A young boy with fever, rash and coughing; what is your diagnosis?

Immunotherapy in airway allergen sensitised patients
• Anticoagulation in nocturnal haemodialysis
• Physical fitness in type 2 diabetes mellitus
• Immunosuppressive therapy in IgA nephropathy
• Myelopathy in systemic lupus
MISSION STATEMENT

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Department of Internal Medicine
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The Netherlands
Tel.: +31 (0)10-703 39 54
Fax: +31 (0)10-703 32 68
E-mail: p.l.a.vandaele@erasmusmc.nl
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Up close and personal with low-molecular-weight heparins (LMWHs)

S. Lugthart

Department of Haematology, Erasmus MC, Rotterdam, the Netherlands, email: s.lugthart@erasmusmc.nl

The fundamental purpose of anticoagulants is to provide the clinician with an essential pharmacological method of treating venous thrombosis. The complications associated with thrombosis can have catastrophic effects, such as severe pulmonary embolism or cerebral vascular accidents. Therefore, the significance of anticoagulants in preventing thrombosis cannot be underestimated.

In the Netherlands it is estimated that 1.8 per 1000 patients annually will be diagnosed with a form of thrombosis. The incidence is increased in older patients (over 75 years) to 6.5 per 1000 patients annually for men and 9.5 for women. The several known risk factors that increase the risk of developing venous thromboembolism are obesity (body mass index (BMI) > 30 kg/m²), elevated homocysteine, hospitalisation, surgery, immobility, cancer and genetic factors.

Accordingly, different classes of anticoagulants have been developed including: unfractionated heparin, low-molecular-weight heparin (LMWH), factor Xa inhibitors, coumarins and new/novel oral anticoagulants (NOACs). Despite their pharmacokinetic differences, inadvertent haemorrhage remains a potential complication for all anticoagulants. In order to prevent profound haemorrhagic adverse outcomes, individualised patient monitoring is highly recommended.

At present, the most commonly used anticoagulants are the LMWHs. They include: dalteparin, enoxaparin, nadroparin, parnaparin, reviparin, bemiparin and tinzaparin. These anticoagulants all contain an active pentasaccharide sequence that binds to antithrombin. The continuous anticoagulant effect is achieved upon binding this heparin, activating antithrombin, dissociating and subsequently binding to additional antithrombin. This binding produces a conformational change, accelerating antithrombin binding and inactivation of coagulation factors XIIa, IXa, XIa, Xa and thrombin.

LMWHs are administered subcutaneously in doses mostly adjusted to the patient’s weight, usually given once daily. Monitoring of LMWHs can be achieved through measuring anti-Xa levels four hours after administration. Routine monitoring is advisable in patients with renal impairment (glomerular filtration rate < 30 ml/min/1.73 m²), obesity (BMI > 30 kg/m²) and elderly patients (over 75 years).

In the first patient group, LMWH levels can accumulate because of impaired renal clearance. Measuring the anti-Xa level removes this uncertainty and allows dosing to be individually tailored, hereby allowing clinicians to minimise the risk of haemorrhagic complications.

However, it is worth noting that anti-Xa tests differ per specific LMWH. LMWHs with longer saccharide fragments (tinzaparin) tend to inhibit thrombin more profoundly compared with LMWHs with shorter fragments (enoxaparin). These shorter fragment LMWHs target the inhibition of factor Xa more specifically. These differences can be expressed using a factor Xa/FIIa ratio. As anti-Xa activity assays are considered the ‘gold standard’ for determining the plasma concentration of LMWH, they do not always correlate well with the in vivo drug effect. Instead, anti-Xa levels can be seen to reflect the pharmacokinetics rather than pharmacodynamics of the relevant LMWH. In a recent study, Thomas et al. suggest that combining anti-Xa levels with an aPTT level can provide essential dosing information in patients at increased risk of anticoagulant-induced haemorrhage, such as in renal impairment.

Nonetheless, in this issue Verhave et al. have demonstrated that in a prophylactic use setting the monitoring of anti-Xa levels alone is sufficient to accurately adjust an individual’s LMWH dose. They studied a small group of patients who were treated with LMWHs for prophylactic use in nocturnal haemodialysis. Their dosing algorithm provides a suitable guideline for future monitoring studies. As differences exist between anti-Xa levels for different LMWHs, more studies are needed to determine the type and frequency of anti-Xa monitoring.

Finally, the antineoplastic effect of LMWHs is an area of research that has received considerable attention. A recent
trial reported that LMWHs (i.e. nadroparin) used in cancer patients increased median survival, which showed a larger effect if life expectancy was greater than six months. Mechanistically, a lot of theories have been proposed but more research is needed. This potentially significant discovery could radically alter our perception of the role of anticoagulants.

In conclusion, LMWHs must be considered an essential pharmacological tool to prevent thrombosis. They remain relatively simple to use, requiring once or twice daily administration. However, in certain patient groups their effect can be unpredictable. Therefore, close monitoring with anti-Xa levels or anti-Xa levels and aPTT in combination can provide invaluable information. Finally, the application of anticoagulants solely for thrombosis prevention arguably underestimates their potential.

REFERENCES
REVIEW

Review on immunotherapy in airway allergen sensitised patients

J.P.M van der Valk*, N.W. de Jong, R. Gerth van Wijk

Department of Internal Medicine, section of Allergology, Erasmus MC, Rotterdam, the Netherlands,
*corresponding author: tel.: +31(0)10-7040100, email: j.p.m.vandervalk@erasusmc.nl

ABSTRACT

Allergen immunotherapy is a more than 100-year-old treatment in particular for birch pollen, grass pollen, house dust mite and cat dander sensitised allergic patients. The mechanism of allergen-specific immunotherapy is complex. Different hypotheses have been postulated to explain the mode of action, such as a decrease of the number of tissue mast cells, eosinophils and basophils, an increase of IgG4 and IgA synthesis, a shift from Th2 to Th1 cells and an increase in the number and function of IL-10 producing T-regulatory cells (T-reg). All these immunological effects may contribute to immune tolerance and long-term changes in the immune system. The efficacy and safety of subcutaneous (SCIT) and sublingual immunotherapy (SLIT) with pollen and house dust mite have been investigated in many trials, meta-analyses and reviews. Nowadays grass pollen SLIT and SCIT, and birch pollen and house dust mite SCIT are implemented in clinical practice to treat therapy-resistant patients. However, the treatment is not effective for all patients and often not without side effects. Therefore, the development of new, safer and more effective immunotherapies is needed. These are approached along novel routes, including improved administration, combined treatment with immune response modifiers, fusion with immune response modifiers, allergen coupled to adjuvants and reconstruction of natural extracts with multiple recombinant allergens or with modified allergens. These developments are promising, but more research is required to implement them in clinical practice.

