs such as methotrexate aiming at significant reduction of inflammation and ultimately remission. However, this certainly has its cost for society as many patients need expensive new treatments with biologic agents such as monoclonal antibodies targeting TNF alpha or anti-B cell therapy to achieve remission. To date no therapy has been proven to be effective in all patients. Consequently, patients can be classified as responder or non-responder. It would be ideal if response to treatment could be predicted prior to starting a new therapy in order to optimise patient care, reduce the risk of adverse effects and last but not least substantially reduce costs.

RA is nowadays thought to consist of different pathogenic subsets leading to common signs and symptoms, clinically defined as RA. It is likely that gaining more insight into the underlying mechanism(s) that cause inflammation in an individual patient will lead to a more rational choice of treatment with a better response rate. Therefore, there is a continuous search for the best biomarkers in combination with clinical variables to predict clinical course and response to therapy.

At present clinicians are only able to make a crude clinical discrimination between early arthritis patients based on the presence or absence of autoantibodies such as rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA) in combination with the presence or absence of bone erosions on X-rays of hands and feet. This classification in RF or ACPA negative vs. positive, and erosive vs. nonerosive disease is important in predicting which early arthritis patients will ultimately develop RA and at least in part influences treatment algorithms. For example, if an RF/ACPA positive RA patient primarily fails to respond to anti-TNF treatment there are some indications that it may be more beneficial to treat this patient with anti-B cell therapy (rituximab) rather than to try another TNF blocker, T cell co-stimulation blockade (abatacept) or anti-IL-6 monoclonal antibodies (tocilizumab), whereas a RF/ACPA negative patient is likely to benefit more from the latter types of therapies. However, more extensive studies are required to decide if this should be common practice.

Immunohistochemical analysis of synovial tissue and molecular biology have defined further subtypes of synovial inflammation that could be associated with a different pathogenesis or response to treatment, whereas the clinical features are identical: pattern of lymphocyte infiltration (diffuse vs organisation in perivascular aggregates with features of germinal centres as seen in lymphoid tissue), high vs low inflammation associated with genes indicative of an activated type I interferon/STAT-1 signal transduction pathway, and heterogeneity in synovial macrophage populations (reviewed by Hamilton and Tak) or expression of cytokines.

Furthermore, a number of studies have been performed to determine whether response to treatment could be predicted by composition of synovial inflammation prior to treatment. One of these studies investigated the response to anti-TNF therapy in relation to baseline TNF expression and the number of macrophages in the synovium, but this could only explain about 10 to 15% of the variance in response to therapy. With the advent of DNA microarray technology, transcriptome analysis has become feasible and is increasingly applied in RA research. One of these studies has demonstrated that almost all patients with a high transcript level of inflammation-related genes responded to anti-TNF therapy. Until now, these approaches are only applicable at the group level and unless a golden
In addition, recent progress in predicting the response to and may therefore be less informative in terms of disease pathogenesis. Nevertheless, a lot of effort has been put into this technique that is thought to be especially suitable for discovery of clinically relevant biomarkers in large cohorts of patients.

In this issue of the *Netherlands Journal of Medicine*, Professor Cor Verweij discusses the heterogeneity of RA and explains the (molecular) subtypes of inflammation that have arisen from genomics research in more detail.14 In addition, recent progress in predicting the response to therapy and personalised medicine using gene expression profiling will be described. These studies are clinically extremely relevant as they may define new biomarkers/disease entities and predict response to treatment. Although DNA microarray technology is suited for picking up signatures of genes associated with RA subsets or response to therapy, this technique also has some pitfalls. Because of its high costs usually only one or two measurements are done per patient. This raises the issue whether the specific molecular profile is stable over time and, in the case of synovial tissue analysis, whether the profile from one biopsy is representative for other sites of synovial inflammation. In addition, this technique does not take into account regulatory mechanisms such as inhibitory micro RNAs or posttranslational modification of proteins. Therefore, to gain more insight into the pathogenesis of this complex multifactorial disease, it is necessary to take it to the next level and perform functional studies on the genes/pathways that emerge from microarray data or genome-wide association studies. This is also crucial in light of the development of new treatments for this disease in order to achieve the ultimate goal of personalised medicine.

It is anticipated that great progress will be made in this field over the next few years, because large clinical datasets of early arthritis and arthralgia patients are currently generated. Genomics research will subsequently identify biomarkers for diagnosis and response to treatment. This will allow early recognition of patients who will develop (chronic) arthritis, and if so, to which therapy they are likely to respond. Therefore, the next era of translational RA research promises to be just as fascinating as it has been in the past 20 years.

### References

Transcript profiling towards personalised medicine in rheumatoid arthritis

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease that is heterogeneous in nature. The heterogeneity is reflected by the variation in responsiveness to virtually any treatment modality. Since our understanding of the molecular complexity is incomplete and criteria for categorisation are limited, we mainly consider the disease RA as group average. A powerful way to gain insight into the complexity of RA has arisen from DNA microarray technology, which allows an open-ended survey to comprehensively identify the genes and biological pathways that are associated with clinically defined conditions. During the last decade encouraging results have been generated towards the molecular description of complex diseases in general. Here, I describe developments in genomics research that provide a framework to increase our understanding of the pathogenesis and the identification of biomarkers for early diagnosis, prognosis and stratification, aimed at a personal medicine approach in RA.

KEYWORDS

Biomarkers, disease subtypes, DNA microarray, genomics, molecular profiling, personalised medicine, pharmacogenomics, rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic chronic inflammatory disease that primarily affects the joints. The aetiology of RA is unknown. Clinical and laboratory observations suggest an immune-mediated attack against self-antigens. This is featured by the connection HLA-DR loci, and the expression of autoantibodies, such as rheumatoid factor (RF) and anticitrullinated protein antibodies (ACPA). The immune-mediated background is substantiated by the ameliorative role of immune-suppressive therapies. Accordingly, initial alterations in the immune system are likely the basis for the development of RA. This is reflected by the finding that ACPA and/or RF are already present prior to the onset of RA. 

Using serum samples stored in a blood bank, Rantapää-Dahlquist and colleagues showed that 34% of the RA patients were positive for ACPA up to nine years prior to diagnosis. In analogy, Nielen and colleagues showed that 49% of the RA patients tested positive for IgM-RF and/or ACPA before onset of disease at a median of 4.5 years before symptom onset. A recent prospective follow-up study of ACPA and/or IgM positive arthralgia patients has shown that ACPA positive patients are more likely to develop arthritis than ACPA negative, IgM-RF positive arthralgia patients (27 vs 6% after a median follow-up of two years). Since not all ACPA and/or RF positive individuals ultimately develop RA the requirements to drive this process are likely to be different between the persons at risk.

Once symptoms are present, RA manifests as a heterogeneous disease with a clinical spectrum ranging from mild to severe disease, and variability in secondary organ system involvement. The heterogeneous nature is reflected by variation in responsiveness to virtually any treatment modality. The heterogeneity most likely has its origin in its multifactorial nature, whereby specific combinations of environmental factor(s) and a varying polygenic background are likely to influence not only susceptibility but also the disease severity and prognosis. Unfortunately, our understanding of the preclinical phase and molecular complexity of RA is incomplete, and criteria for subtyping of patients, for example to select those patients who will benefit from a specific treatment, is currently lacking.

By definition, nearly every aspect of a disease phenotype should be represented by pathophysiological processes
driven by genes and proteins that are expressed in the patient. These genes and proteins typically represent a molecular signature that is associated with disease characteristics and subtypes and thus defines the samples unique biology. A very powerful way to gain insight into the molecular signature underlying pathophysiological processes has arisen from DNA microarray technology, which allows an open-ended survey to identify comprehensively the fraction of genes that are differentially expressed in blood and tissue samples among patients with clinically defined disease and could serve a role as clinically relevant biomarker.

Initially, several pitfalls were experienced using this multistage and relatively expensive technology, which highly depends on perfectly standardised conditions. Factors that could influence the sensitivity and reproducibility range from sample processing differences, variation in amount and quality of starting RNA, amplification and labelling strategies and dyes, to probe sequence and hybridisation conditions. In addition the lack of standardised approaches for normalisation and usage of data analysis algorithms could influence the outcome. Therefore, verification of results became an essential step in microarray studies. In order to set quality criteria for performing and publishing microarray studies, standards for microarray experiments and data analysis were created.6

Nowadays, after a decade of technical and analytical improvements, the technology and algorithms for data analysis have been shown to be robust and reproducible across properly designed and controlled experiments, and different research groups. The availability of the Paxgene whole blood isolation system, which directly lyses aspirated blood cells and stabilises the RNA in the aspiration tube, excludes ex-vivo processing artifacts. These developments make transcriptomic profiling superior to a proteomics approach for biomarker discovery. However, careful standardisation is still required for cell subsets and tissues that are obtained via ex-vivo manipulation. The differentially expressed genes may then be used to provide insight into biological pathways contributing to disease and to identify classifiers for early diagnosis, prognosis, and response prediction.7,8 This review describes developments in transcriptomics research to identify novel pathways that contribute to disease and to uncover clinically relevant biomarkers (figure 1). Ultimately this information may help clinicians to improve disease management.

**Molecular Markers for Heterogeneity Between Synovial Tissues**

The first study on gene expression profiling in RA concerned synovial tissue biopsies using a combination of subtractive hybridisation and high-density cDNA arrays.9 This study highlighted the increased expression of genes involved in chronic inflammation such as immunoglobulins and HLA-DR in RA synovium when compared with normal synovium. Comparative analysis of synovial tissue specimen from RA and osteoarthritis (OA) patients revealed that these diseases were characterised by distinct synovial gene signatures.10 In particular genes involved in B and T cell regulation were upregulated in RA tissues.9 Histogramical analysis confirmed that in RA synovium was characterised by a higher amount of infiltrating T cells and B cells when compared with OA synovium.

A large-scale gene expression profiling study of 30 synovial tissue specimens from patients with erosive RA revealed considerable heterogeneity among patients.11-13 Systematic characterisation of the differentially expressed genes highlighted the existence of at least two molecularly distinct forms of RA tissues. One group, referred to as the RA high inflammation group, was characterised by genes involved in inflammation and adaptive immune response. The genes involved in the high inflammation tissues consist of
immunoglobulin genes and genes indicative for an activated IFN/STAT-1 pathway. Seven of these (TIMP2, PDGFRA, GBP1, Fos, CTSL, TUBB and BHLHB2) were also described by Devauchelle and colleagues. Two of these (GBP1 and CTSL) are known to be regulated by type I IFN.

The second group of RA tissues was characterised by a low inflammation gene signature that was reminiscent of that of tissues from patients with OA. While inflammation and immune-related genes were decreased, these tissues showed an increased expression of genes involved in tissue remodelling activity, which is associated with fibroblast dedifferentiation. Remarkably, the high and low inflammation tissues revealed reciprocal expression of specific matrix metalloproteinases (MMP). Whereas levels of MMP11 and 13 were increased in low inflammation tissues, levels of MMP1 and 3 were increased in high inflammation tissues. Tsubaki and colleagues demonstrated that tissue heterogeneity within RA can already be observed in the early phase of RA (duration of less than one year after diagnosis). Analogous to the previous study using biopsies from long-standing RA patients, the early RA patients could be divided in at least two different groups based on their gene expression profiles.

In approximately 5 to 10% of synovial tissues T cells, B cells, and follicular dendritic cells (FDCs) are spatially organised into structures resembling lymph nodes with germinal centres (GC). The remainder of the tissues lack FDCs and show either a diffuse or an aggregated T-cell and B-cell infiltrate. Histological analyses revealed that the differences observed in global gene expression among the rheumatoid synovia are related to differences in cell distribution. Tissues that contain ectopic GC-like structures were selectively present in the high inflammation tissues. The GC-containing tissues revealed increased Ig transcript expression in accordance with the presence of B cells and/ or plasma cells, which may reflect local production of antibodies. These tissues also showed enhanced expression of the chemokines CXCL12 and CCL19 and the associated receptors CXCR4 and CXCR5, which are important for the attraction of T cells, B cells, and dendritic cells. In addition genes involved in JAK/STAT signalling, T-cell and B-cell specific pathways, Fc-receptor type I signalling in mast cells, and IL-7 signal transduction (e.g. IL-7 receptor α (IL-7R α)/IL-2Rγ chains and IL-7) were elevated. These findings suggest a role for the IL-7 pathway in synovial lymphoid neogenesis in RA, analogous to its role in the development of normal lymphoid tissue. Tissues with a diffuse type of infiltrate showed a profile that indicated repression of angiogenesis and increased extracellular matrix remodelling. Overall, the gene expression profiling of rheumatoid synovium has provided insight into the molecular basis of the heterogeneous nature of synovial disease pathogenesis in RA (figure 2). It remains to be determined if a specific molecular profile applies to all affected synovia in a single patient, and if the profile is stable during the course of disease.

**GENE EXPRESSION IN MESENCHYMAL CELLS DERIVED FROM AFFECTED TARGET TISSUES**

Fibroblast-like synoviocytes (FLS) are major players in joint destruction in RA. These cells are considered to be sentinel cells that contribute to leucocyte migration and local immune response through the production of various immune modulators.

Gene expression profiling analysis of rheumatoid FLS revealed the overexpression of genes responsible for tumour-like growth when compared with FLS derived from traumatic control patients. Moreover, an increased expression was observed for PDGFRα, PAI-1 and SDF1A. Other investigators studied the influence of tumour necrosis factor-α (TNF) on FLS, since TNF showed to be of primary importance in the pathogenesis of RA. These studies are instrumental to define TNFα response signatures that can be used to monitor the pharmacodynamics of TNF blockade.