KEYWORDS

Immunotherapy, SCIT, SLIT

INTRODUCTION

Immunotherapy with inhalation allergens is over 100 years old. The treatment is indicated for patients with birch pollen, grass pollen, house dust mite or cat dander allergy. The therapy induces allergen-specific immune tolerance for inhalation allergic patients and leads to a reduction of allergic symptoms in patients with rhinitis, conjunctivitis and allergic asthma. Immunotherapy is the only available effective treatment to target the disease instead of the symptoms. Whether immunotherapy is prescribed depends on the severity of symptoms, the effect of elimination measures, or medication and the desire of the patient to stop long-term pharmacological treatment. Currently, immunotherapy can be given either subcutaneously (SCIT) or sublingually (SLIT). Grass pollen has been registered for subcutaneous and sublingual administration, whereas birch pollen, house dust mite and cat dander have been registered for subcutaneous use only. Allergens are administered subcutaneously under medical supervision. The therapy has a build-up phase with, in general, weekly injections with an increasing concentration of the allergen. The duration of this build-up phase depends on the frequency of injections and ranges from 3-6 months. The build-up phase is followed by monthly maintenance injections for 3-5 years. Another option is to use an accelerated schedule (rush immunotherapy) for the build-up phase. This approach shortens the build-up phase substantially. A series of injections is administered in one visit. SLIT uses sublingual administration of drops or tablets under the tongue and is usually taken at home, except for the first dose. The safety profiles of SCIT when using the conventional schedule or the rush schedule are comparable. The safety profile of SLIT is superior to SCIT. Which route of immunotherapy is used depends on several factors such as vaccine availability, patient characteristics, cost, and physician and patient preferences.1,2
MECHANISM

The mechanisms of allergen-specific immune response are complex and not fully understood. For this response, an early and a late phase can be distinguished. In the early phase of immunotherapy a decrease occurs in the number of tissue mast cells, eosinophils and basophils along with a reduction in mediator release. Basophil decrease is caused by up-regulation of H2 receptors, which causes inhibition of FcεRI-mediated release of histamine and other mediators. Also an increase of IgG4 and IgA synthesis is observed in the early phase of immunotherapy. IgG4 blocks the interaction between IgE and the allergen and the presentation of the allergen to T cells. In the late phase, after one to several months of immunotherapy, a shift occurs from Th2 to Th1 cells, as well as an increase in the number and function of two types of T-regulatory cells (T-reg) cells. The two types of T-reg cells are the natural T-reg (nT-reg) cells and inducible T-reg (iT-reg) cells. iT-reg cells are IL-10 secreting cells derived from naïve CD4+ T lymphocytes and are an important factor in peripheral tolerance induction. IL-10 inhibits the production of IgE, enhances IgG4 and is directly involved in the suppression of allergen-specific T-effector cells. nT-reg cells (CD4+, CD25+ and FOXP3+ (Forkhead box protein 3)) originate from the thymus and show a corresponding function to the iT-reg cells. nT-reg cells express high levels of IL-10 and TGF-beta. T-reg cells induce IL-10 secreting dendritic cells. These dendritic cells play a role in the activation and differentiation of T-cells into different subtypes. These are able to cause inflammatory response or immune tolerance depending on their maturity stage. iT-reg cells induce suppression of IgE production by effector B-cells. Recently, IL-10 secreting B-cells have been detected in venom-allergic patients after immunotherapy. IL-10 secreting natural killer cells have also been reported to play a role in the immune tolerance by antigen-specific T-cell suppression and via a decrease in IgE production. However, more research is needed to reveal their specific role. All these immunological effects, i.e. decrease in the number of tissue mast cells, eosinophils and basophils, an increase of IgG4 and IgA synthesis, a shift from Th2 to Th1 cells and an increase in the number and function of IL-10 producing T-reg cells, may contribute to long-term immune tolerance and to a change in the immune system (figure 1). The immunological mechanisms in SCIT and SLIT are considered to be identical.

Figure 1. The mechanism of immunotherapy is shown in this figure. The number of mast cells, basophils and eosinophils decreases and the production of IgG4 and IgA increases. A shift from Th2 cells to Th1 cells and an increase of T-reg cells occurs during immunotherapy. IL-10 plays a important role in this induction of immune tolerance.
Table 1. High-evidence studies on the efficacy and safety of immunotherapy

<table>
<thead>
<tr>
<th>Study year</th>
<th>Author</th>
<th>Type of article (n = studies)</th>
<th>Study allergen</th>
<th>Administration</th>
<th>Efficacy</th>
<th>Safety</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>Calderon et al.</td>
<td>Cochrane meta-analysis (n=15)</td>
<td>Tree, grass and weed pollen</td>
<td>SCIT</td>
<td>Significant reduction in symptom score and medication usage</td>
<td>Adrenalin administered in 0.13% of actively treated group</td>
</tr>
<tr>
<td>2011</td>
<td>Radulovic</td>
<td>Meta-analysis (n = 49 RCTs)</td>
<td>Grass pollen</td>
<td>SLIT</td>
<td>Significant reduction in symptom score and medication requirement</td>
<td>None of the trials reported severe allergic reactions</td>
</tr>
<tr>
<td>2012</td>
<td>Di Bona et al.</td>
<td>Meta-analysis (n = 36 RCTs)</td>
<td>Grass pollen</td>
<td>SCIT</td>
<td>Significant clinical benefit from SCIT</td>
<td>0.86 AE/patient receiving SCIT; 12 anaphylactic reactions</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SLIT</td>
<td>Mild-to-moderate benefit from SLIT</td>
<td>2.13 AEs/patient receiving SLIT; one anaphylactic reaction</td>
</tr>
<tr>
<td>2009</td>
<td>Compalati et al.</td>
<td>Meta-analysis (n = 17)</td>
<td>HDM</td>
<td>SLIT</td>
<td>Reduction of symptoms and medication score</td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>Calderon et al.</td>
<td>Evidence-based analysis (n = 44)</td>
<td>HDM</td>
<td>SCIT</td>
<td>Significant favourable effect of SCIT vs placebo on symptom score</td>
<td>Safety aspects poorly described Serious side effects which required adrenaline were reported</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SLIT</td>
<td>Significant difference in symptom score in benefit of SLIT vs placebo in some studies</td>
<td>Safety aspects are reported very rarely</td>
</tr>
<tr>
<td>1997</td>
<td>Varney et al.</td>
<td>Double-blind, randomised, placebo-controlled study</td>
<td>Cat dander</td>
<td>SCIT</td>
<td>Mean number of symptoms, peak flow response, and conjunctival provocation sensitivity decreased</td>
<td>No serious immunotherapy-related side effects were reported</td>
</tr>
</tbody>
</table>

AE = adverse event; HDM = house dust mite; RCT = randomised controlled trial; SCIT = subcutaneous immunotherapy; SLIT = sublingual immunotherapy.

EVIDENCE FOR EFFICACY AND SAFETY OF SCIT AND SLIT

Pollen

Many studies have been performed in patients with a seasonal allergy on the efficacy of SCIT and SLIT. Three meta-analysis on this topic have appeared (table 1).