Profiling studies of FLS derived from 19 RA patients revealed considerable heterogeneity. The distinct FLS subtypes were associated with a specific phenotypic characteristic of the synovial tissue from which they were
derived. The FLS subtype that reveals similarity with ‘myofibroblasts’ was associated with high-inflammation tissues. The myofibroblast is a specialised fibroblast, which expresses α-smooth muscle actin (SMA), an actin isoform typical of vascular smooth muscle cells. These myofibroblast-like FLS showed a markedly increased expression of transforming growth factor β (TGFβ) response genes. Among these response genes were SMA, SERPINE1, COL4A1 (type IV collagen-α chain), IER3 (immediate early response 3), TAGLN (transgelin), and the gene for activin A as a potential agonist for the induction of the TGFβ response programme. Similar cells have recently been identified in the human TNF-α transgenic mouse model of arthritis. Studies from the field of oncology indicate that myofibroblasts present in tumours play a crucial role in angiogenesis and cell trafficking through the production of extracellular matrix proteins, chemokines and growth factors. Hence, it is hypothesised that the increased presence of this specific type of fibroblast, which is characterised by increased expression of SMA among other genes, is selectively associated with high-inflammation tissues and contributes to angiogenesis and cell trafficking in RA synovium. FLS that are characterised by increased expression of growth-related genes and Igf2 and IGFBP5, were associated with low-inflammation tissues. These data support the notion that cellular variation between target tissues is reflected in the phenotypic characteristics of the stromal cells (figure 2).

**GENE EXPRESSION IN PERIPHERAL BLOOD CELLS**

Knowing the systemic nature of RA and the communication between the systemic and organ specific compartments, we and others also studied whole blood and/or peripheral blood mononuclear cells (PBMC) to obtain disease-related gene expression profiles. The peripheral blood may not directly have implications for our understanding of disease pathogenesis, but is especially suitable to analyse gene expression profiles that provide a framework to select clinically relevant biomarkers.

Accordingly, several investigators studied gene expression levels in peripheral blood cells to address the question whether disease characteristics are detectable from gene expression levels in peripheral blood cells. Bovin and colleagues identified 25 genes discriminating between PBMC of RA patients (n=14) and healthy controls (n=7). These genes reflected changes in the immune/inflammatory responses in RA patients, such as the calcium-binding proteins S100A8 and S100A12. No significant differences between RF-positive and RF-negative RA were observed. Szodoray and colleagues studied gene expression differences in peripheral blood B cells from eight RA patients and eight healthy controls. A total of 305 genes were upregulated, whereas 231 genes were downregulated in RA B cells. In a larger study with 29 RA patients and 21 healthy controls, Batiwalla and colleagues identified 81 differentially expressed genes, including glutaminyl cyclase, IL1RA, S100A12 and Grb2-associated binding protein (GAB2) as the main discriminators. This profile correlated with an increased monocyte count. These findings indicate that there are clear differences in peripheral blood markers between RA patients and healthy controls that may have diagnostic potential.

Other investigators addressed the issue of heterogeneity in peripheral blood gene expression profiles among RA patients. Olsen and colleagues studied gene expression differences in PBMC between early (disease duration less than two years) and established RA (with an average disease duration of 10 years). Out of 4300 genes analysed, nine genes showed a threefold increased expression in the early RA group. These genes included colony-stimulating factor 3 receptor, cleavage stimulation factor, and TGFβ receptor II, which affect B-cell function. A total of 44 genes, which are involved in immunity and cell cycle regulation, were expressed at threefold lower levels. The observation that a quarter of the early arthritis genes overlapped with an influenza-induced gene set led the authors to suggest that the early arthritis signature may partly reflect the response to an unknown infectious agent. We studied gene expression profiles of whole blood cells of 35 RA patients and 15 healthy individuals. This analysis confirmed previous observations of increased expression of, for example, the calcium-binding proteins S100A8 and S100A12 by RA blood cells. The significantly differential expressed genes represent specific biological processes related to immune defence, including type I IFN-response genes, indicative that this pathway is also activated systemically in RA. This type I IFN signature may be a direct reflection of increased type I IFN activity or other ligands known to activate the IFN/STAT-1 pathway. Upregulation of IFN-response genes has now been observed in peripheral blood cells and/or target tissue of (a subset of) patients with other autoimmune diseases such as SLE, scleroderma, Sjogren’s syndrome, multiple sclerosis, and type 1 diabetes. These findings suggest that an activated IFN-response gene expression programme is a common denominator in rheumatic diseases, and autoimmune diseases in general. Type I IFNs (IFNα and IFNβ) are early mediators of the innate immune response that influence the adaptive immune response through direct and indirect actions on dendritic cells (DCs), T and B cells, and natural killer cells. A likely candidate in RA is IFNβ, which is highly produced in the

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Verweij. Transcript profiling towards personalised medicine in rheumatoid arthritis.
Pharmacogenomics in RA Towards Personalised Medicine

Therapies to target the proinflammatory mediator TNF-α, B and T lymphocytes are approved worldwide for the treatment of RA. Clinical experience showed that the targeted therapies with biologicals are effective for most but not all of the RA patients, reflecting that there are ‘responders’ and ‘nonresponders’. Given the destructive nature of RA, the risk of adverse effects, and considerable costs for therapy, there is a strong need to make predictions on success before the start of therapy. If we rely solely on clinical or radiographic manifestations we will probably be responding too late to maximise protection. However, clear criteria for such classification are still lacking.

Ideally, a molecular biomarker signature as a predictor for therapy responsiveness should be obtained prior to the start of therapy in a readily available biosample, such as peripheral blood. Ultimately, this may lead to a personalised form of medicine, whereby a specific therapy will be applied that is best suited to an individual patient. I will present the results of pharmacogenomic studies to provide insight into the pharmacology of TNF blockade by soluble antagonists such as etanercept, infliximab or adalimumab, which are effective for approximately two thirds of the patients, and to predict the response to therapy. In essence similar studies can be carried out for therapies directed against T and B lymphocytes. The term pharmacogenomics emerged in the late 1990s and is associated with the application of genomics in drug development. Pharmacogenomics is defined as: ‘The investigation of variations of DNA (genetics) and RNA (transcriptomics) characteristics as related to drug response’.

The concept of a personalised form of medicine has attracted interest in the search for molecular criteria to dissect TNF responders from nonresponders in RA. Initial pharmacogenomics approaches aimed to understand the pharmacological effects of TNF blockade in the peripheral blood compartment in order to gain a comprehensive understanding of the mode of action. Pharmacogenomics studies revealed a similar change in the expression of a pharmacogenomic response gene set in the peripheral blood compartment of all RA patients treated with infliximab, irrespective of clinical response. This result suggests the presence of bioactive TNF in the circulation irrespective of clinical response.

Detailed analyses in search of (subtle) differences in the pharmacogenomic response profiles between responders and nonresponders identified informative sets of genes whose expression changes during therapy and were associated with clinical response. Koczán and colleagues determined the pharmacogenomic differences after 72 hours in 19 RA patients (12 responders and 7 nonresponders) following administration of etanercept. They report on an informative set of genes including NFKBIA, CCL4, IL8, IL1B, TNFAIP3, PDE4B, PPR15 and ADM involved in NF-kB and cAMP signalling whose expression changes after 72 hours that is associated with good clinical responses (disease activity score (DAS)28 >2.2). We showed that patients who developed an increased type I IFN response after one month of treatment had a worse clinical response to treatment. This was reflected by less improvement in DAS and higher tender joint counts and higher health assessment questionnaire-disability scores after treatment. Likewise, all patients without an anti-TNF induced increase in type I IFN gene activity had a good or moderate response to treatment as assessed by the EULAR response criteria. Comparative analysis did not reveal an overlap between the three gene sets.

No significant gene expression differences between responders and non-responders were found at baseline. Lequerre and colleagues studied in 13 patients (6 responders and 7 nonresponders) who started with an infliximab/methotrexate combination. Treatment response was determined after three months based on a difference in disease activity score (DAS)28 score ≥2.2 to define responders. In a validation study with 20 patients (10
responders and 10 nonresponders) a set of 20 transcripts in PBMC, which covered a diverse set of proteins and functions, was selected as classifiers.

At the synovial tissue level Lindberg and colleagues found 279 genes that were significantly differently expressed between the good responding and nonresponding patients. Among the identified genes was MMP-3. We found that a number of genes involved in biological processes related to inflammation were upregulated in patients who responded to infliximab therapy, compared to those who did not show clinical improvement were identified. These results indicate that patients with a high level of tissue inflammation are more likely to benefit from anti-TNF treatment.

Overall, the data reveal the presence of TNF bioactivity in all patients treated with TNF antagonists irrespective of the clinical response. The results suggest subtle pharmacological differences between responders and non-responders. However, the identification of biomarkers before the start of therapy in order to predict the response to anti-TNF treatment in RA has not revealed consistent results, yet. Therefore, additional studies using large cohorts of patients and more stringent response criteria are necessary.

**Preclinical Diagnosis of RA**

In order to induce remission and thereby prevent irreversible joint damage in RA, early diagnosis and a timely start of effective treatment is of high importance. Ideally, early diagnosis in the asymptomatic/preclinical phase is required. Several studies have documented the appearance of ACPA and RF prior to the onset of RA. Since not all ACPA and/or RF positive individuals ultimately develop RA other processes are involved. Hence, either additional factors are needed to result in a chronic inflammatory response ultimately leading to RA or some individuals may have a protective immune profile which suppresses disease development despite the presence of autoantibodies. To understand the differences between persons at risk who do and who do not develop RA, we analysed the gene expression profiles of blood samples of a unique cohort of ACPA/RF positive arthralgia patients at risk for RA (n=109) who were clinically followed for progression to arthritis. We demonstrated the heterogeneous nature of ACPA and/or RF positive arthralgia patients at risk for development of RA and identified sets of genes whose expression profiles segregate arthralgia patients at risk for RA into different subgroups. Subgroups that are characterised by a gene signature of IFN-mediated immunity, cytokine activity, or haematopoiesis all contain at-risk persons who have developed arthritis. These gene expression characteristics increase the risk for arthritis development approximately fourfold, independent of ACPA status. Interestingly, the group of patients characterised by increased expression of genes involved in humoral immunity is devoid of patients who have developed arthritis in the follow-up period. These results indicate that predisposition for the development of arthritis can be used to predict the diagnosis of arthritis in ACPA and/or RF positive individuals at risk.

On the basis of our data, we propose three levels involved in susceptibility to RA. First, some genes predispose the individual to autoimmunity. Second, this altered immunoreactivity is directed to particular antigens, i.e. citrullinated antigens, which affect B- and T-cell recognition of epitopes. Third, other genes act on the progression of autoimmunity to target tissues. Our results imply that, among others, IFN-mediated immunity and cell trafficking specify the processes relevant to progression to arthritis besides autoantibody positivity.

These results suggest that higher-order combinatorial searches may improve the predictive performance of autoantibody status towards diagnosis of preclinical RA.

**Conclusions**

Gene expression profiling approaches have fuelled insight into the complexity of RA pathogenesis and provide a framework to identify biomarkers as a promising tool for future clinical applications. Molecular profiling of blood cells and synovial tissues has already revealed important pathways contributing to the spectrum of diversity in RA. Until now, studies have been carried out using cohorts of relatively limited size. For the future the clinical implications of these observations require further definition and independent validation in large well-powered cohorts.

Pharmacogenomics studies are just emerging. The results of these studies look promising, but full confirmation of the biomarker profiles in independent uniform cohorts is of the utmost importance to create value for prediction of therapy response in RA to pave the way to more individualised treatment strategies. However, caution must be taken in the interpretation of these results because of small sample size and differences in treatment response measurements. To increase the sample size collaborative efforts from different groups are essential. To maximise the usage of information from different sources standardised procedures for sample processing, technology and data analysis and the algorithms used are needed. Moreover, full and open access to genomics data is important. This will ultimately allow a multidisciplinary approach, whereby clinometric, cytometric, metabolomic, genomic, proteomic, and imaging data from different laboratories are integrated to assign and validate clinically relevant markers for future clinical applications.
that reflect disease pathogenesis (diagnosis), prognosis, heterogeneity, and facilitate selection of patients with a high likelihood to respond to therapy.

Gene profiling in persons at risk to develop RA has revealed gene signatures in the peripheral blood that pose an increased risk above ACPA and RF. This finding forms the basis to envision predictive models based on preclinical expression profiling as an ‘evolving’ evidence-based process for determining the risk of developing RA.

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Verweij. Transcript profiling towards personalised medicine in rheumatoid arthritis.


Noninvasive haemodynamic monitoring using finger arterial pressure waveforms

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ABSTRACT

Haemodynamic monitoring may potentially lead to improved quality of care in haemodynamic compromised patients. However, the usefulness of invasive techniques using the pulmonary artery catheter is questioned. Noninvasive techniques which provide data on haemodynamics might provide a good alternative. New techniques have been developed in recent years to monitor cardiac output and other parameters of cardiac performance continuously and noninvasively. Recently, a new technique has become available that assesses these haemodynamic data from finger arterial pressure waveforms obtained noninvasively. Although an invasively derived calibration is still needed to obtain absolute data on cardiac output, relative changes in cardiac output can be accurately monitored using this method. Currently, the device can be used in patients to continuously monitor haemodynamic data and guide therapy. Furthermore, it might have a role in clinical research to noninvasively assess cardiac output, as a surrogate endpoint, before and after interventions. Although this new method seems promising, the clinical value has to be proven.

KEYWORDS

Blood pressure, cardiac output, heart failure, noninvasive haemodynamic monitoring

INTRODUCTION

Haemodynamic assessment is of particular importance in the evaluation of haemodynamically compromised patients. Cardiac output is considered one of the most important parameters when assessing the effects of treatment in a patient with haemodynamic instability but measurement is often precluded by the invasiveness and complexity of the established cardiac output monitoring techniques. Furthermore, although cardiac output seems to be an important parameter in patient management there is no structural evidence that cardiac output measurements improve morbidity and mortality.

In 1970 Swan and Ganz introduced the pulmonary artery catheter1 and since then measurement of cardiac output by the pulmonary artery catheter and the thermodilution method has become the gold standard against which new methods are compared. Recently, many questions have been raised regarding the safety, validity and adequate clinical response to the physiological parameters measured, and whether outcome is improved or worsened using the pulmonary artery catheter.2 In heart failure, functional status as a result of pulmonary artery catheter-guided therapy might improve but no clear benefit has been shown on mortality. Thus, no clear benefit has been shown in guiding therapy while invasive monitoring leads to catheter-related adverse events. As a result, usage of the pulmonary artery catheter has decreased.3 Although, new strategies for monitoring heart failure patients have been developed in combination with implantable devices4 5 these techniques have to prove their clinical benefit and are not applicable in patients without a device.

HAEMODYNAMIC MONITORING USING FINGER ARTERIAL PRESSURE WAVEFORMS

A relatively new method which noninvasively measures blood pulse curves and that was primarily developed to measure blood pressure has recently been further developed to obtain cardiac output measurements (Nexfin™). This monitor measures blood pressure...
noninvasively and continuously. Pressure is measured in the finger arteries and consecutively reconstructed to brachial artery values. This methodology builds on the volume clamp technique as proposed by Peñáz and the physiological criteria of Wesseling to calibrate the blood pressure measurement. In 1986, the first commercially available device was introduced. Further development resulted in several devices for specific settings, including space research. The noninvasiveness of the method allows easy access to arterial blood pressure. Methods have been validated in both clinical and research settings.

The pressure wave is further used as input to a dedicated algorithm to calculate stroke volume and cardiac output. The original concept of the pulse contour method for estimation of beat-to-beat stroke volume was described by Frank in 1899. Wesseling et al. further developed this method (Wesseling’s cZ method). Wesseling’s method relates cardiac output to the area under the systolic portion of the arterial pressure wave (A_syst). Dividing A_syst by aortic impedance provides a measure of stroke volume. In this model the mean arterial pressure is used to correct the pressure-dependent nonlinear changes in cross sectional area of the aorta. The heart rate is used to correct for pressure reflections from the periphery. The corrections for arterial pressure and heart rate are age dependent which is taken into account. This method has been further developed using the input of noninvasive finger arterial pressure waveforms. Other parameters which can be assessed are heart rate, total peripheral resistance and maximum dP/dt.

Figure 1 shows an example of optimising cardiac resynchronisation therapy in a patient aged 56 years with systolic heart failure due to ischaemic heart disease. Beat-to-beat stroke volume and blood pressure were measured using the advanced developments of Wesseling’s cZ method while changes in atrioventricular delay were made and an optimal setting was found. Figure 2 shows the possibility to perform measurement during exercise.

The derived parameters might be used in the assessment and management of patients with (acute) haemodynamic changes.

**Figure 1.** Optimalisation of AV delay with cardiac resynchronisation therapy using noninvasively derived stroke volume

**Figure 2.** Noninvasive haemodynamic parameters during exercise in a patient with heart failure

BP = blood pressure; HR = heart rate; BPM = beats per minute; SV = stroke volume (obtained with NexfinTM, BMEYE BV, Amsterdam).
DISCUSSION AND CONCLUSION

Parameters of cardiac performance can be obtained continuously and noninvasively using finger arterial pressure waveforms. These parameters can be assessed during interventions in rest and during exercise. At this moment alternative techniques that can continuously and noninvasively monitor cardiac output in the conscious patient are scarce. Extensive descriptions of invasive and noninvasive methods to assess cardiac output can be found in a recent review. Other pulse contour techniques exist but these are still invasive using an (radial) arterial input curve. With transthoracic echocardiography, cardiac output can be obtained, but continuous monitoring is time consuming and beat-to-beat monitoring is not possible. Another noninvasive method for continuous cardiac output monitoring is oesophageal Doppler monitoring using a probe inserted in the oesophagus. However, the technique can only be applied for continuous haemodynamic monitoring in sedated and mechanically ventilated patients, which applies for transoesophageal echocardiography as well. Another possible true noninvasive alternative might be impedance cardiography. This technique uses four pairs of electrodes placed on the thorax and neck and a set of ECG leads. High frequency, low amplitude alternating currents are transmitted after which thoracic impedance can be measured. Changes in thoracic impedance are related to fluid changes in the thorax and this way stroke volume can be assessed. An extensive description of impedance cardiography can be found in the review by Mathews and Singh. This technique seems ideal for continuous online and intermittent cardiac output monitoring. In heart failure the technique may be hampered by thoracic fluid and therefore haemodynamic data could be less reliable. Relative changes in cardiac output can be accurately monitored noninvasively with data obtained from the finger arterial pressure waveforms. However, an invasively derived calibration of cardiac output is needed to obtain more reliable absolute data on cardiac output. Besides being noninvasive, easy and fast assessment (first data within one minute) of haemodynamic parameters on a beat-to-beat basis is allowed. Blood pressure, cardiac output and dP/dt can be assessed noninvasively by this new monitor. These parameters can be assessed continuously and might be used in clinical practice to guide patient treatment. Potential areas where noninvasively obtained haemodynamic parameters might be used are guiding of inotropic and cardiac pacing therapy, dialysis and perioperative management. However, in most clinical situations no or scarce evidence exists on which parameters are the best to monitor and how this should influence therapeutic decisions leading to improved morbidity and mortality. The advantage of intensive noninvasive monitoring during sepsis seems obvious. Even so, the possibility to prevent infections using noninvasive monitoring during haemodynamic disturbances in acute heart failure and perioperative management is clear. This new monitor could be used in physiological research and in clinical research to noninvasively assess changes in cardiac output as a surrogate endpoint. Although accuracy and reproducibility have been investigated in some small trials, these should be further assessed thoroughly in various patient groups in different clinical conditions before this technique can be widely used. Moreover, further development to obtain absolute values of cardiac output without invasive calibration might give a substantial added value to the method. Finally, the usefulness in clinical practice has to be investigated in trials to assess applicability and effects on patient management. The new monitor is not hampered by the safety concerns of invasive monitoring and seems to be a promising technique that may be useful both in clinical research and in patient management.

REFERENCES


Type 1 diabetes and autoimmune polyglandular syndrome: a clinical review

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ABSTRACT

Type 1 diabetes mellitus (T1DM) results from autoimmune destruction of insulin-producing β cells and is characterised by the presence of insulitis and β-cell autoantibodies. Up to one third of patients develop an autoimmune polyglandular syndrome. Fifteen to 30% of T1DM subjects have autoimmune thyroid disease (Hashimoto’s or Graves’ disease), 5 to 10% are diagnosed with autoimmune gastritis and/or pernicious anaemia (AIG/PA), 4 to 9% present with coeliac disease (CD), 0.5% have Addison’s disease (AD), and 2 to 10% show vitiligo. These diseases are characterised by the presence of autoantibodies against thyroid peroxidase (for Hashimoto’s thyroiditis), TSH receptor (for Graves’ disease), parietal cell or intrinsic factor (for AIG/PA), tissue transglutaminase (for CD), and 21-hydroxylase (for AD). Early detection of antibodies and latent organ-specific dysfunction is advocated to alert physicians to take appropriate action in order to prevent full-blown disease.

Hashimoto’s hypothyroidism may cause weight gain, hyperlipidaemia, goitre, and may affect diabetes control, menses, and pregnancy outcome. In contrast, Graves’ hyperthyroidism may induce weight loss, atrial fibrillation, heat intolerance, and ophthalmopathy. Autoimmune gastritis may manifest via iron deficiency or vitamin B12 deficiency anaemia with fatigue and painful neuropathy. Clinical features of coeliac disease include abdominal discomfort, growth abnormalities, infertility, low bone mineralisation, and iron deficiency anaemia. Adrenal insufficiency may cause vomiting, anorexia, hypoglycaemia, malaise, fatigue, muscular weakness, hyperkalaemia, hypotension, and generalised hyperpigmentation. Here we will review prevalence, pathogenetic factors, clinical features, and suggestions for screening, follow-up and treatment of patients with T1DM and/or autoimmune polyglandular syndrome.

KEYWORDS

Autoantibodies, autoimmune polyglandular syndrome, type 1 diabetes mellitus

INTRODUCTION

Type 1 diabetes mellitus (T1DM), arising through a complex interaction of immune, genetic and environmental factors, results from autoimmune destruction of insulin-producing β cells. T1DM is characterised by the appearance of insulitis and the presence of β-cell autoantibodies.3 In up to one third of patients the autoimmune attack is not limited to β cells, but expands into an autoimmune polyglandular syndrome.2-8 Of type 1 diabetic subjects, 15 to 30% have autoimmune thyroid disease (Hashimoto’s or Graves’ disease), 5 to 10% are diagnosed with autoimmune gastritis and/or pernicious anaemia (AIG/PA), 4 to 9% present with coeliac disease (CD), 0.5% have Addison’s disease (AD), and 2 to 10% show vitiligo (table 1).2-8

In this clinical review we will discuss prevalence, predisposing factors (age, immune, genetic, environmental), clinical presentation, and suggestions for screening, follow-up and treatment of patients with type 1 diabetes and/or autoimmune polyglandular syndrome.

AUTOIMMUNITY

Organ-specific autoimmunity is frequent in T1DM subjects. This might be due to the fact that these patients show multiple immunological abnormalities. These include an imbalance in B and T lymphocytes, or an increased tendency to react strongly against certain antigens or a (genetically determined) poor ability to develop tolerance to autoantigens.9 Individuals with one autoimmune disease are known to be at increased risk for other autoimmune processes.1

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Most of the autoimmune endocrine disorders appear initially as infiltration of the gland by lymphocytes and macrophages. This may lead to destruction and atrophy of the gland with deficiency of its hormone. The destructive process is presumed to be T-cell mediated. Antibodies to certain antigens of the gland, mostly intracellular enzymes, appear in the blood. These include thyroid peroxidase (for Hashimoto’s thyroiditis), thyroid stimulating hormone (TSH) receptor (for Graves’ disease), parietal cell or intrinsic factor (for autoimmune gastritis/pernicious anaemia), endomysial or tissue transglutaminase (for CD), and 21-hydroxylase antibodies (for AD). The role of such antibodies remains unclear, but they are important as diagnostic messengers and appear commonly before clinical hormone deficiency. Thus screening for these antibodies allows early detection and the potential to prevent significant morbidity related to unrecognised disease. However, the frequency of screening for and follow-up of patients with positive autoantibodies remain controversial.

There are several direct links between genetics and autoimmune disease: the developmental maturation of T cells in a genetically susceptible individual occurs through molecular interactions between the T-cell receptor and the HLA-antigen complex. Selection of T cells with receptors likely to contribute to autoreactivity may preferentially occur in the context of specific HLA-DQ alleles that are prone to diabetes, autoimmune thyroid disease, AD, CD, and other auto-immune diseases. Indeed, disease-prone HLA molecules may be ineffective at binding and presenting peptides derived from tissue-specific antigens, and such a poor presentation in the thymus could impair mechanisms of negative selection allowing autoreactive T cells to survive the passage through the thymus. Subsequent activation of these T cells in the context of recognising islet-associated antigens can trigger a poorly regulated immune response that results in progressive tissue destruction.

Other important genetic factors include the cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), the MHC I-related gene A (MIC-A) and the protein tyrosine phosphatase nonreceptor type 22 (PTPN22). The CTLA-4, being expressed on activated CD4+ and CD8+ T-cell membranes, inhibits T-cell activation by binding costimulatory molecules. Polymorphisms within the CTLA-4 gene have been associated with T1DM and autoimmune thyroid disease, particularly the CT60 A/A polymorphism, and with Addison’s disease. The MIC-A protein is expressed in the thymus and is thought to interact with a receptor, NKG2D, which may be important for thymic maturation of T cells. NKG2D regulates the priming of human naive CD8+ T cells, providing a possible explanation for the association with autoimmune diseases. The PTPN22 gene is expressed in T cells, encoding for lymphoid tyrosine phosphatase (LYP). A specific polymorphism at position 620 (Arg → Trp) may decrease that ability of LYP to interact with its target molecules and down-regulate T-cell receptor signalling. This has been observed in Graves’ disease and weakly in Addison’s disease.

**Autoimmune Polyglandular Syndromes**

In 1866, Ogle was the first to describe the association between diabetes mellitus and Addison’s disease. However, the adrenal insufficiency in this case was due to tuberculosis. In 1910, Parkinson first described a patient with coexisting diabetes and pernicious anaemia. In 1926, Schmidt reported two cases of Addison’s disease associated with lymphocytic thyroiditis. In 1931, Rowntree and Snell

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**Table 1. Prevalence of organ-specified autoantibodies and autoimmune diseases**

<table>
<thead>
<tr>
<th>Disease or AB</th>
<th>General population</th>
<th>Type 1 diabetes mellitus</th>
<th>Coeliac disease</th>
<th>Addison’s</th>
<th>Hypothyroidism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1 diabetes mellitus</td>
<td>2-3%</td>
<td>1-8%</td>
<td>85-90%</td>
<td>│</td>
<td></td>
</tr>
<tr>
<td>anti-islet AB</td>
<td>1-3%</td>
<td>1-8%</td>
<td>8-12%</td>
<td>│</td>
<td></td>
</tr>
<tr>
<td>Coeliac</td>
<td>0.5%</td>
<td>0.5-1%</td>
<td>0.5%</td>
<td>99%</td>
<td>5%</td>
</tr>
<tr>
<td>transglutaminase AB</td>
<td>0.5-1%</td>
<td>0.6%</td>
<td>0-7.3%</td>
<td>83-90%</td>
<td></td>
</tr>
<tr>
<td>Addison's</td>
<td>2-0.05%</td>
<td>0-1%</td>
<td>0-5%</td>
<td>3-12%</td>
<td>14-21%</td>
</tr>
<tr>
<td>21-hydroxylase AB</td>
<td>0-0.6%</td>
<td>0-0.6%</td>
<td>0-7.3%</td>
<td>18%</td>
<td>47-83%</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>5-9%</td>
<td>30%</td>
<td>30%</td>
<td>14-21%</td>
<td></td>
</tr>
<tr>
<td>tPO</td>
<td>2-10% in adults</td>
<td>15-30% in adults</td>
<td>5-22%</td>
<td>23-40%</td>
<td></td>
</tr>
<tr>
<td>Graves'</td>
<td>0.1-2%</td>
<td>6-10%</td>
<td>6-10%</td>
<td>10-20%</td>
<td></td>
</tr>
<tr>
<td>TSH receptor AB</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Pernicious anaemia/ autoimmune gastritis</td>
<td>2% for AIG</td>
<td>5-10% for AIG</td>
<td>5-10% for AIG</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>autoimmunity gastritis</td>
<td>0.15-1% for PCA</td>
<td>2-4% for PCA</td>
<td>2-4% for PCA</td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>PCA</td>
<td>2.5-12%</td>
<td>12-12%</td>
<td>12-12%</td>
<td>6%</td>
<td>2%</td>
</tr>
</tbody>
</table>

**Note:** aTPO = antibody; AIG = autoimmune gastritis; PCA = parietal cell antibodies; T1DM = type 1 diabetes mellitus.
described the unprecedented findings of an association between Addison’s disease, Graves’ disease and T1DM. A year later Gowen reported the first case of Addison’s disease, Hashimoto’s thyroiditis and T1DM. The presentation of these autoimmune diseases as associated disorders led to the definition and classification of autoimmune polyendocrine syndromes I and II, by Neufeld, Maclaren and Blizzard in 1980.5 Both are associated with type 1 diabetes in about 20% of cases.3

The autoimmune polyendocrine syndrome I (APS-I), also known as autoimmune polyendocrinopathy, candidiasis, and ectodermal dysplasia (APECED), is a rare polyendocrine autoimmune disease caused by mutations of the autoimmune regulator gene (AIRE).3,26 It is inherited in an autosomal recessive manner. APS-I is defined by the presence of two or three of the following components: mucocutaneous candidiasis, adrenal insufficiency and/or hypoparathyroidism. It usually manifests in infancy at age 3 to 5 years or in early adolescence. The female-to-male ratio varies between 0.8/1 and 2.4/1. The autoimmune polyendocrine syndrome II (APS-II) is defined as the association of an autoimmune endocrine disorder with an additional autoimmune disease but not meeting criteria for APS-I and not having an identified mutation of the AIRE gene. APS-II is a complex polygenic disorder. By definition, patients with T1DM and an additional autoimmune disease meet the criteria for APS-II. In the majority of T1DM patients, the associated autoimmune disease follows the onset of diabetes. The prevalence of APS-II is 1/20,000 with a female preponderance (male/female ratio = 1/3). This syndrome has a peak incidence between the ages of 20 and 60 years, mostly in the third or fourth decade.