The Cochrane meta-analysis of Calderon et al. (2007) evaluated the safety and efficacy of allergen (tree, grass and weed) SCIT for patients with seasonal allergic rhinitis. In the symptom score meta-analysis 15 studies (n = 597 immunotherapy, n = 466 placebo) and in the medication score meta-analysis 13 studies (n = 549 immunotherapy, n = 414 placebo) were included. The authors conclude that...
a significant reduction in symptom score and medication usage can be achieved with immunotherapy. With regard to the safety aspects of this treatment, adrenaline was administered to only 0.13% of the actively treated patients to cope with adverse events due to the immunotherapy. The publication by Radulovic et al. (2011) is a systematic review on the efficiency and safety of SLIT. A total of 49 RCTs were used for the meta-analysis to evaluate the effect on symptom score (n = 2333 immunotherapy, 2236 placebo) and 38 RCTs (n = 1737 immunotherapy, 1642 placebo) to evaluate the effect on medication score. A significant reduction in symptom score and medication requirements was measured in the actively treated group compared with the placebo group. Severe systemic reactions such as anaphylaxis were not reported in any of these trials. Grazax® and oralair® are used for SLIT therapy in clinical practice since they were registered in Europe in 2006 and 2008, respectively. The meta-analysis by Di Bona et al. (2012) has demonstrated the effect of grass pollen SCIT and SLIT on symptom score and medication score in 36 RCTs (immunotherapy n = 3014, placebo n = 2768). This study evaluated the standardised mean difference between SLIT, SCIT and placebo: there appears to be a significant clinical benefit for patients treated with SCIT and a mild-to-moderate benefit for patients treated with SLIT, both compared with placebo. Data on medication score have been analysed for 20 SLIT RCTs and 11 SCIT RCTs. These analyses showed a significantly better pooled mean estimation of treatment with SLIT and SCIT on the medication score compared with placebo. This study reported a total of 960 adverse events (AEs) due to the immunotherapy (0.86 AEs/patient for SCIT and 2.13 AEs/patient for SLIT). Paradoxically, the total number of AEs reported was higher in the SLIT group compared with SCIT group. However, these AEs varied from mild to severe and in total 12 anaphylactic reactions were reported for SCIT-treated patients and one for SLIT-treated patients. In summary, SCIT and SLIT generally achieve a reduction in symptom score and medication usage, but these treatments are not without local and systemic side effects. SCIT has been found effective and safe for birch pollen and grass pollen sensitised patients, whereas SLIT was only effective and safe for grass pollen sensitised patients.

House dust mites
Only a few meta-analyses have been performed on the effectiveness of house dust mite immunotherapy. First, Compalati et al. (2009) performed a meta-analysis on the efficacy and safety of house dust mite SLIT in allergic rhinitis patients and allergic asthma patients. The data from eight of the 12 selected RCTs (n = 382 allergic rhinitis patients and n = 476 allergic asthma patients) in allergic rhinitis patients were included in this meta-analysis, revealing a reduction of symptoms and medication score. Similar results have been found with allergic asthma patients, based on nine out of the 12 selected RCTs. These led the authors to conclude that there is promising evidence of efficacy of house dust mite SLIT for patients with allergic rhinitis and allergic asthma, but more high-quality studies are required to substantiate this. Another study was initiated as an evidence-based analysis of house dust mite allergen SLIT and SCIT for allergic asthma and allergic rhinitis patients. Nine out of 19 selected studies on SCIT (n = between 22 and 132 randomised participants per study) in patients with allergic asthma were found to reveal a significant difference in mean ± SD symptom score for symptom-related parameters. Unfortunately, the safety aspects were rather poorly described in these studies: nearly all serious side effects, which required adrenaline, were reported to occur during the rush dosing phase. Only two of the 14 selected studies (n = between 15 and 465 randomised participants per study) on SLIT in allergic asthma patients demonstrated a significantly better symptom score of SLIT over placebo. Safety aspects were scarcely reported in these studies. However, a few studies have specifically mentioned that no adverse events occurred. Three out of seven studies (n = between 32 and 145 randomised participants per study) on SCIT in allergic rhinitis patients reported a significantly favourable effect of SCIT versus placebo on the symptom score. The quality of safety reporting was low in most of these studies. However, anaphylactic reactions were reported in only one study. Two out of 15 studies (n = between 15 and 257 randomised participants per study) on SLIT in allergic rhinitis patients showed significant benefit of SLIT versus placebo on symptom score. The conclusion was that there were too many shortcomings in primary and secondary efficiency criteria in all of the considered studies to allow a solid meta-analysis on the efficiency of house mite dust immunotherapy. However, they were able to establish statistically that SCIT shows a significantly favourable effect compared with SLIT and placebo. The safety aspects were described only moderately. No serious AEs due to the immunotherapy were reported in the actively treated group.

Cat dander
To our knowledge, the only published study on the effectiveness of cat immunotherapy was performed by Varney et al. This is a double-blind, randomised, placebo-controlled study with 28 cat allergic patients, investigating the efficacy of immunotherapy with standardised cat dander extract. This study established that immunotherapy with standardised cat dander is an effective treatment for cat allergic patients. No serious immunotherapy-related side effects were reported in this study.
In conclusion, the efficacy and safety of pollen, house dust mite and cat dander SCIT and SLIT have been investigated in many trials, meta-analyses and reviews and are implemented in clinical practice to treat therapy-resistant patients, because of the good safety profile and effectiveness in most patients.

**Prevention**

There is evidence that immunotherapy can prevent the development of asthma and new sensitisations. The first prospective long-term follow-up study on this topic is the Preventive Allergy Treatment (PAT) study. Children with seasonal rhinoconjunctivitis (n = 259) were selected from three paediatric allergy centres and randomised into a group receiving grass and/or birch pollen specific immunotherapy (SIT) for three years and an open placebo group. The SIT group showed significantly fewer asthma symptoms and improved results of methacholine bronchial provocation testing after three years compared with the placebo group. The subsequent follow-up of the PAT study performed by Jacobsen et al. evaluated the even longer-term clinical effect and the preventive effect on developing asthma in 147 patients treated with grass and/or birch pollen SIT. A significant improvement of rhinoconjunctivitis and conjunctival sensitivity persisted seven years after a three-year SIT treatment (ten-year follow-up.) Also significantly less patients in the SIT group developed asthma evaluated by clinical symptoms after ten years.

The retrospective study by Purello-D’Ambrosio et al. based on 8396 mono-sensitised patients, treated with immunotherapy or pharmacotherapy (groups are well balanced in numbers), demonstrated that 27% of the patients treated with immunotherapy were poly-sensitised after seven years and even 77% of the pharmacological group. Asthmatic patients were more prone to develop poly-sensitisation than rhinitis patients.

**New developments**

Immunotherapy in its current form is not without side effects and patient adherence still remains a problem. Immunotherapy is cost-effective for both SCIT and SLIT compared with symptomatic therapy from around six years (threshold of £20,000-30,000 per quality-adjusted life years, QALY), but these costs remain significant. Furthermore, there are still problems with the efficacy. The extrinsic factors that might play a role in the effectiveness/ineffectiveness of immunotherapy are the doses of the allergen used, the allergens present in the preparation, the route of administration, the indication for which it is prescribed and therapy adherence. It is not clear which intrinsic factors in the immune mechanisms are important per patient for establishing whether the immunotherapy actually translates into clinical tolerance/improvement of symptoms or failure of immunotherapy non-responders. The costs, ineffectiveness of immunotherapy in some patients and the safety aspects stimulate researchers to develop new immunotherapy strategies. Targets are numerous: novel route of administration, combined treatment with immune response modifiers, fusion with immune response modifiers and allergen coupled to adjuvants. Other targets are reconstruction of natural extracts with multiple recombinant allergens (allergens produced by recombinant DNA technology) or with modified allergens (altered composition of allergens) or the use of peptides of allergens as extracts (table 2).