**TYPExE 1 DIABETES**

T1DM is a T-cell mediated autoimmune disease that develops in genetically susceptible individuals and results in destruction of insulin-producing β cells.1,27 Several lines of evidence support the autoimmune nature of the β-cell destructive process:

- Infiltration of the pancreatic islets by lymphocytes and macrophages (insulitis);38
- Presence of autoantibodies to islet cell antigens (ICA), tyrosine phosphatase IA-2 (IA2A), glutamic acid decarboxylase-65 (GADA), insulin (IAA), and zinc transporter ZnT8 (Slc30A8);1,27,29
- A preferential occurrence of T1DM in persons carrying specific allelic combinations at immune response loci within the HLA gene complex;9
- Increased prevalence of organ-specific autoimmune disorders in T1DM;4,8
- The disease can be transferred by spleen or bone marrow cells;39
- Animal models of T1DM (NOD mouse, BB rat) that show a defect in immunoregulation contributing to the onset of disease.

One or more β-cell autoantibodies are present in approximately 90% of new-onset patients with type 1 diabetes.73 They appear to develop sequentially. Insulin autoantibodies are often the first expressed, especially in younger children.34,35 GADA positivity is suggested to represent a propensity for general autoimmunity, while IA2A positivity may be a more specific marker of β-cell destruction.4,34 Beta-cell autoantibodies also represent important preclinical markers of the disease as they may be present for years before the diagnosis of diabetes.34,35 The risk of diabetes for a first-degree relative depends on the number and type of antibodies that are present. Family members who express IAA, GADA and IA2A have a 75% risk of developing T1DM within the next five years, as compared with a 10 to 25% five-year risk in those expressing only one of the antibodies. Data from the Belgian Diabetes Registry show a five-year risk for T1DM of 34% in subjects positive for 23 antibodies. Progression to diabetes amounted to 12% within five years among siblings positive for IAA, 20% for ICA, 19% for GADA but 59% for IA-2A. IA-2A were detected in 1.7% of all siblings and in 56% of the prediabetic subjects on first sampling.36

Among genes associated with T1DM, the HLA gene complex on chromosome 6p21 (IDDM1) is the genetic factor with the strongest association.37 The IDDM2 gene located on chromosome 11p15 in the upstream region of the insulin gene also confers susceptibility to T1DM.38,39 IDDM1 and IDDM2 are estimated to contribute to about 40 to 50% and 10%, respectively, of familial clustering of type 1 diabetes.40 Multiple additional genes also contribute to diabetes susceptibility. One of these is IDDM12 on chromosome 2q33, which contains two autoimmune disease candidate genes: CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) and CD28, encoding for T-cell receptors involved in controlling T-cell proliferation.41 Other important genes include the MHC I-related gene A (MIC-A) and the protein tyrosine phosphatase nonreceptor type 2A (PTPN22),42 as discussed above. Ninety percent of Caucasian T1DM patients express the HLA DR3 and/or DR4 alleles. These HLA alleles are expressed in 40% of the general white population.37,44 Expression of HLA DR2 is decreased in persons with diabetes. Certain combinations of HLA alleles are found with a frequency greater than expected and are thus not randomly distributed within the general population. This phenomenon is called linkage disequilibrium. Particularly, HLA DQA1*0301-DQB1*0302 (in linkage disequilibrium with DR4) and DQA1*0301-B*0702 (in linkage disequilibrium with DR3) haplotypes confer a high

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diabetogenic risk. The absolute risk of a child with this DR3/DR4 genotype developing T1DM from the general population is similar to a first-degree relative of a T1DM patient (1 in 20). DQB1*0602 and DQA1*0102 alleles are associated with dominant protection.

The Eisenbarth model of T1DM as a chronic autoimmune disorder begins with environmental triggers in genetically susceptible persons, progressing to autoimmunity with appearance of β-cell antibodies, further evolving towards metabolic dysregulation with loss of first-phase insulin response (FPIR), increasing HbA1c within the normal range, impaired fasting glycaemia (IFG) or impaired glucose tolerance (IGT), and finally resulting in overt diabetes and loss of C-peptide. The first-phase insulin response (FPIR) measured by iv glucose tolerance testing is the sum of insulin levels the first and third minute after administration of an iv glucose load. Many subjects with T1DM had a low FPIR before the diagnosis of diabetes, and this may persist for years before clinical disease onset. These data suggest that subjects go through a phase of decreasing β-cell mass. The exact β-cell mass at diagnosis is poorly defined. For patients with long-term type 1 diabetes, it is usually decreased to less than 1% of normal. Data from the Belgian Diabetes Registry demonstrate that residual β-cell function can also be analysed according to the measurement of C-peptide release as induced by a hyperglycaemic clamp procedure. Low first phase C-peptide response specifically predicts impending diabetes while a low second phase response probably reflects an earlier disease stage. Clamp-derived parameters may improve selection and monitoring of first-degree relatives in future prevention trials.

A typical type 1 diabetes patient presents with hyperglycaemia and polyuria, polydipsia and weight loss. Ketoadiposis is common. We suggest to verify autoantibody status at the moment of diagnosis of type 1 diabetes. The association between T1DM and organ-specific autoimmune disease can be explained by sharing a common genetic background (HLA antigens), but also by a defective immunoregulation.

Autoimmune thyroid disease (AITD) include Hashimoto’s thyroiditis and Graves’ disease. Hashimoto’s thyroiditis, first described in 1912, is the most prevalent autoimmune disease associated with T1DM. Hashimoto’s thyroiditis is defined by the presence of thyroid peroxidase (TPO) or thyroglobulin antibodies, and elevated TSH concentrations in the absence of medications. Although many subjects with Hashimoto’s thyroiditis are hypothyroid, there is a subgroup of thyroid autoantibody-positive cases who are euthyroid. It may take years for those subjects to develop thyroid disease. The 20-year follow-up study of the Whickham cohort demonstrates that TPO antibodies are predictive of thyroid failure with an annual incidence of 4.3% in subjects with an initial elevated TSH level (>5.0 µU/ml). TSH is also predictive of thyroid failure. At a TSH level of 5 µU/ml the probability of hypothyroidism is 0.5% per year. Higher initial serum TSH or thyroid antibody concentrations predicted higher rates of progression. Thyroid peroxidase antibodies (aTPO) are present in 15 to 30% of adults and in 5 to 22% of children with type 1 diabetes, compared with 2 to 10% and 1 to 4%, respectively, in matched controls (table 1). The prevalence of subclinical hypothyroidism in type 1 diabetic patients is estimated at 13 to 20%, compared with 3 to 6% in a nondiabetic population, with a preponderance for older female patients. Up to 50% of TPO-antibody-positive T1DM patients progress to overt autoimmune thyroid disease. Conversely, 2.3% of children with AITD have islet cell antibodies compared with 0% of controls. Cross-sectional analysis has shown that hypothyroidism is present in 4 to 18% of subjects with T1DM. Long-term follow-up suggests that as many as 30% of patients with T1DM will develop AITD. T1DM subjects with GADA positivity, in contrast to IA2A-positive patients, are more prone to have aTPO. The association of GADA with thyrogastric antibodies might be explained by the fact that GAD-65 is not exclusively present in the brain and pancreas but can also be found in the thyroid gland and stomach. T1DM patients with persisting ICA positivity for more than three years or with GADA+ are at increased risk of thyrogastric autoimmunity. Other factors such as age, diabetes duration, and gender (female preponderance) influence the link between T1DM and AITD.

At-risk haplotypes for Hashimoto’s thyroiditis are the HLA DQA1*0501 (linked to DR4), DQB1*0201 (linked to DR5), and DQB1*0301 (linked to DR3). The HLA haplotype DR3-DQB1*0201 contributes to the genetic susceptibility to T1DM, AITD and autoimmune polyglandular syndrome II. Other loci (VNTR and CTLA-4) may also contribute to the clustering and may influence disease phenotype and severity, as discussed above.

A symmetric, painless goitre is usually the first presentation in Hashimoto’s thyroiditis, although about 10% of patients have atrophic thyroid glands. Treatment of hypothyroidism is important because the decrease in basal metabolism may cause weight gain, hyperlipidaemia, atherosclerotic heart disease, goitre, and may affect diabetes control, growth, menses, and increase the risk of adverse pregnancy outcome. Even more, the presence of autoantibodies may be associated with an increased likelihood of spontaneous abortion, even in the absence of overt disease. Although thyroid lymphoma is very rare, the risk of this disease is increased 67-fold in patients with Hashimoto’s thyroiditis.

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Treatment of Hashimoto’s hypothyroidism consists of supplemenation of levothyroxine sodium. The goal of replacement therapy is to normalise serum TSH levels.\textsuperscript{14,56} Current recommendations from the American Diabetes Association are to screen TiDM patients for dysthyroidism using TSH after stabilisation at onset of diabetes, or in case of symptoms of hypothyroidism or hyperthyroidism, and every one to two years thereafter.\textsuperscript{66} Since patients who are TPO-antibody positive have an 18-fold increased risk of developing thyroid disease compared with patients who are TPO-antibody negative, we and others suggests to screen TiDM patients using TPO autoantibodies, TSH and T4 levels at onset of TiDM and yearly thereafter.\textsuperscript{57}

Autoimmune hyperthyroidism is less common. Robert Graves first identified the association of goitre, palpitations, and ophthalmos in 1835, although Caleb Parry had published details of a case ten years earlier.\textsuperscript{65} Graves’ hyperthyroidism is caused by thyroid stimulating antibodies that bind to and activate the TSH receptor on thyroid cells (TSH-receptor antibodies).\textsuperscript{66} These antibodies not only cause hypersecretion of thyroid hormone, but also promote hypertrophy and hyperplasia of thyroid follicles, resulting in a goitre. Graves’ disease shares many immunological features with Hashimoto’s thyroiditis, including high serum concentrations of antibodies against thyroglobulin and thyroid peroxidase. Thyroid function tests reveal a suppressed TSH level, elevated levels of serum T4 and T3, and positive TSH receptor antibodies.\textsuperscript{66} Graves’ disease affects approximately 0.5\% of the general population and is the underlying cause of 50 to 80\% of cases of hyperthyroidism.\textsuperscript{66} Subclinical hyperthyroidism can be diagnosed in 6 to 10\% of TiDM patients, compared with 0.1 to 2\% in the nondiabetic population (table 1).\textsuperscript{59,69}

The incidence of overt hyperthyroidism in persons with a suppressed serum TSH is calculated at 2 to 4\% per year. Women are five to ten times more at risk of developing Graves’ disease than men. Stress may trigger the disease. At-risk haplotypes for Graves’ disease are DQA1*0501 (linked to DR3 and to DR5), and DQB1*0302 (linked to DR4).\textsuperscript{67,68} The severity and duration of Graves’ disease and the age of the patient determine the manifestations of hyperthyroidism: nervousness, emotional lability, disturbed sleep, fatigue, palpitations and atrial fibrillation, heat intolerance, weight loss, and Graves’ ophthalmomyopathy.\textsuperscript{66} Among patients treated with insulin for diabetes, hyperthyroidism increases insulin requirements. Hyperthyroidism may aggravate glucose intolerance by multiple mechanisms, which include increased hexose intestinal absorption, increased glucose production (gluconeogenesis and glycolysis), and decreased responsiveness to insulin.

Current treatment of Graves’ hyperthyroidism consists of antithyroid drugs (propylthiouracil or methimazole), radioactive iodine, and surgery. Antithyroid drugs are effective in controlling hyperthyroidism because they inhibit thyroid hormone production and may have an immunosuppressive effect, causing a decrease in the levels of TSH receptor antibodies. No consensus exists regarding the treatment of subclinical hyperthyroidism despite arguments suggesting that therapy with antithyroid drugs may be indicated to prevent atrial fibrillation in older subjects.\textsuperscript{59}

We screen TiDM patients for dysthyroidism on an annual basis, using TSH, T4 and TPO levels. In case of a suppressed TSH level, TSH receptor antibodies are looked for. General guidelines based on expert opinion for the management of hyperthyroidism have been published by both the American Thyroid Association and the American Association of Clinical Endocrinologists.\textsuperscript{70,71}

**COELIAC DISEASE**

Coeliac disease (CD) is defined as a pertinent intolerance to dietary gluten. In 1888, Samuel Gee first described the clinical features of coeliac sprue.\textsuperscript{72} Dicke observed that the ingestion of certain cereal grains, including wheat and rye, was harmful to children with coeliac disease and demonstrated that the alcohol-soluble, or gliadin, component of the water-insoluble protein, or gluten, moiety of wheat produced fat malabsorption in patients with coeliac disease.\textsuperscript{73} CD results from a T-lymphocyte-driven autodestructive process within the gastrointestinal mucosa as response to certain dietary cereals.\textsuperscript{74,75} CD is characterised by inflammation, villous atrophy and crypt hyperplasia of the small bowel mucosa. These mucosal lesions recover when gluten is withdrawn from the diet. Diagnosis is made by biopsy of the mucosa of the proximal small intestine. Presence of circulating antibodies against gliadin (AGA), endomysium (EmA) and tissue transglutaminase (tTGA) further support diagnosis.\textsuperscript{76} These antibodies ultimately disappear in most patients with coeliac disease who follow a gluten-free diet. Susceptibility to coeliac disease is determined to a significant extent by genetic factors localised within the HLA region. Approximately 90\% of coeliac disease patients share the HLA DR3/DR2 configuration.\textsuperscript{77} A weak association between EmA-IgA and HLA DQA1*0501-DQB1*0302 has been reported.\textsuperscript{72} The prevalence of tTGA has been reported to be as high as 32\% in HLA DQ2 homozygous TiDM patients, as compared with 2\% in patients without HLA DQ2 or DQ8.\textsuperscript{78} The prevalence of HLA DQ2 in the population is 20 to 30\% and only a minority of these will ever develop coeliac disease. This implies the involvement of additional, non-HLA linked, genes in the pathogenesis of coeliac disease. MIC-A polymorphisms have been linked to celiac disease, as described above.
The coexistence of T1DM and CD could be explained by the sharing a common genetic factor in the HLA region, or by molecular mimicry by which gliadin or tissue transglutaminase activates T cells that are cross-reactive with various autoantigens. During active β-cell destruction, transglutaminase C, which is expressed in pancreatic islets, might be presented in an immunogenic form. In view of the high frequency of coeliac disease in patients with the HLA DQA1*0501-DQB1*0201 haplotype, this presentation may be facilitated by these alleles. Such inflammatory responses may have the capacity to persist in genetically susceptible hosts and lead to chronic organ-specific autoimmune disease. Furthermore, it has been suggested that in the development of autoimmunity in T1DM, the failure to achieve tolerance to autoantigens derives from the gut.

The prevalence of coeliac disease in Western countries is estimated at around 0.5%. It ranges between 1 to 8% in patients with T1DM (Table 1). The coexistence of T1DM and CD was first described by Walker-Smith in 1969. About 5% of patients with CD have autoimmune thyroid disease. Conversely, up to 2 to 4% of patients with autoimmune thyroid disease are affected by coeliac disease.

Clinical features of coeliac disease may be subtle and include mild abdominal discomfort and bloating, weight loss, fatigue, but also growth abnormalities mimicking constitutional growth delay, infertility, recurrent atrophic stomatitis, low bone mineralisation and hypocalkaemia with vitamin D deficiency and compensatory hyperparathyroidism, and rarely enteroenteropathic-associated T-cell lymphoma. Iron or folic acid deficiency with or without anaemia is the most common laboratory finding. Hypoglycaemia and a reduction of insulin requirements may indicate the presence of coeliac disease in type 1 diabetes. Some report no effect, whereas others report improved control with less hypoglycaemic episodes after gluten-free diet.

Coeliac disease is considered sufficiently prevalent and the benefits of diagnosis and treatment by gluten withdrawal are such that it is advocated to screen all T1DM patients for this disorder. When serological screening is used, most cases of CD will be detected within one year after onset of T1DM. Current (ADA) recommendations for screening subjects with T1DM are to obtain autoantibodies at diagnosis and with symptoms of CD. Barker et al. propose measuring TGA autoantibodies every two years. When positive, subjects should have a small intestinal biopsy to confirm diagnosis. Others advocate that serological screening for coeliac disease in T1DM should be carried out every fifth year due to the possibility of latent coeliac disease, but prospective studies are lacking to substantiate this policy. At this moment there remains controversy as to whether asymptomatic coeliac disease - when detected - should be treated with a gluten-free diet. Large clinical trials are needed to address this question. We suggest to test at onset of T1DM and then yearly for three years, and five yearly thereafter, or at any other time if there are clinical indications, because the test may later become positive.

**Autoimmune Gastritis**

In 1849, Thomas Addison was the first to report a patient with autoimmune atrophic gastritis. He described a ‘very remarkable form of anaemia’, which was later called pernicious anaemia that was linked to atrophy of the gastric mucosa. Autoimmune gastritis is characterised by atrophy of corpus and fundus mucosa, and presence of circulating autoantibodies to the parietal cell (PCA) and to their secretory product, intrinsic factor (AIF). PCA and AIF are present in 60 to 85% and 30 to 50% of patients, respectively. The prevalence of PCA positivity increases with age: from 2.5% in the third decade to 12% in the eighth decade in the general population. In T1DM patients, PCA are found in 10 to 15% of children and in 15 to 25% of adults. Chronic autoaggression to the gastric proton pump, H+/K+ ATPase, may result in decreased gastric acid secretion, hypergastrinaemia, and iron deficiency anaemia. In a later stage of the disease, pernicious anaemia results from vitamin B12 deficiency, which is ten times more common in type 1 diabetic than nondiabetic subjects. Finally, in up to 10% of patients, autoimmune gastritis may predispose to gastric carcinoid tumours or adenocarcinomas.

Endoscopic features of AIG include a shiny and red mucosa, a thin stomach wall and flattened or absent rugal folds. In biopsy specimens, lymphocytic infiltrates are present in the submucosa and lamina propria. In the next stage, intestinal metaplasia or enterochromaffin-like cell hyperplasia can be seen. Autoimmune gastritis (AIG) and pernicious anaemia (PA) are common autoimmune diseases with respective prevalences of 2% and 0.15 to 1%, in the general population, increasing with age. In patients with T1DM the prevalence is three to fivefold increased with respective frequencies of 5 to 10% and 2 to 4%. Pernicious anaemia occurs in 2 to 12% of patients with autoimmune thyroid disease, in 6% of those with Addison’s disease, in 9% of those with primary hypoparathyroidism, and in 3 to 8% of those with vitiligo (Table 1). Iron deficiency anaemia is present in 20 to 40% of patients with autoimmune gastritis, whereas pernicious anaemia can be diagnosed in up to 15 to 25% of patients. The progression of AIG to pernicious anaemia is likely to span 20 to 30 years. Finally, gastric carcinoid tumours are
observed in 4 to 9% of patients with AIG/PA, which is 13 times more frequent than in controls.103,104 Patients with AIG/PA also have a three to sixfold increased gastric cancer risk, ranging from 0.9 to 9%.105,106

A genetic predisposition to AIG/PA has been suggested by its familial occurrence.108 However, the link between AIG/PA and particular HLA haplo/genotypes is weak. In TiDM patients, a weak association between PCA positivity and the HLA DQA1*0501-Br1*0101 haplotype, linked to HLA-DR3, has been observed.64 Patients who manifest both pernicious anaemia and endocrine disease often have a DR3/DR4 genotype.109 In mouse models, four distinct genetic regions that confer genetic susceptibility to autoimmune gastritis have been identified (Gasa1-4).110 Three of these four susceptibility loci are nonmajor histocompatibility complex genes that colocalise with those of TiDM.111,112 This is the strongest concordance identified between any two autoimmune diseases so far. In patients with TiDM, immunological risk factors that have been associated with PCA positivity include persistent ICA positivity,4,7–8 GADA positivity,4,62 and aTPO positivity.4,62 The association with GADA might be explained by the fact that GAD-65 is not only present in the pancreas and brain but can also be found in the thyroid gland and stomach. PCA are more frequent in type 1 diabetic patients than in their first-degree relatives, even after HLA matching, suggesting that the diabetic condition itself plays an important role.108 No gender associations were found for PCA. Up to 50% of patients with autoimmune gastritis have aTPOs.116,118 These results support the recommendation of screening patients with autoimmune thyroid disease for AIG.

Clinical presentation varies widely. Iron deficiency anaemia presents as a hypochromic microcytic anaemia. Symptoms include pallor, fatigue and reduced exercise performance. Patients with pernicious anaemia present with a macrocytic anaemia and a low vitamin B12 which may lead to a painful neuropathy.96 Early detection of PCA, AIG and associated pathology is important, not only for prevention of iron deficiency anaemia, but also for prevention of pernicious anaemia which may cause neurological damage and may lead to (pre)malignant gastric lesions. No clear guidelines for the management are available, but we suggest to examine gastrin, iron, vitamin B12 levels and perform a complete blood count at yearly intervals. It seems prudent to test PCA status at the onset of diabetes and then yearly for three years, then five yearly thereafter.96 Particularly TiDM patients with positive GADA and aTPO should be screened. It is controversial whether patients with AIG/PA should be placed under a surveillance programme with regular gastroscopies. Performing gastroscopy is indicated at least once in patients with PCA positivity, anaemia or high gastrin levels.96 Since both AIG and pernicious anaemia predispose to gastric carcinoid tumours, which manifest only late in the disease process, a good follow-up is warranted. The possible adverse impact on the health of the patient provides a strong rationale for screening, periodic surveillance by gastroscopy with biopsy, early diagnosis, prevention and/or treatment.96

ADDISON’S DISEASE

Addison’s disease (AD) is the most frequent cause of primary adrenal insufficiency. It results from destruction of the adrenal cortex with an ensuing deficiency of cortisol, aldosterone, and in females of adrenal androgens.115 In 1849, Thomas Addison described a group of patients with severe anaemia and coexistent adrenalitis and/or adrenal atrophy and some had coexisting vitiligo.116 The suprarenal syndrome was named Addison’s disease by Wilks in 1862. Primary adrenal insufficiency (Addison’s disease) has been reported to affect 1 in 10,000 in the general population.116

Autoimmune adrenal insufficiency results from destruction of the adrenal cortex by cytotoxic T lymphocytes.117 In the active phase of the disease, there is a widespread mononuclear cell infiltrate. There is loss of normal three-layer structure of the adrenal cortex and adrenocortical cells show necrosis and pleiomorphism. The cytochrome P450 enzyme 21-hydroxylase has been identified as a major antigen for the antibodies.55 Also in AD, screening for 21-hydroxylase antibodies (21-OHAA) identifies subjects who are at risk to develop AD before clinical presentation. Clinical disease can either present as isolated AD or in combination with other autoimmune diseases as part of the ‘APS’.118 In TiDM the prevalence of antiadrenal cortical antibodies (AAA or 21-OH antibodies) ranges between 0.7 to 3%, compared with 0 to 0.6% in first-degree relatives and controls (table 1).4,5,8,115,117–120 A small number of autoantibody-positive subjects have been followed for the development of AD, and a yearly incidence of AD of approximately 2% has been observed.122 AAA are more frequent in female subjects9 and in TiDM patients with persisting ICA positivity.4,7 Furthermore, AD is frequently associated with other autoimmune endocrinopathies, particularly with Hashimoto’s thyroiditis (Schmidt’s syndrome). Barker et al. reported in their patient group that 70% of patients with 21-OHAA also expressed thyroid autoimmunity.124 Genetic susceptibility for AD has, not surprisingly, also been linked to the MHC complex on chromosome 6. The HLA DRB1*0404/DQ8-DRB1*0101/DQ2 genotype occurs at an increased frequency in individuals with isolated AD and in those with AD and TiDM.125 A second locus within the MHC, the MHC class I-related MIC-A, has been

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linked to AD. Homozygosity for MICA5.1 (allele 5.1) was associated with an 18-fold increased risk for AD.

Adrenal insufficiency may cause persistent vomiting, anorexia, hypoglycaemia, unexplained weight loss in an adult, malaise, ill-defined fatigue, muscular weakness, hypotension, and craving for salt. The most specific sign of primary adrenal insufficiency is generalised hyperpigmentation of the skin and mucosal surfaces, which is due to the high plasma concentrations of melanocyte-stimulating activity of β lipotropin, which derives from the same precursor as ACTH. Laboratory tests can aid in the diagnosis: hyponatraemia, hyperkalaemia, and acidosis. In addition, hypocorticism may, by reducing the insulin needs, cause frequent attacks of hypoglycaemia. Patients with symptomatic adrenal insufficiency should be treated with hydrocortisone and with fludrocortisone as a substitute for aldosterone. Patients with suspected AD and hypothyroidism should be evaluated and treated for adrenal insufficiency prior to replacement of thyroid hormone to avoid an Addisonian crisis, since thyroxine seems to be a good practice. If autoantibodies are positive, one has to evaluate morning baseline ACTH, cortisol and 21-OHAA at diagnosis of T1DM and then every two years seems to be a good practice. If autoantibodies are positive, one has to evaluate morning baseline ACTH, cortisol and PRA levels (supine position) and perform a corticotropin stimulation test. However, in the absence of symptoms, an annual basal cortisol and ACTH may be sufficient.

VITILIGO

Vitiligo is an autoimmune-mediated hypomelanotic disorder, with a prevalence of approximately 0.5% in the general population. Half of the patients present with vitiligo before adulthood. Vitiligo is characterised by circumscribed depigmented macules resulting from the loss of epidermal melanocytes. The initial cause is still unclear, but involves immunological factors, oxidative stress, and a sympathetic neurogenic disturbance. Most cases with vitiligo have antibodies against melanocytes. In populations of European origin, variants in the gene encoding NACHT leucine-rich-repeat protein-1 (NALP1) have been observed in vitiligo-associated multiple autoimmune diseases. A significant association of allele HLA DR4 and vitiligo has also been reported.