Novel routes are, for example, intralymphatic or epicutaneous administration, which have both already scored in efficiency and safety in clinical trials. Pretreatment patients with anti-IgE omalizumab before immunotherapy, and an optional period during immunotherapy, caused a significant decrease of systemic allergic reactions and a decrease in the need for rescue medication to suppress symptoms. The fusion of allergens with immune response modifiers should ensure a more effective form of immunotherapy with fewer side effects. The fusion with human Fcy receptors inhibits basophil and mast cell degranulation by cross-linking of Fcy and FcRRI receptors. The major cat allergen Felis domesticus 1 (Fel d 1) was fused to TAT peptide and to a part of the human variant chain, creating a transporter (MAT) vaccine (MAT-Fel d 1.) MAT-Fel d 1 on intralymphatic administration has proved to be safe and effective for immunomodulation. Allergens are also coupled to GpG oligonucleotide, virus-like particles, monophosphoryl lipid A, carbohydrate-based particles and hepatitis B PreS to cause a shift in immune reaction, to increase the efficiency or to decrease the side effects of the immunotherapy. These forms of immunotherapy show promising results in humans and mouse models. The efficacy and safety of immunotherapy with a mixture of grass pollen components (allergenic proteins in allergens) to allergic patients demonstrated a strong IgG response. The idea behind immunotherapy with modified allergens is to induce T-cell tolerance and to avoid IgE-related side effects. The allergens can be administered in allergen fragments, fusions, hybrids or chimeras. Fel d 1-derived cat peptides have been proven safe and well tolerated by patients with a cat allergy.

In summary, these are approached along novel routes including improved administration, combined treatment with immune response modifiers, fusion with immune response modifiers, allergen coupled to adjuvants and reconstruction of natural extracts with multiple recombinant allergens or with modified allergens. These are promising new developments, but more research is required to implement these in clinical practice.
CONCLUSION

Allergen immunotherapy has been prescribed now over a long period for birch pollen, grass pollen, house dust mite and cat allergic patients. The efficacy and safety have been proven in many years of practice, despite the fact that the immunological reactions are not fully understood. This treatment has a lot of benefits for patients, such as decrease of symptoms, decrease of medication usage, long-term effect and prevention of new sensitisation or asthma. Therefore, immunotherapy should be prescribed more often as therapeutic option, in particular if you take the high prevalence of airway allergy into consideration.

However, the development of new immunotherapy methods will continue to improve the prolonged period of the treatment, the side effects and the ineffectiveness for some patients. Despite the fact that the results of the new developments are promising, more research is needed to implement these new technics in clinical practice.

DISCLOSURES

The authors declare no conflicts of interest.

Table 2. New developments in immunotherapy

<table>
<thead>
<tr>
<th>New methods</th>
<th>Specified</th>
<th>Expected value</th>
<th>Status efficacy and safety</th>
</tr>
</thead>
<tbody>
<tr>
<td>Different routes of administration</td>
<td>Intralymphatic</td>
<td>Inducing faster immune tolerance</td>
<td>Proven in humans[24,25]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Causing less side effects</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Lasting longer</td>
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<tr>
<td></td>
<td></td>
<td>Improvement of compliance</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Epicutaneous</td>
<td>Improvement of compliance</td>
<td>Proven in humans[23,26]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reducing risk of allergen leaking into the circulation/</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>provoking systemic reactions</td>
<td></td>
</tr>
<tr>
<td>Allergens combined with immune response modifiers</td>
<td>Anti-IgE Omalizumab</td>
<td>Reducing systemic reactions</td>
<td>Proven in humans[27,28]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Obtaining a higher percentage patients on maintenance target dose</td>
<td></td>
</tr>
<tr>
<td>Allergen fusion with immune response modifiers</td>
<td>Human Fcy receptor</td>
<td>Inhibition of basophil and mast cell degranulation by cross-linking of Fcy</td>
<td>Proven in mouse models[29]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and FcεRI receptors</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Transactivator of transcription peptide</td>
<td>Targeting allergen to the MHC class II pathway</td>
<td>Proven in humans[9]</td>
</tr>
<tr>
<td>Allergens coupled with adjuvants</td>
<td>gpG oligonucleotide</td>
<td>Causing a shift in immune reaction to increase the efficiency and decrease the</td>
<td>Promising results in humans</td>
</tr>
<tr>
<td></td>
<td>Virus-like particles</td>
<td>side effects</td>
<td>and mouse models[25]</td>
</tr>
<tr>
<td></td>
<td>Monophosphoryl lipid A</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carbohydrate-based particles</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hepatitis B PreS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reconstruction of natural extracts with multiple</td>
<td>Mixture of grass pollen components</td>
<td>Increase efficacy and safety by inducing more specific immune tolerance</td>
<td>Proven in humans[32]</td>
</tr>
<tr>
<td>recombinant allergens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reconstruction of natural extracts with modified</td>
<td>Allergen fragments, fusions, hybrids and chimeras</td>
<td>Inducing T–cell tolerance and avoiding IgE related side effects by using more</td>
<td>Promising results in humans</td>
</tr>
<tr>
<td>allergens</td>
<td></td>
<td>rational and safer allergen preparations</td>
<td>and mouse models[27]</td>
</tr>
<tr>
<td>Allergen peptides</td>
<td>Fel d 1 –derived cat peptides</td>
<td>Improvement of safety and reduce the duration of treatment</td>
<td>Proven in humans[31]</td>
</tr>
</tbody>
</table>
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Anticoagulation with dalteparin and nadroparin in nocturnal haemodialysis

G. Verhave1, M.C. Weijmer2, B.C. van Jaarsveld3*

1Department of Internal Medicine, Gemini Hospital, Den Helder, the Netherlands, 2Department of Internal Medicine, Sint Lucas Andreas Hospital, Amsterdam, the Netherlands, 3Department of Nephrology, VU University Medical Centre Amsterdam and Diapriva Dialysis Centre Amsterdam, the Netherlands, *corresponding author: tel.: +31(0)20-4444444, fax: +31(0)20-4442675, email: b.jaarsveld@vumc.nl

ABSTRACT

Background: Low-molecular-weight heparins (LMWHs) are increasingly used as anticoagulant during haemodialysis. The aim of this study is to establish the efficiency and duration of anticoagulation with dalteparin and nadroparin administration in patients treated with nocturnal haemodialysis.

Methods: All patients were treated with nocturnal in-centre haemodialysis, 3-4 times a week. Anticoagulation was obtained with dalteparin (n = 15) or nadroparin (n = 10). Anti-factor-Xa activity was measured during a midweek dialysis session at t = 0, 4 and 8 hours.

Results: The LMWH dose necessary to prevent extracorporeal circuit clotting was higher for dalteparin than for nadroparin. In the dalteparin group, anti-Xa activity was almost negligible at the start of dialysis whereas most patients on nadroparin still had anti-Xa activity at the start of dialysis (0.08 (IQR 0.05-0.11) IU/ml), reflecting the effect of previous LMWH administration. After eight hours of dialysis, median anti-factor-Xa activity was 0.49 (IQR 0.22-0.57) after dalteparin and 0.69 (IQR 0.55-0.83) after nadroparin (p = 0.01). When a target range of 0.2-0.6 IU/ml was applied, the present dosing method led to over-anticoagulation in more than half of the patients.

Conclusion: Administration of two doses of LMWH is an effective method of anticoagulation in nocturnal, eight-hour haemodialysis. With two doses of dalteparin, a larger proportion of patients reached but did not exceed target levels of anticoagulation, compared with two doses of nadroparin. Nadroparin caused prolonged anti-Xa activity with measurable anticoagulation up to the next dialysis session. The measurement of anti-Xa activity is advocated for dose assessment of LMWH, when LMWH is used as anticoagulant during nocturnal haemodialysis.

KEYWORDS

Nocturnal haemodialysis, long intermittent nocturnal haemodialysis, LMWH, dalteparin, nadroparin, anti-Xa activity, anticoagulation

INTRODUCTION

During haemodialysis, systemic anticoagulation is necessary to avoid clotting of blood in the extracorporeal circuit. For this purpose, low-molecular-weight heparins (LMWHs) have emerged in recent years as a good alternative to unfractionated heparin. LMWHs form a heterogeneous group of preparations with variable molecular weights and half-lives. Half-life in renal failure is extended, as most LMWHs are cleared by renal excretion. Due to the lower molecular weight, LMWHs inactivate thrombin less than unfractionated heparin, and exert their action mainly by inhibiting factor Xa. This specific property can be expressed as the anti-factor Xa to thrombin ratio (anti-Xa/IIa). LMWHs have proven to be safe and well tolerated for the use in haemodialysis patients. In comparison with unfractionated heparin, LMWHs have a more rapid onset of action, cause less platelet activation and less fibrin deposition on dialyser surfaces, enabling better urea clearance. A further advantage is the simplicity of use: a single bolus injection at the start of a dialysis session results in enough anticoagulation to cover a four-hour period. The downside is their reduced reversibility by protamine and the lack of a bedside monitoring test, although such a test is being developed. Because of the variable duration of action, the dose of LMWH one should administer is established empirically during subsequent haemodialysis sessions by titrating the
dosages according to visibility of clot in the dialyser. With the growing use of intensive haemodialysis techniques, such as nocturnal haemodialysis, there is a need for detailed data on anticoagulant activity with different LMWHs during long dialysis sessions. Therefore, we studied the anti-Xa activity after dalteparin and nadroparin, the preparations most commonly used in the Netherlands, in patients on nocturnal haemodialysis.

SUBJECTS AND METHODS

Patients
Nocturnal haemodialysis patients were included from two different dialysis units, using dalteparin as anticoagulant (centre A) and using nadroparin as anticoagulant (centre B). All patients had end-stage renal disease (ESRD) and were treated with intermittent nocturnal in-centre haemodialysis for at least three months. Exclusion criteria were known malignancy and haemorrhagic or thrombotic coagulation disorders. Written informed consent was obtained from all patients.

Haemodialysis protocol
Patients were treated 3-4 nights a week with a session length of eight hours. Both centres used polysulfone filters, in centre A a polysulfone APS 18U filter (Asahi) on a Nikkiso DBBo 05 monitor, and centre B a polysulfone Fx8 or Fx10 filter (Fresenius) on a Fresenius 4008 monitor. The extracorporeal systems were primed with isotonic saline without anticoagulant. Blood flow varied between 100-300 ml/min and dialysate flow was 300-500 ml/min, depending on the patient’s needs.

Dose finding of LMWHs
Both dalteparin (Fragmin®, Pfizer, New York City, US) and nadroparin (Fraxiparin®, GlaxoSmithKline, Brentford, UK) were given as a bolus injection into the arterial tubing line prior to haemodialysis, followed by a second bolus injection four hours after the start of haemodialysis. The dosing algorithm prescribed a second bolus of half the dose of the first one when a patient started his first session of nocturnal haemodialysis. Hereafter, the individual LMWH dose requirement was adjusted by a stepwise protocol, based on judgement of the extracorporeal circuit at the end of treatment by experienced haemodialysis nurses (no clotting in air traps, clean dialyser, time to stop bleeding from access needle sites). The study was performed when patients were on stable anticoagulant dosages for at least four weeks, during a midweek session after a one-night dialysis-free interval.

Blood sampling
Blood samples were taken at the start of haemodialysis treatment, and at t = 4 and t = 8 hours. The first sample was drawn from the arterial dialysis needle after clean puncture of the fistula and before application of LMWH. To avoid artificial coagulation activation, the first 5 ml of blood were discarded. During extracorporeal circulation and at the end of dialysis, blood was collected from the arterial line of the circuit into a 4.5 ml vacutainer (Becton Dickinson & Company, Franklin Lakes, US) containing 0.5 ml of 3.2% sodium citrate, and processed without delay. Laboratory assessment of anti-factor-Xa activity was performed using the Biophen Heparin (LRT) chromogenic assay.

Statistical analysis
Differences between the two groups were evaluated by two-sided Student’s t-test (for data with a Gaussian distribution) or Mann-Whitney test (for data with a non-Gaussian distribution). Differences in proportions between the two groups were evaluated by Fisher’s exact test. Computations were performed using IBM SPSS Statistics software (version 22).

RESULTS
Patient demographics, medication use, and dialysis adequacy are shown in table 1. Mean age was 55.1±17.1 years and dialysis vintage was about 1.5 years. Dialysis access consisted of a native fistula in 18 patients and a PTFE graft in seven patients. No premature interruptions of the haemodialysis sessions occurred by circuit clotting in any of the patients. No prolonged bleeding times from access sites nor other bleeding complications were noted.

Dose of LMWH
After individual adjustment, the LMWH dose necessary to prevent extracorporeal circuit clotting was higher for dalteparin than for nadroparin. At t = 0, the mean dalteparin dose was 6667±1543 IU (95±18 IU/kg), compared with 4845±1870 IU (72±23 IU/kg) for nadroparin (p = 0.01). At t = 4 h, the mean dalteparin dose was 308±1238 IU (45±16 IU/kg), compared with 275±181 IU (41±11 IU/kg) for nadroparin (NS, table 2).

Anti-Xa activity
In the dalteparin-treated patients, anti-Xa activity was almost negligible at the start of dialysis (IQR 0.00-0.02 IU/ml). In the nadroparin-treated patients, most patients had some degree of anti-Xa activity at the start of dialysis: 0.08 (IQR 0.05-0.11) IU/ml. This implies that anticoagulant activity by nadroparin persisted up to 40 hours after the previous dialysis session. Figure 1 shows the mean anti-Xa activity at start of dialysis, halfway and at the end of dialysis. After four hours, median anti-factor-Xa activity was 0.34 IU/ml (IQR 0.25-0.51) in the dalteparin group and 0.55 IU/ml (IQR 0.32-0.73) in the...
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After eight hours these values were 0.49 (0.22-0.57) and 0.69 (0.55-0.83) respectively. Patients on nadroparin had higher levels of anti-Xa activity throughout the complete dialysis session.