Patients with vitiligo should be asked whether any family member has a history of vitiligo, a thyroid disorder or other autoimmune disease such as T1DM or pernicious anaemia. Also, because vitiligo is associated with an increased risk for Hashimoto’s thyroiditis, the TSH level and TPO antibodies should be measured annually. A high index of suspicion for other autoimmune diseases is warranted. Autoimmune thyroiditis, autoimmune gastritis, pernicious anaemia, and T1DM are found more commonly in vitiligo patients compared with the background population, with frequencies of 30, 15, 5, and up to 10%. Treatment of vitiligo may include narrow-band UVB radiation as first-line therapy for widespread disease. In the case of localised disease, a topical corticosteroid or calcineurin inhibitor can be applied. Camouflage techniques can also be useful. Guidelines for the management of vitiligo have been published by the British Association of Dermatologists.

CONCLUSIONS

Type 1 diabetic patients exhibit an increased risk of other autoimmune disorders such as autoimmune thyroid disease, coeliac disease, autoimmune gastritis, Addison’s disease, and vitiligo. Approximately 15 to 30% of patients with T1DM have thyroid antibodies, and up to 50% of such patients progress to clinical autoimmune thyroid disease. The prevalence of autoimmune gastritis and pernicious anaemia is 5 to 10% and 2.6 to 4% respectively. Approximately 4% of T1DM patients have concomitant coeliac disease and 0.5% have Addison’s disease. Early detection of antibodies and latent organ-specific dysfunction is advocated to alert physicians to take appropriate action in order to prevent full-blown disease. Patients and family members should be educated to be able to recognise signs and symptoms of underlying disease. It is important to keep in mind that genetic background may affect the risk for autoimmune disease, and this may vary dependent on the region where the patient is coming from. A different genetic background may influence the need for and organisation of screening strategies.

In clinical practice we screen for TPO antibodies, PCA, EmA-IgA and 21-OHAA at diagnosis of T1DM. After diagnosis, regular screening of autoantibodies is warranted (figure 1):

- We screen T1DM patients for dysthyroidism on an annual basis, using TSH, T4 and TPO levels. When TPO antibodies are positive and thyroid function is still normal, we screen patients for overt thyroid
dysfunction on a more frequent basis (every 6 months to 1 year). When aTPO are negative, we still test thyroid function on an annual basis. In case of a suppressed TSH level, TSH receptor antibodies are looked for. Clinical signs and symptoms of dysthyroidism warrant earlier testing.

- Due to the possibility of latent coeliac disease, we suggest measuring tTGA and EmA autoantibodies at onset of T1DM and then yearly for three years, and five yearly thereafter, or at any other time if there are clinical indications, because the test may later become positive. When positive, subjects should have a small intestinal biopsy to confirm diagnosis. Clinical suspicion should be high in case of growth failure or delayed puberty in children, osteopenia, anaemia, menstrual irregularity, and glycaemic instability.

- Testing PCA status yearly after T1DM onset is recommended for three years, then five yearly thereafter. Particularly those patients with positive GADA and aTPO should be screened. We also examine gastrin, iron, and vitamin B12 levels and perform a complete blood count at yearly intervals. Performing gastroscopy is indicated in patients with PCA positivity, anaemia or high gastrin levels.

- For Addison's disease, in line with our screening procedure for other autoimmune disorders, we screen for 21-OHAA at onset and yearly for three years, then five-yearly thereafter, or in case of clinical suspicion. If autoantibodies are positive, an annual check-up of basal cortisol and ACTH (at minimum) should be performed. Clinical suspicion is warranted in case of unexplained weight loss, refractory hypoglycaemia, hyperpigmentation or unexplained hypotension.

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**Figure 1. Flowchart for screening and follow-up of associated autoimmune disorders in patients with T1DM**

<table>
<thead>
<tr>
<th>Type 1 diabetes mellitus</th>
<th>ICA, GADA, IAA2A, 1AA, ZnT8, HLA DR3/4 or Dq2/8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At diagnosis, screen for:</strong></td>
<td></td>
</tr>
<tr>
<td>AITD</td>
<td>AIG/PA</td>
</tr>
<tr>
<td>TSH/FT4/TPO</td>
<td>Yearly for the first 3 years, 5-yearly thereafter</td>
</tr>
<tr>
<td>Yearly of 6-monthly if TPO-positive</td>
<td></td>
</tr>
<tr>
<td>TSH ↑</td>
<td>TSH ↓</td>
</tr>
<tr>
<td>FT4 ↓</td>
<td>TSH rec AB</td>
</tr>
<tr>
<td>Levothyroxine</td>
<td>Start with methimazole or PTU</td>
</tr>
<tr>
<td>Fe or Vit B12 suppl</td>
<td>Avoid gluten</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Screen for AITD and AIG</td>
</tr>
<tr>
<td></td>
<td>↓ Cortisol or ↑ ACTH or ↑ PRA</td>
</tr>
<tr>
<td></td>
<td>Corticotropin-stimulation test positive</td>
</tr>
</tbody>
</table>

AB = antibody; AD = Addison's disease; AIF = antibodies to intrinsic factor; AITD = autoimmune thyroid disease; AIG = autoimmune gastritis; CD = coeliac disease; ECL = endocrine cell hyperplasia; EmA-IGA = anti-endomysium antibodies; IAA = insulin autoantibodies; ICA = islet cell antibodies; FT4 = free T4; GADA = glutamic acid decarboxylase-65 antibodies; PA = pernicious anaemia; PCA = parietal cell antibodies; PRA = plasma renin activity; PTU = propylthiouracil; TPO = thyroid peroxidase antibodies; TSH = thyroid-stimulating hormone; tTGA = tissue transglutaminase antibodies; 21-OHAA = 21-hydroxylase antibodies.
NOTE
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ABSTRACT

Background: In 2007 a national guideline on perioperative nutrition was issued in the Netherlands. As external indicator for adequacy of nutritional therapy, the percentage of malnourished patients who reach at least 1.2 grams of protein on day 4 after admission was chosen by the Netherlands Health Care Inspectorate.

Methods: We developed an algorithm that allows users to ask for advice on which artificial nutritional formula to prescribe and at which rate, assuring provision of adequate amounts of both protein and energy. Feedback on nutritional therapy is given to the users on a daily basis, and to the management per quarter. Both the advice and the feedback have been integrated in our data management system. The advice module is also available on-line.

Results: In the baseline situation over the first four quarters (2006) an average of 30.2% of patients who had a full day 4 in our unit reached the protein indicator. In the last six quarters post-implementation, the average percentage reached was 56.5% with values consistently over 50%. Changes were statistically significant at third quarter of 2007 (p<0.05) and thereafter (p<0.001). Results for day 7 of admission were unaffected, which indicates that targets were reached earlier during hospital stay.

Conclusion: Our study shows that integration of nutritional advice and automatically generated feedback to users in a data management system consistently improves delivery of (early) nutrition.

KEYWORDS

Balanced protein/energy provision, decision support, optimal nutrition, patient data management system, quality indicator

INTRODUCTION

In 2007 a national guideline on perioperative nutrition was issued in the Netherlands. The guideline defines optimal nutrition in terms of energy and protein for hospitalised patients as provision of energy as calculated with the Harris and Benedict 1984 formula + 30%. For long-term acute patients in the intensive care unit the guideline advocates tailoring energy provision towards the total energy expenditure determined by indirect calorimetry (resting energy expenditure + 10%). For protein the guideline aims to provide an amount of 1.2 to 1.5 grams/kg pre-admission weight. The evidence supporting optimal nutrition is based on surrogate outcomes that have not been validated against patient-oriented, clinically meaningful outcomes.1

The aims of nutritional support have been defined as 1) to preserve or restore lean body mass, 2) to maintain immune function and 3) to avert metabolic complications. Provision of nutritional support is aimed at reduction of disease severity, diminishment of complications, decreased length of stay in the ICU and a more favourable patient outcome." To preserve lean body mass, historic studies in healthy volunteers have shown that when individuals keep meeting their increasing energetic needs by increasing exercise, protein provision based on body weight suffices when 1.0 gram per kg is provided.2 Also in disease state, a fixed amount of protein (1.2 to 1.5 gram) per kg body weight has proven to be valid to minimise catabolism.3

So, optimal nutrition requires both an energy target and a minimal amount of protein provision per kg bodyweight. For practical registration reasons, the Netherlands Health Care Inspectorate has proclaimed the provision of at least 1.2 grams of protein/kg pre-admission body weight an external quality indicator for adequate nutritional therapy for all malnourished perioperative patients in...
Dutch hospitals. The indicator should be reached on day 4 of admission; the day of admission is day 1. Choosing a minimum amount of protein on day 4 as indicator for adequacy of nutritional therapy implicitly presumes that provision of such an amount of protein will also guarantee provision of enough energy and that reaching the nutritional goal at an early moment after admission will be followed by a sustained intake during admission from day 4 on. In this article we aim to share our implementation strategies and results to optimise nutritional therapy by integrating knowledge in a patient data management system. We provide a review of the recent literature supporting evidence that reaching the surrogate endpoints is clinically relevant in terms of mortality.

MATERIALS AND METHODS

Our department is a 28-bed level 3 intensive care unit in an academic hospital. It is a mixed medical-surgical unit. The protocol for nutritional therapy in the unit aims at early feeding, preferably by the enteral route. Enteral nutrition is started as soon as the patient’s condition allows it. The feeding rate is increased every 12 hours depending on retention until the target volume is reached. Post-pyloric feeding is initiated early if gastric retention (>250 ml every 6 hours) prohibits reaching the intended volume of nutrition. Parenteral nutrition is only prescribed if severe intestinal dysfunction does not allow enteral nutrition. The hospital’s nutritional support team visits the ICU twice a week on a consultation basis.

Calculations based upon the guideline

In 2006 our local protocol already aimed at reaching the goals as defined in the national guideline that was published in 2007: for calculation of the resting energetic needs, the Harris and Benedict formula (1984) is used with a 20% supplementation. An extra 10% is added to compensate for activity and thus to meet the total energy expenditure, recalculated to a weight corresponding to the guideline, recalculated to a weight corresponding with a BMI of 27.5 kg/m². Of note, the recent literature offers different recommendations for protein provision in different patient groups (e.g. adapted for low/high BMI, burns, trauma, age, and patients on renal replacement therapy), but for practical reasons the Dutch guideline lacks recommendations on protein provision for specific patient groups apart from the high BMI groups.

Patient data management system

The unit is equipped with a patient data management system (Metavision, MD-sof®, Israel). This system can be configured towards the needs that the users specify. Two fulltime ICT technicians with a nursing background are responsible for development and maintenance of the system and its applications. In order to facilitate the users and to provide optimal nutrition for our patients, the following steps were taken: for every nutritional formula available in the unit, the ingredients (amount of kcal/l, protein in gram/l) were entered in the system, using calculations based on the volume delivered. Energy and protein contents from other sources (propofol, glucose, albumin) are entered in the same way. A nutritional section was added to the forms in the data management system, providing a quick insight into nutritional parameters and balances.
Height, weight, gender and age are entered in the patient data management system upon admission of the patient and the estimated energy expenditure is automatically calculated and used by the system. After indirect calorimetric measurement the calculated value is replaced by the most recent result of measured resting energy expenditure +/− 10%. For patients with a BMI >30 kg/m², body weight is automatically recalculated to a body weight corresponding with a BMI of 27.5 kg/m², and the latter value is used to compute the amount of protein to be prescribed. Access to the algorithm is available to all users of the patient data management system through an advice button in the nutritional section of the system. Clicking the button provides the user with advice on which nutritional formula to use and on the amount of millilitres per hour. Furthermore, the user is given insight into the amount of energy and protein per 24 hours and the amount of protein/kg bodyweight that will be delivered if the advice is followed.

Feedback to users, nutritional support team, management
On a daily basis, at seven o’clock in the morning, an automated query is performed on all 28 beds in the unit. The query compares the amount of energy and protein provided in the last 24 hours with the results of the algorithm calculation per patient. The actual nutritional therapy given at the time of query is compared with what should have been prescribed. The results of the query are sent by e-mail to medical staff members and to the nutritional support team and are available before the early morning rounds start. In the automatically generated daily doctors notes the attending physician is confronted with deviations from the intended nutritional therapy, and a suggestion to prescribe therapy as indicated by the algorithm is made.

Every three months, a query is run to determine the percentage of patients reaching at least 1.2 grams of protein/kg on day 4. The day of admission is day 1. Irrespective of the admission time, day 2 is defined as the next 24 hours from 00.00 hours on. The results of this query are communicated to the medical and nursing staff and are part of the monthly staff meetings. For the present study also the percentage of patients reaching the same protein target at day 7 of hospital stay is added for comparison. Also the mean length of stay on the ICU is added for the same quarterly periods of 2006 to third quarter of 2009.

Implementation strategy
The abovementioned steps were developed over time. The main intervention for users of the patient data management system was the availability of the advice button. All the workers in the unit were given background information on the rationale of optimal nutritional therapy and on the use of the advice button. The training lasted 20 minutes and was given by members of the nutritional support team. For new co-workers the use of the nutritional part of the data management system is an integral part of their initial training.

Statistical analysis
Percentages of patients reaching protein intake by day 4 have been compared within quarters between years (i.e. quarter 4 of 2007 with quarter 4 of 2006), using a χ² test (SPSS 17, SPSS Inc., Chicago, USA).

RESULTS
The results are shown graphically in figure 1. In the baseline situation over the first four quarters (2006) an average of 30.2% of patients who had a full day 4 in our unit reached the protein indicator. In the last six quarters post-implementation, the average percentage was 56.5% with values consistently over 50%. Changes were statistically significant at third quarter of 2007 (p<0.05) and thereafter (p<0.001). During the steady increase in the percentage of patients reaching the protein target at day 4 (2007 and 2008), also a steady decrease in length of stay on the ICU (LOS ICU) is observed. However, there is no continuous trend in LOS ICU across 2006 up to the third quarter of 2009. Furthermore, during the post-intervention period, the percentage protein target reached at day 4 approaches and finally equals the percentage protein target reached at day 7. This indicates that nutritional targets have been reached earlier, which is in accordance with current ICU guidelines.

Reaching 90% of the energy goal over the same periods rose from 46 and 66.9% respectively (data not shown).