After exclusion of patients receiving vitamin K antagonists from the analysis, anti-Xa activities were essentially the same compared with the values of the complete patient group (data not shown).

Desired anti-Xa activity

Although there are no guidelines for the desired ranges of anti-Xa activity after LMWH administration in haemodialysis, various sources report target ranges between 0.2 and 0.6 IU/ml.6-8 When these ranges are applied to our patients, it appears that with the use of dalteparin most patients remained within the target range throughout nocturnal dialysis (figure 2). With the use of nadroparin, about half of the patients were already over-anticoagulated halfway through the dialysis session, and 75% of patients had anti-Xa activity > 0.60 at the end of dialysis.

**DISCUSSION**

Administration of two doses of LMWH is an effective way of anticoagulation in nocturnal haemodialysis: anticoagulation was sufficient throughout the whole dialysis session in all patients, showing that both treatments were effective without signs of clotting or

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**Table 1. Patient characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total n = 25</th>
<th>Dalteparin n = 15</th>
<th>Nadroparin n = 10</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>84</td>
<td>93</td>
<td>70</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55.1±17.1</td>
<td>53.9±17.0</td>
<td>57.0±18.0</td>
<td>NS</td>
</tr>
<tr>
<td>Dry weight (kg)</td>
<td>69.8±16.8</td>
<td>70.3±14.8</td>
<td>69.0±20.3</td>
<td>NS</td>
</tr>
<tr>
<td>Vintage nocturnal haemodialysis (months)</td>
<td>19.3±10.8</td>
<td>20.5±12.4</td>
<td>17.4±8.1</td>
<td>NS</td>
</tr>
<tr>
<td>Use of acenocoumarol (%)</td>
<td>20</td>
<td>27</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>Use of aspirin (%)</td>
<td>12</td>
<td>13</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>Haemoglobin (mmol/l)</td>
<td>7.4±0.8</td>
<td>7.2±0.8</td>
<td>7.6±0.8</td>
<td>NS</td>
</tr>
<tr>
<td>Thrombocytes (10^9/l)</td>
<td>228±56</td>
<td>239±53</td>
<td>212±60</td>
<td>NS</td>
</tr>
<tr>
<td>Blood flow (ml/min)</td>
<td>211±42</td>
<td>203±22</td>
<td>224±61</td>
<td>NS</td>
</tr>
<tr>
<td>Urea before dialysis (mmol/l)</td>
<td>20.9±5.6</td>
<td>23.4±3.1</td>
<td>17.2±6.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Urea after dialysis (mmol/l)</td>
<td>4.8±2.3</td>
<td>5.4±1.6</td>
<td>3.8±2.9</td>
<td>NS</td>
</tr>
<tr>
<td>Urea reduction rate</td>
<td>0.78±0.10</td>
<td>0.77±0.10</td>
<td>0.79±0.15</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Table 2. Dose of LMWH and anti-Xa activity during nocturnal haemodialysis**

<table>
<thead>
<tr>
<th>Dose of LMWH</th>
<th>Total n = 25</th>
<th>Dalteparin n = 15</th>
<th>Nadroparin n = 10</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMWH dose t = 0 (IU)</td>
<td>6667±1543</td>
<td>4845±1870</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>LMWH dose/kg t = 0 (IU/kg)</td>
<td>95±18</td>
<td>72±23</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>LMWH dose t = 4 h (IU)</td>
<td>308±1238</td>
<td>2755±831</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>LMWH dose/kg t = 4 h (IU/kg)</td>
<td>45±16</td>
<td>4±11</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>anti-Xa t = 0 (IU/ml)*</td>
<td>0.02 (0.01-0.06)</td>
<td>0.01 (0.00-0.02)</td>
<td>0.08 (0.05-0.11)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>anti-Xa t = 4 h (IU/ml)*</td>
<td>0.36 (0.28-0.56)</td>
<td>0.34 (0.25-0.51)</td>
<td>0.35 (0.12-0.73)</td>
<td>0.07</td>
</tr>
<tr>
<td>anti-Xa t = 8 h (IU/ml)*</td>
<td>0.36 (0.39-0.69)</td>
<td>0.49 (0.22-0.57)</td>
<td>0.69 (0.55-0.83)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*median (IQR); LMWH = low-molecular-weight heparin.
bleeding. The use of nadroparin led to higher levels of anti-Xa activity with longer duration of action, compared with anticoagulation with dalteparin. This is the first study to assess the level of anticoagulation with LMWH in eight-hour dialysis, and the first one to describe different types of LMWH in this dialysis modality.

Marked differences exist between several LMWH preparations due to different methods of depolymerisation. As a result, they vary in molecular weight, elimination rate and anticoagulatory effect. Dalteparin has a higher molecular weight than nadroparin (table 3). This leads to combined hepatic and renal clearance, whereas nadroparin is eliminated by the kidney only. Elimination half-life is longest and antiXa/IIa ratio is highest for nadroparin. In short, the properties of dalteparin lie in between those of unfractionated heparin and nadroparin. Although national pharmaceutical guidelines advise similar doses for both LMWHs (70 IU/kg),6,9 it is apprehensible from the above information that the required dose of nadroparin will be lower than that of dalteparin. This is consistent with data in healthy volunteers, in whom a 1.54 times greater anti-Xa activity for nadroparin was found compared with dalteparin.10 Despite the lower dose of nadroparin in our study, however, the level of anticoagulation measured by anti-Xa activity throughout dialysis was higher for nadroparin. This must be caused by slower elimination of nadroparin compared with dalteparin. As data on LMWH concentrations in dialysate are lacking, exact clearance of these drugs during haemodialysis is not known. Regarding anticoagulant effect during haemodialysis, Nigten et al. measured serial anti-Xa activity during conventional haemodialysis and extrapolated that the elimination of dalteparin follows a linear process.7

We measured detectable anti-factor Xa levels as long as 40 hours after the previous dialysis session in almost all of our patients on nadroparin. One could argue that this level of anticoagulation is not relevant, as our patients did not experience any bleeding complications. On the other hand, if a range of 0.2-0.6 IU/ml is considered adequate, the average dosing in our entire population was too high. Many patients reached anti-factor Xa levels > 0.6 IU/ml.
which seems to be more than needed for appropriate clot prevention. Our study has several limitations. First, nocturnal haemodialysis was performed in two different centres. Theoretically, it could have been possible that local habits in adjusting the LMWH dose influenced the results, but we consider this highly unlikely. Furthermore, the difference in dose and anti-Xa activity between dalteparin and nadroparin was large, and plausible in light of pharmacokinetic data. Second, the dosing of LMWH was adjusted according to subjective endpoints. However, this is the common way to adjust doses of anticoagulants, as most centres do not formally monitor dosage-response effects. Thirdly, different dialysis filters and extracorporeal systems were used in the two groups, but both were low-flux polysulfone filters, and no difference in LMWH clearance has been shown in four different types of low-flux filters. Finally, the present study included both patients without and with oral anticoagulant drugs in both groups; however, when we excluded these patients from our analysis the outcome was not influenced. The results of this study can be of use for the execution of long haemodialysis, either in-centre or at home. Compared with anticoagulation with continuous infusion of heparin, LMWH has the advantage of easy and quick administration, and less risk of dosing mistakes. For in-centre nocturnal haemodialysis, a regimen with two doses of dalteparin (at t = 0 and t = 4 hours) could be used, which is easy to use for dialysis nurses and guarantees adequate anticoagulation throughout the dialysis session.

**Table 3. Characteristics of heparin and low-molecular-weight heparins**

<table>
<thead>
<tr>
<th></th>
<th>Mean molecular weight (Dalton)</th>
<th>Elimination process*</th>
<th>Elimination half-life with normal GFR</th>
<th>Anti Xa/IIa ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin</td>
<td>5000-15,000</td>
<td>Hepatic</td>
<td>Dose-dependent 0.5-2.5 h</td>
<td>1.0</td>
</tr>
<tr>
<td>Dalteparin</td>
<td>5600</td>
<td>Slightly hepatic, mainly renal</td>
<td>2.3-2.8 h</td>
<td>2.0-2.7</td>
</tr>
<tr>
<td>Nadroparin</td>
<td>4500</td>
<td>Solely renal</td>
<td>3.7 h</td>
<td>3.2-3.7</td>
</tr>
</tbody>
</table>

*for heparin given after iv administration; for LMWH after subcutaneous administration; GFR = glomerular filtration rate.

**Figure 3. Algorithm for dosing of nadroparin and dalteparin in nocturnal dialysis; doses are adjusted to the available quantities in pre-packaged syringes: dalteparin is available in syringes with 25,000 IU/ml or 12,500 IU/ml and nadroparin syringes contain 9500 IU/ml**
without the disadvantage of extensive anticoagulation after dialysis. Of course, administering a second dose halfway through dialysis is not suitable for home haemodialysis, as the patient or his partner would have to wake up in the middle of the night to administer the second dose. Because of the prolonged action, one could choose to administer a single dose of nadroparin, with a possibility of extended anticoagulation after haemodialysis. This has been applied by others in five-hour sessions with a single high nadroparin dose of 12,500 IU. Based on our findings we propose an algorithm for dosing of nadroparin and dalteparin in nocturnal dialysis (figure 3). We acknowledge that further studies are needed to ensure adequate anticoagulation during eight-hour sessions with a single dose of nadroparin.

In conclusion, adequate and safe prevention of clot formation during long nocturnal haemodialysis can be established by using LMWH. In the present study, the use of dalteparin led to a large proportion of patients reaching and not exceeding target ranges of anticoagulation, whereas nadroparin showed prolonged duration of action up to the next dialysis session. Knowledge of these data is valuable in assessing adequate dosing of LMWH, and we advocate the measurement of anti-Xa activity when LMWH is used during nocturnal haemodialysis.

DISCLOSURES

The authors declare no conflicts of interest.

REFERENCES

ABSTRACT

Background: The disease-related components such as physical fitness and daily energy expenditure may change in each progressive period of type 2 diabetes. The purpose of this study was to compare the maximal aerobic capacity (VO₂ max), muscle strength, trunk flexibility, total energy expenditure, daily physical activity, resting metabolic rate (RMR), body composition, and body fat distribution in newly diagnosed type 2 diabetic patients with those of healthy controls.

Methods: Eighty patients (40 male, 40 female) with type 2 diabetes and 80 (40 male, 40 female) controls were included in this study. All participants completed the measurements.

Results: It was determined that the VO₂ max, muscle strength, daily number of steps, and total energy expenditure were lower, and the body fat percentage, and central obesity were higher in male and female type 2 diabetic patients, when compared with the controls. In addition, the lean body mass was decreased in male diabetic patients, compared with the controls. The fasting plasma glucose showed negative correlations with the maximal aerobic capacity, daily number of steps, and muscle strength in the patients in both genders. RMR and trunk flexibility values were not significantly different between the patients and the controls in either gender.

Conclusion: We suggest that using exercise intervention especially comprised of strength training and aerobic activities, including not only daily slow activities but also moderate to vigorous activities, as a lifestyle modification in newly diagnosed type 2 diabetic patients might be helpful for the development of earlier and more successful illness management strategies.

KEYWORDS

Type 2 diabetes, cardiorespiratory fitness, muscle strength, resting metabolic rate, body fat, lean body mass

INTRODUCTION

Type 2 diabetes mellitus has reached epidemic proportions worldwide, and is associated with obesity, and metabolic and cardiovascular disease. In the treatment of diabetes mellitus, reducing the risk of severe complications with irreversible organ damage is essential over the long-term. A multidisciplinary approach is also vital for health care professionals in the management of type 2 diabetes. There are several lines of scientific evidence demonstrating the role of physical inactivity in the aetiology, as well as the beneficial effects of exercise in both the prevention and treatment of type 2 diabetes and its related morbidity. A sedentary person may become even more metabolically unfit over the years; therefore, various forms of physical activity may be necessary to short-circuit unhealthy molecular signals causing metabolic disease.

Physical effort is an integral part of programs for the prevention and treatment of diseases. Health-related physical fitness includes maximal aerobic capacity (VO₂ max), muscle strength, flexibility, and body composition parameters. However, daily energy expenditure parameters are closely related to physical fitness. Identifying which health-related physical fitness and energy expenditure parameters are impaired in patients with newly diagnosed type 2 diabetes could contribute to the development of more beneficial physical rehabilitation strategies.
Thus, the aim of this study was to investigate health-related physical fitness parameters, such as maximal aerobic capacity, muscle strength, flexibility, body composition, and body fat distribution, and daily energy expenditure parameters, such as total energy expenditure, daily physical activity and resting metabolic rate (RMR) changes in newly diagnosed type 2 diabetic patients, and compare them with healthy controls.

MATERIALS AND METHODS

This study was designed as a comparative association analysis between patients with type 2 diabetes and healthy controls regarding daily energy expenditure, physical fitness, and anthropometric parameters. The study protocol was approved by the local Ethics Committee of Clinical Research, and all patients and controls participated voluntarily and provided written informed consent.

Subjects

Patients who were referred to the Department of Internal Medicine at the Afyon Kocatepe University Faculty of Medicine were preselected for the study following the diagnosis of type 2 diabetes by a specialist. The patients were considered to have type 2 diabetes mellitus if: the fasting plasma glucose (FPG) level was ≥ 126 mg/dl (7.0 mmol/l), and/or the second hour plasma glucose level was ≥ 200 mg/dl (11.1 mmol/l) following an oral glucose tolerance test, and/or random plasma glucose level was ≥ 200 mg/dl (11.1 mmol/l), and/or the value of the HbA1c was ≥ 6.5%, in addition to hyperglycaemia symptoms. The healthy controls were recruited from voluntary hospital staff and individuals who accompanied relatives during their hospital visits. Inclusion criteria required that patients had newly diagnosed type 2 diabetes, and no exercise-related risks. Exclusion criteria were acute infection, dehydration, cardiopulmonary and renal diseases, malignancy, the use of drugs that might affect heart rate (such as beta blockers), musculoskeletal disorders, psychiatric diseases (such as severe affective disorder and psychosis), and other metabolic diseases. In all, 80 patients (40 male, 40 female) with type 2 diabetes and 80 healthy controls matched for age and body mass index (BMI) (40 male, 40 female) were included in this study. Because of their biological differences, the analysis between patients with type 2 diabetes and healthy controls was approved by the local Ethics Committee of Clinical Research, and all patients and controls participated voluntarily and provided written informed consent.