DISCUSSION
In the present study, the percentage of patients reaching the indicator for early and adequate nutritional support as defined by the Netherlands Health Care Inspectorate rose from 30.2% in the pre-implementation phase to 56.5% in the post-implementation phase. This effect shows sustainability over the period of 18 months. By comparison with the same results on day 7 it can be shown that protein targets were met at an earlier stage of ICU stay, which is in accordance with ICU guidelines. Although the indicator measures only protein provision, the underlying algorithm that we use ensures provision of adequate amounts of energy. Defining optimal nutrition in quantifiable terms enables the development of decision support in a patient data management system and for assurance of nutritional...
therapy. Key points in reaching this goal are availability to the users of the advice button, allowing them to choose the right sort and amount of nutrition as a target from the moment of admission onwards; daily automatic feedback by e-mail on nutritional therapy to staff members and nutritional support team and integration of deviances of what should be provided in the daily medical notes. On a higher level, insight is given by three-monthly reports on the percentage of patients who reach the indicator.

Theoretically and practically, a 100% score of reaching the indicator seems impossible. As optimal nutritional therapy is volume dependent and retention is a main problem, patients who need large volumes will take longer to reach their goal. Furthermore, early in the course of admission patients will frequently undergo diagnostic or therapeutic procedures or surgery, which leads to interruption of nutrition. Haemodynamic instability or limited treatment options hamper a further attempt to optimise nutritional therapy. In the present study no analysis of these and other factors that may explain the fact that the goal was not always reached, was performed.

An earlier study on the effects on nutritional therapy by the introduction of a patient data management system showed positive effects. Configuration of a nutritional page in the patient data management system made it possible to compute and display the daily energy target, the amount of nutrition actually delivered and to compute energy balances, which led to enhanced compliance with the feeding protocol. Provision of energy improved significantly in the groups that were supported by structured information from the data management system compared with the pre-implementation groups. No data on protein provision are given in this study.

Negative effects of undernutrition have been reported in earlier studies: In the earliest study on cumulative energy balances in surgical intensive care patients, excess mortality was shown (although no statistical analysis was performed in that study) when the energetic deficit, as determined by the cumulative difference between energy expenditure measured by indirect calorimetry and the actual provision of energy exceeded 10,000 kcal during the ICU admission period. A cumulative deficit of the supply of energy during the ICU stay has been shown to correlate with more complications and a prolonged length of stay.

Despite the introduction of protocols aimed at the delivery of adequate amounts of energy and protein to intensive care patients, several studies have shown that implementation of evidence-based protocols does not result in a significant increase in nutrients delivered per day. In these studies, emphasis was put on early initiation of nutrition, choice of the route of administration, stepwise increasing of the feeding rate, dealing with intolerance of enteral nutrition, gastric residues and management of diarrhoea. Although nutrition was initiated earlier in time the mean amounts of energy and protein provided per day were not significantly different; in the intervention group values for energy and protein were 1241 kcals and 50.1 grams, whilst in the control group 1065 kcals and 44.2 grams, respectively.
were given. Likewise, more days on enteral nutrition were reached in the ACCEPT trial but the amount of energy and protein provision per day did not increase. Barr did not achieve statistically earlier nutrition and the percentage of patients who reached the caloric target on day 4 of nutritional support did not increase despite the adherence to evidence-based guidelines. The likelihood that patients would receive enteral nutrition increased by adhering to the protocol and for this group a reduced risk of in-hospital death of 56% was found. In all three studies impediments for reaching adequate nutrition were the focus of attention, rather than a well-defined target for the amount of nutrition to be delivered. Nurses or doctors were not supported by a patient data management system to provide bedside advice for goals of nutritional therapy in either of the studies.

Recently, Anbar et al. provided preliminary evidence in a group of 50 patients with an expected ICU stay of >3 days that provision of energy according to indirect calorimetry led to cumulative positive energy balances whereas the control group (targeted at 25 kcal/kg) had negative cumulative energy balances; hospital morbidity and hospital mortality decreased in the intervention group.

Pichard et al. demonstrated that provision of >1500 kcal/day, besides parenteral glucose, in the first three days of admission reduces ICU mortality and hospital mortality. Early provision of energy diminishes the cumulative caloric deficit.

To the best of our knowledge, only two studies where both effects of energy supply and protein provision were studied showed beneficial effects of providing energy and protein closer to nutritional goals on mortality. Positive effects on 60-day mortality were demonstrated in a group of 2772 mechanically ventilated patients from 167 intensive care units and 37 countries. For a maximum of 12 days, the type and amount of nutrition was recorded. An increase of 1000 kcal/day was associated with reduced mortality (odds ration (OR) 0.76, confidence interval (CI) 0.61 to 0.95; p = 0.014) and a similar trend was seen for an additional intake of 30 g of protein associated with an adjusted OR of 0.84 (CI 0.74 to 0.96; p = 0.008). These effects were largely found in the BMI groups ≤25 kg/m² and in BMI group ≥35 kg/m². The average provision of energy and protein were 1034 kcal/day and 47 g/day, respectively. Overall, patients received 59.2% of the energy and 56% of the protein prescribed. The second study was performed in 243 sequential mixed medical-surgical patients where the caloric goal was guided by the result of indirect calorimetry and aimed to provide at least 1.2 grams of protein/kg/day. Cumulative balances were calculated for the period of mechanical ventilation.

Outcome parameters were ICU, 28-day and hospital mortality. In this study, female patients who reached their nutritional goals compared with those who did not showed a hazard ratio (HR) of 0.199 for ICU mortality (CI 0.048 to 0.831; p = 0.027), a HR of 0.079 for 28-day mortality (CI 0.013 to 0.467; p = 0.005) and a HR of 0.328 for hospital mortality (CI 0.113 to 0.952; p = 0.04). Achievement of energy goals whilst not reaching protein goals did not affect ICU mortality; the HR for 28-day mortality was 0.120 (CI 0.027 to 0.528; p = 0.005) and 0.318 for hospital mortality (CI 0.107 to 0.945; p = 0.039). No difference in outcome related to optimal feeding was found for men.

CONCLUSION

Our study shows that support by a patient data management system can almost double the percentage of patients who are adequately fed on day 4. Recent literature supports the view that surrogate endpoints for optimal nutrition as formulated in the Dutch guideline affect clinically important endpoints in terms of decreased mortality. Providing the users with an advice button that returns advice for one of three enteral nutritional formulas together with the desired pump setting in ml/hour, and guaranteeing adequate energy provision in combination with the desired 1.2 to 1.5 grams of protein/kg/day led to improved nutrition. Furthermore, making use of the possibilities of the patient data management system enabled threefold automated feedback on nutritional therapy on different levels: a daily dataset of all patients for staff members and the nutritional support team, a report for individual feedback to the attending physician in the medical notes, and a quarterly report of the percentage of patients who reached the indicator as feedback to all the workers in the ICU.

ACKNOWLEDGEMENTS

The authors wish to thank iMD-soft®, Israel for their financial support of this study; Jan Peppink, senior informatics specialist for his enthusiastic and ongoing support in this project; to the nurses of the department of intensive care who have adopted the idea of optimal nutrition and initiate early nutrition; to the medical staff who have given support to implementation and use of computer-aided prescription. Finally, the nutrition support team and the department of dietetics are to be thanked for their support.

NOTES

Part of the results were presented at the Intensivistendagen, Ede, the Netherlands on 11 February 2009. The implementation project was supported by a grant from iMD-soft®, Israel.
The first author, R.J.M. Strack van Schijndel, died on 12 September 2009. This manuscript has been written by him in his last days.

REFERENCES


ERRATUM

Unfortunately in the article ‘Internal medicine residents’ knowledge about sepsis: effects of a teaching intervention’, which was published in the October issue of the Netherlands Journal of Medicine on pages 312-315, the name of one of the authors was spelled incorrectly. The correct spelling should be T. van Achterberg. We apologise for any inconvenience caused.

Strack van Schijndel, et al. Computer-aided support and nutrients in the ICU.

DECEMBER 2009, VOL. 67, NO 11

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Glycogenic hepatopathy: a rare cause of elevated serum transaminases in diabetes mellitus

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ABSTRACT

Glycogenic hepatopathy (GH) is a rare cause of serum transaminase elevations in type 1 diabetes mellitus (DM). We describe a 29-year-old woman with a history of poorly controlled type 1 DM who presented with hepatomegaly and severe transaminase flares. Liver histology confirmed GH, with glycogen accumulation due to severe fluctuations in both glucose and insulin. GH can be regarded as an adult variant of Mauriac’s syndrome. Despite severe laboratory abnormalities, it does not cause liver cirrhosis. Treatment consists of improving glycaemic control.

KEYWORDS

Glycogenic hepatopathy, liver glycogenosis, Mauriac syndrome, type 1 diabetes mellitus

INTRODUCTION

Elevated serum transaminases in type 1 as well as type 2 diabetes mellitus (DM) are most frequently caused by non-alcoholic fatty liver disease (NAFLD), with possible progression to liver cirrhosis.1 Glycogenic hepatopathy (GH) is a rare cause of elevated serum transaminases, mostly confined to type 1 diabetics. We present a case of GH in a patient with poorly controlled type 1 DM. The recovery of severe transaminase elevations in this patient illustrates the more benign course of GH as compared with NAFLD.

CASE REPORT

A 29-year-old female with a 14-year history of poorly controlled type 1 DM (HbA1c between 7.2 and 15.3%), complicated by recurrent ketoacidotic dysregulations, developed severe increases in transaminase levels that were followed by recovery to near normal levels (figure 1) during periods of better metabolic control. Physical examination revealed hepatomegaly, which was confirmed by abdominal tomography. Laboratory analysis was compatible with major aminotransferase disturbances (figure 1), with concurrent increases in gamma-glutamyl transferase (1467 U/l, normal <35) and alkaline phosphatase (316 U/l, normal <120). Liver synthetic capacity as measured by serum albumin (35 g/l, normal 35 to 35) and coagulation tests (activated partial prothrombin time 23 sec, normal <35) remained normal. Her medical history included an eating disorder (anorexia nervosa) and repeated bouts of pancreatitis and abscess formation in liver, m. psoas and the peritoneal cavity caused by Staphylococcus aureus and Candida albicans. Despite extensive investigation no immune deficiency was found. When she was 29 years old she had a flare with grossly elevated liver enzymes and a liver biopsy was performed. Pathological examination of the liver biopsy specimen showed extensive glycogen accumulation suggesting an inherited glycogen storage disorder (GSD). However, GSD type Ia, Ic, Ic and III were excluded by mutational analysis and enzyme studies.

What was known on this topic?

Glycogenic hepatopathy (GH) has been described in literature, but reports remain scattered. Only recently, we are beginning to acknowledge and understand this entity.

What does this add?

Intermittent elevated liver transaminases in diabetes mellitus are not necessarily caused by non-alcoholic fatty liver disease, but can be due to GH, a condition with a far better prognosis. Clinicians’ awareness of GH should prevent diagnostic delay, as was the case in our patient, and will provide better insight into the prevalence of GH.
The flares with gross elevations of the serum transaminases persisted, but liver ultrasound consistently showed normal liver parenchyma hepatopetal flow in the portal and hepatic veins. Remarkably, transaminase flares followed glucose dysregulations, with recovery several days after glucose normalisation.

At the age of 30 years liver biopsy was repeated. In line with the previous biopsy there was glycogen accumulation, characterised by hepatocyte swelling, accentuation of cell membranes due to cytoplasmic rarefaction and strongly positive periodic acid Schiff (PAS, stains polysaccharides) staining (figure 2). After diastase digestion, which selectively degrades glycogen, PAS staining was no longer positive, confirming that glycogen accumulation was responsible for the findings.

From the results above we can conclude that this patient is most likely suffering from an acquired GSD. In this patient, it is most probably related to the poorly controlled type 1 DM in a condition called glycogenic hepatopathy (GH). In retrospection, this diagnosis could have been established from the first liver biopsy.

**DISCUSSION**

GH was first described by Mauriac in diabetic children as part of a syndrome including growth retardation, cushingoid habitus and delayed puberty. It is now becoming clear that the liver defects observed in Mauriac’s syndrome can occur without the syndromal features in adults with type 1 DM. GH is most probably a rare condition and many clinicians are unaware of this differential diagnosis. As a result, incidence and prevalence are unknown and it most likely goes unrecognised, as illustrated by our patient.

The key finding in GH is glycogen accumulation in the liver causing hepatomegaly and elevated liver enzymes, especially transaminases. A literature survey on reports of GH shows that especially hepatomegaly and elevated transaminases are very frequent findings (table 1). All patients with GH are on insulin therapy and virtually all patients have type 1 DM, although GH has been reported in type 2 DM. Liver biopsy shows ballooning of hepatocytes and glycogen deposition causing cytoplasmic rarefaction and cell membrane accentuation. PAS staining is strongly positive.

An essential element in the pathophysiology of GH is wide fluctuations in both glucose and insulin levels. High serum glucose levels cause an insulin independent inflow of glucose in hepatocytes where it is rapidly phosphorylated, trapping it in the cell. Subsequent treatment of high glucose levels with insulin causes the trapped glucose to polymerise to glycogen. Glycogen production persists for some time after insulin levels have declined. The alternation of high glucose and insulin levels in poorly controlled DM causes glycogen accumulation. Therefore, it comes as no surprise that this syndrome was first described in 1930, only shortly after insulin treatment had become available.
It is unclear why only a small subset of patients develop GH. It could be speculated that defects exist in genes that encode regulatory proteins, such as laforin,19 causing mild or no abnormalities in normal individuals, but marked glycogen storage under certain conditions such as glucose and insulin fluctuations. These proteins could regulate the activity of glycogen synthase and/or glucose-6-phosphatase.17

Treatment of GH consists of improving glycemic control. Adequate management of glucose and insulin levels can result in complete remission of clinical, laboratory and histological abnormalities.10 Unfortunately, in our patient, despite treatment with continuous subcutaneous insulin infusion, poor glycemic control and elevated liver enzymes persisted due to her eating disorder.

An important differential diagnosis to consider in diabetics with liver disturbances is NAFLD. NAFLD can develop in both type 1 and type 2 DM, regardless of insulin treatment.2 Persistence and relatively mild disturbances in liver enzymes favour NAFLD, whereas transaminase flares are more compatible with GH. Because liver ultrasound is similar in NAFLD and GH, a final distinction between GH and NAFLD can only be made with a liver biopsy.1 It is important to distinguish both conditions as NAFLD can progress to cirrhosis whereas GH has a much better prognosis as liver fibrosis does not develop. Especially with transaminase flares, liver biopsy should be considered. Clinicians’ awareness of GH will cause less diagnostic delay and more insight into the prevalence of this presumably rare but completely reversible disorder. To further enhance our knowledge on this syndrome we wish to establish a registry for such patients and we welcome information on additional patients.

### Conclusions

GH can cause severe, but reversible elevations of serum transaminase levels in patients with poorly controlled type 1 diabetes due to liver glycogen accumulation. It is important to distinguish it from NAFLD because the prognosis differs. Especially in patients with transaminase flares, liver biopsy should be considered. Clinicians’ awareness of GH will cause less diagnostic delay and more insight into the prevalence of this presumably rare but completely reversible disorder. To further enhance our knowledge on this syndrome we wish to establish a registry for such patients and we welcome information on additional patients.

### References


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**Table 1. Case reports on glycogenic hepatopathy**

<table>
<thead>
<tr>
<th>Total no. of patients (n)*</th>
<th>42 (100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes mellitus</td>
<td></td>
</tr>
<tr>
<td>- Type 1</td>
<td>40 (95%)</td>
</tr>
<tr>
<td>- Type 2</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Hepatomegaly (n=37)</td>
<td>34 (92%)</td>
</tr>
<tr>
<td>Transaminases (n=37)</td>
<td></td>
</tr>
<tr>
<td>- Normal</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>- Mild increase (&lt;3 times normal)</td>
<td>14 (38%)</td>
</tr>
<tr>
<td>- Strong increase (≥3 times normal)</td>
<td>21 (57%)</td>
</tr>
<tr>
<td>Alkaline phosphatase (n=33)</td>
<td></td>
</tr>
<tr>
<td>- Normal</td>
<td>9 (27%)</td>
</tr>
<tr>
<td>- Mild increase (&lt;3 times normal)</td>
<td>18 (55%)</td>
</tr>
<tr>
<td>- Strong increase (≥3 times normal)</td>
<td>6 (18%)</td>
</tr>
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<td>Gamma-GT (n=7)</td>
<td></td>
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<tr>
<td>- Normal</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>- Mild increase (&lt;3 times normal)</td>
<td>4 (57%)</td>
</tr>
<tr>
<td>- Strong increase (≥3 times normal)</td>
<td>3 (43%)</td>
</tr>
</tbody>
</table>

*Derived from publications on glycogenic hepatopathy, in English, between 1990 and 2009.

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A 79-year-old woman with a history of coronary heart disease, hypertension, and severe cognitive impairment related to Alzheimer disease, presented with constipation and incoercible vomiting in the previous five days. Previous medication included alprazolam, simvastatin, low-dose aspirin, bisoprolol, and omeprazole. On physical examination her blood pressure was 135/50 mmHg, and temperature 36.5°C. Abdominal palpation was not painful, no guarding, tenderness or abdominal wall hernias were noticed. Abdominal distension and augmented bowel sounds were present. A digital rectal examination was unremarkable.

Laboratory investigations showed 11,200 leucocytes/mm$^3$ with 85% neutrophils, and normal liver enzymes, amylase, electrolytes, renal function and urinalysis. Electrocardiogram did not show any changes suggestive of cardiac ischaemia.

A plain abdominal X-ray showed a pelvic calcification, and was otherwise normal (figure 1). An abdominal computed tomography (CT) scan showed a pelvic calcification (figure 2), and abnormalities in the gallbladder (figure 3).

**What is your diagnosis?**

See page 398 for the answer to this photo quiz.
Discussion

Abdominal CT scan showed radiological signs of small bowel obstruction, a pelvic ectopic gallstone located in the ileum (figure 2), pneumobilia, and a cholecystoduodenal fistula (figure 3). After the diagnosis of gallstone ileus, a laparotomy with enterolithotomy without fistula repair or cholecystectomy was performed. Recovery was uneventful, and the patient was discharged six days later.

Gallstone ileus is an infrequent cause of intestinal obstruction accounting for 1 to 3% of all mechanical intestinal obstruction, and it is more frequently observed in elderly women. Gallstone ileus results from the formation of a fistula between the biliary tract and the intestine. Most fistulas occur in the duodenum; however, fistulas may also occur to the stomach, colon, and jejunum. The point of obstruction is usually in the terminal ileum because of its smaller diameter and weaker peristalsis, but it can occur throughout the gastrointestinal system. Most gallstones that enter the intestinal tract are eliminated without consequences; obstruction may occur with larger stones, usually greater than 2 to 2.5 cm.1

Plain abdominal radiographs have been considered the fundamental tool to recognise gallstone ileus. The main radiological signs are known as the Rigler triad, composed of pneumobilia, ectopic stone and mechanical ileus, which is considered pathognomonic for this entity. However, the complete triad is observed in only a very low percentage of the patients.2 In the case presented here, pneumobilia and intestinal obstruction signs were absent, and the ectopic stone was located in the pelvis, suggesting an alternative diagnosis.1 Abdominal CT scan is considered the most important diagnostic procedure. In addition to the Rigler triad, CT allows the exact location of the ectopic stone and direct visualisation of the fistula.4

Proper therapy of gallstone ileus includes laparotomy and enterolithotomy. A one-stage procedure including cholecystectomy with fistula repair may be performed in healthy patients without serious inflammatory changes in the right upper quadrant. This procedure prolongs the duration of surgery and can increase morbidity and mortality. Enterolithotomy alone may be enough for elderly patients or those with comorbid conditions. In selected patients enterolithotomy may be performed laparoscopically.1

References


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A patient with an inguinal mass: a groin hernia?

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**Case Report**

A 66-year-old man was admitted to our hospital with a possible left-sided groin hernia. He had no history of trauma and no urinary tract symptoms. His previous medical history revealed insulin dependent type 2 diabetes. On physical examination a firm non-tender mobile mass of 3 to 4 cm was found above the left testis. No hernia was felt. Routine laboratory tests were normal. Ultrasound of the scrotum and inguinal region showed a non-specific, well-circumscribed mass of 3 x 4 cm on the left side. The lesion itself showed increased vascularity. Magnetic resonance imaging of the scrotum and inguinal region was performed (figure 1).

**What is your diagnosis?**

See page 400 for the answer to this photo quiz.
DIAGNOSIS

Magnetic resonance imaging of the inguinal region revealed a circumscribed tumour in the spermatic cord without signs of growth into circumferential tissues. The staging CT scan of the chest, abdomen and pelvis did not reveal any local or distant metastases. A radical orchidectomy along with excision of the spermatic cord mass (figure 2) was performed. The patient’s postoperative recovery was uneventful. Microscopic examination revealed a leiomyosarcoma with intermediate grade of malignancy and with negative margins. Clinical and radiological follow-up at 12 months showed no recurrence.

Leiomyosarcoma of the spermatic cord is rare, about 110 cases have been reported in literature. The clinical presentation is often an inguinal or scrotal mass, painful or painless, and is sometimes accompanied by a hydrocele. A review of paratesticular sarcomas in adults showed a peak incidence in the sixth and seventh decade. The most common means of spread is lymphatic, haematogenous (lung) and furthermore by local extension to the scrotum, inguinal canal or pelvis. Radical orchidectomy is the standard primary surgical procedure and the importance of adequate surgical margins has been well documented. To reduce local recurrence, adjuvant radiotherapy can be applied. A reported survival rate is 50 to 80% but probably half of the patients experience tumour recurrence. Therefore, thorough clinical and radiographical long-term follow-up is essential. In clinical practice, spermatic cord leiomyosarcoma, although rare, should be in the differential diagnosis for a firm palpable mass in the scrotum or inguinal region especially in older men.

REFERENCES

A 42-year-old man was admitted to the hospital because of watery diarrhoea and weight loss. He had been diagnosed with seronegative arthritis ten years earlier, for which he was taking methotrexate. At that time he had no other complaints, notably no diarrhoea. We cannot rule out the possibility that the arthritis was the primary presentation of the disease. He was not on NSAIDs. On physical examination no abnormalities were found. His laboratory studies showed an iron deficiency anaemia with normal white cell account and a C-reactive protein of 128 mg/l (normally <5 mg/l). As inflammatory bowel disease was suspected, colonoscopy was performed, revealing normal mucosa. Wireless capsule endoscopy (WCE) showed markedly abnormal mucosa, with a pseudopolypoid appearance and numerous small white spots (figure 1).

WHAT IS YOUR DIAGNOSIS?

See page 402 for the answer to this photo quiz.
DIAGNOSIS

The clinical presentation and images obtained with WCE support the diagnosis of Whipple's disease.1 Histological findings from duodenal biopsies revealed periodic acid Schiff (PAS)-positive macrophages typical for this disease (figure 2). Bacterial polymerase chain reaction (PCR) was positive for Tropheryma whipplei. The patient was treated with a third-generation cephalosporin (ceftriaxone) intravenously for two weeks, and after discharge he continued treatment with trimethoprim-sulphamethoxazole for one year. Within two weeks his diarrhoea had disappeared. After two months, he had no more complaints of arthralgia and had gained weight. Whipple's disease is a rare, systemic disease caused by Tropheryma whipplei. Its clinical manifestation is diarrhoea, weight loss and fever. Extraintestinal disease often involves the brain, the heart and the joints.2 Patients without the classic symptoms of gastrointestinal disease may be misdiagnosed. Patients who are not treated or insufficiently treated can experience fatal outcome or irreversible neurological damage.

The diagnosis of Whipple's disease is made by the presence of PAS-positive macrophages in histological specimens from the small bowel. Organisms at different stages of degeneration are seen within phagosomes in macrophages which also contain abundant irregular membranous inclusions representing remnants of the bacterial capsule, which are the equivalent of the PAS-positive material seen at light microscopy. PCR assay for Tropheryma whipplei is positive, but can also be positive in asymptomatic subjects. There are several publications describing capsule endoscopy in Whipple's disease.3 Recommended therapy is a third-generation cephalosporin for 10 to 14 days followed by long-term treatment for one year with trimethoprim-sulphamethoxazole.4 Follow-up of patients with Whipple's disease could be based on quantitative PCR. If patients have a good clinical response, they can simply be followed up with duodenal biopsies 6 months and 12 months after diagnosis. Antibiotic treatment can generally then be stopped if no PAS-positive material is identified.4

REFERENCES

Case report

A 60-year-old woman, who had received a post-mortem donor renal transplant six weeks ago because of unknown end-stage renal disease, was re-admitted because of fever. Her post-transplant course had been complicated by steroid-induced diabetes mellitus and bladder retention for which she had learned self-catheterisation. Immunosuppressive therapy consisted of prednisolone, tacrolimus and mycophenolate-sodium. On admission, she complained of general malaise and fever for the last 24 hours. On physical examination, she was disoriented and hypotensive with a temperature of 38.4°C. The abdomen was distended and high pinched bowel sounds were heard. The renal transplant in the right iliac fossa was slightly tender on palpation. Laboratory investigation demonstrated a rise in plasma creatinine from 93 µmol/l to 539 µmol/l; C-reactive protein was 330 mg/l and leucocytes were 15.8 x 10⁹/l. Urinary sediment showed >20 leucocytes/high power field. An abdominal X-ray was performed because concomitant ileus was suspected (figure 1).

What is your diagnosis?

See page 404 for the answer to this photo quiz.

Emphysematous pyelonephritis in a renal transplant patient

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Figure 1. Abdominal X-ray (137 x 161 mm)
DIAGNOSIS

The abdominal X-ray revealed subcapsular and parenchymatous air accumulation in the renal transplant (figure 2, black arrows). This is compatible with the diagnosis of emphysematous pyelonephritis. She was admitted to the intensive care unit, where she became anuric and dependent on vasopressive medication. Despite antibiotic treatment with vancomycin and gentamicin, based on previous urine cultures, a transplantectomy had to be performed 24 hours after admission, due to ongoing haemodynamic instability. Cultures from blood, urine and the transplant tissue showed growth of *Escherichia coli*, susceptible to the prescribed antibiotics.

After transplantectomy, the clinical condition of the patient improved rapidly. However, the postoperative course was complicated by recurrent abscesses for which prolonged treatment with antibiotics and repetitive drainage were necessary.

Emphysematous pyelonephritis is a rare, but serious complication after renal transplantation. Only 15 cases, not including our patient, have been reported so far. It is a gas-producing infection of the kidney and peri-renal tissue, most often caused by *E. coli* or *Klebsiella pneumoniae*. As with emphysematous pyelonephritis of native kidneys, the presence of diabetes mellitus is known to be a major risk factor for its development in renal transplant recipients. In 13 of the 15 reported cases diabetes was present, as in our patient. The mortality is high. Nephrectomy is almost always necessary.

REFERENCE

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The Netherlands Journal of Medicine publishes papers in all relevant fields of internal medicine. In addition to reports of original clinical and experimental studies, reviews on topics of interest or importance, case reports, book reviews and letters to the editor are welcomed.

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

Acknowledgements: All funding sources should be credited here. Also a statement of conflicts of interest should be mentioned.
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Journal abbreviations should conform to the style used in the Cumulated Index Medicus. Examples:

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Recheck references in the text against the reference list after your manuscript has been revised.

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Figures must be suitable for high-quality reproduction (>300 DPI). Submit line drawings made in Word or other computer programmes but not in a PowerPoint file. Colour figures are occasionally possible and will be charged to the authors. Legends for figures should be typed, with double spacing, on a separate page.

Case reports

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

Mini reviews

Mini reviews are concise notes that bring the reader up to date with the recent developments in the field under discussion. The review article should mention any previous important reviews in the field and contain a comprehensive discussion starting with the general background of the field. It should then go on to discuss the salient features of recent developments. The authors should avoid presenting material which has already been published in a previous review. The manuscript should be divided as follows: title page, abstract and main text. The text may be subdivided further according to the areas to be discussed. The text should not exceed 2500 words.

Letters to the editor (correspondence)

Letters to the editor will be considered by the editorial board. Letters should be no more than 400 words. Please use SI units for measurements and provide the references conform the Vancouver style (N Engl J Med. 1991;324:424-8). No more than one figure is allowed. For letters referring to articles previously published in the Journal, the referred article should be quoted in the list of references.

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