Physiological measurements

Before the exercise test, the risk of exercise for the participants was assessed according to American College of Sports Medicine criteria. The maximum volume of oxygen consumed to produce energy was estimated with the Astrand test protocol, a valid submaximal exercise test for estimating \( VO_{max} \) (aerobic exercise capacity, or cardiorespiratory fitness). \(^7\) The Astrand test was performed on a computerised cycle ergometer (Monark 899E, Monark Exercise AB, Vansbro, Sweden). The subjects were asked to perform a six-minute submaximal exercise test, reaching a steady state heart rate. \(^8\) \( VO_{max} \) was expressed in litres per minute \( VO_{max} \) (l/min) and millilitres per kilogram of body weight \( VO_{max} \) (ml/kg/min).

Handgrip strength was measured with a digital dynamometer (Grip Strength Dynamometer T.K.K.5401, Takei Co., Tokyo, Japan). The patients were asked to hold the dynamometer parallel to the side of the body and squeeze the handgrip dynamometer as hard as possible while taking care not to hold their breath (Valsalva manoeuvre). The test was repeated three times with each hand, and the highest of the three scores was recorded. \(^9\) Back-leg strength was determined during the maximal isometric strength of the trunk muscles in the standing posture with 30 degrees of lumbar flexion using a digital back muscle strength meter (Back Strength Dynamometer T.K.K.5402, Takei Co., Tokyo, Japan). Participants were asked to stand upright on the base of the dynamometer with their arms straight and their fingers extended downward as far as possible on the fronts of their thighs. Then the participants bent forward slightly, grasped the bar, and lifted it as high as possible while keeping their legs straight and their feet flat on the base of the dynamometer. The test was repeated three times, and the highest of the three scores was recorded. \(^10\)

Trunk flexibility was assessed with a digital flexibility testing device (Standing Trunk Flexion meter T.K.K.5403, Takei Co., Tokyo, Japan) after stretching. The participants were asked to stand barefoot on a specially designed measuring bench, placing their toes even with the front edge of the bench. The mobile part of the scale was raised to the uppermost distance (20 cm). While standing on the bench, participants were asked to bend over and reach down as far as possible, without bouncing, while keeping their knees locked. The test was repeated three times, and the highest of the three scores was recorded. \(^11\) Daily physical activity and related parameters were monitored with a metabolic Holter monitor (SenseWear Armband MF-SW, BodyMedia Inc., Pittsburgh, PA, USA) 24 hours per day for seven days. The participants wore the armband over the triceps muscle of the left arm, at the midpoint between the acromion and olecranon. \(^12\) The metabolic Holter monitor continuously recorded an array of physiological data from the four sensors contained on the armband (skin temperature, galvanic skin response, heat flux, and near body temperature), as well as by triaxial accelerometry. These physiological data as well as the participant characteristics (including gender, age, height, weight, smoking status, and handedness) were then processed by the manufacturer’s software using...
advanced algorithms to calculate and report the total energy expenditure, daily number of steps, and daily sleep duration in a free-living environment. RMR was measured using an indirect calorimeter (Quark b’, Cosmed, Rome, Italy) with a computerised metabolic card, which analysed oxygen consumption and carbon dioxide production. The participants were asked not to eat for 12 hours and not to exercise for 24 hours before the test. After resting for 15 minutes, the measurements were applied to the subjects in a silent, unlit laboratory, which was at room temperature. The participants were asked to put on a face mask, lay in a supine position, and not move their arms or legs during the test.13

Anthropometric measurements
Body composition parameters were determined with a bioelectrical impedance analysis (BIA) system (Bodystat 1500, Bodystat Ltd., Douglas, Isle of Man, UK). The basic premise of the BIA procedure is that the volume of fat-free tissue in the body is proportional to the electrical conductivity of the body.14 Some precautions were taken before the measurements. The participants were instructed to avoid eating or drinking for four hours, using diuretics for seven days, participating in strenuous exercise for 24 hours, and consuming alcohol for 48 hours prior to the test procedure.14,15 The data were analysed using the manufacturer’s software, and body fat percentage and lean body mass were determined for each patient. Circumference measurements were taken at anatomical positions with a 7 mm wide tape measure. The tape measure was held parallel to the ground and completely surrounded the part of the body, but it did not compress the subcutaneous fat tissue.14 The waist, abdomen, and hip sites, which reflect central obesity, were used for circumference measurements.16 The waist-to-hip ratio was also calculated. BMI was calculated as the body weight divided by the square of the height (kg/m²).

Biochemical measurements
The peripheral venous blood samples were taken from all participants in the study after overnight fasting of at least eight hours. The blood samples were centrifuged for 5 minutes at 4000 rpm to obtain the serum samples; while the FPG and lipid levels which contained total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), and triglycerides (TG) were analysed in an autoanalyzer (Cobas 6000-c501, Roche Applied Sciences, Basel, Switzerland) using diagnostic kits (Roche Diagnostics, Mannheim, Germany). The HbA1c (glycated haemoglobin) analyses were performed using the electrophoretic method.

RESULTS
In all, 85 participants were invited to participate in the study. Five of them refused because they did not want to perform the exercise and other tests. None of the patients reported any problems during strength or aerobic exercise tests. The mean HbA1c (%) values were 9.1±2.7 and 8.1±2.5 in the male and female patients with type 2 diabetes, respectively. The mean values for age, BMI, body fat percentage, VO₂ max, strength, flexibility, RMR, daily number of steps, and total energy expenditure for the male and female patients and controls are shown in tables 1 and 2. The mean values for the RMR and trunk flexibility did not differ significantly between the male patients and controls. However, the body fat percentage, waist/hip ratio, abdominal circumference, and daily sleep duration were significantly higher, whereas the lean body mass, maximal aerobic capacity values (VO₂ max [l/min] and VO₂ max [ml/kg/min]), all strength measurements (back-leg strength, right and left handgrip strength), daily number of steps, and total energy expenditure were significantly lower in the male patients, compared with the controls. The mean values for the RMR, trunk flexibility, lean body mass, and daily sleep duration did not differ significantly between the female patients and the controls. However, the body fat percentage, waist/hip ratio, and abdominal circumference were significantly higher, whereas the maximal aerobic capacity values (VO₂ max [l/min] and VO₂ max [ml/kg/min]), all strength measurements, daily number of steps, and total energy expenditure were significantly lower in the female patients than in the controls. Tables 3 and 4 demonstrate the mean values of the blood pressure, FPG, and lipid profile in the male and female patients with type 2 diabetes and the healthy controls. The mean values for the diastolic blood pressure, TC, HDL-C, LDL-C, VLDL-C, and TG did not differ significantly.
between the male patients and the controls; nevertheless, the systolic blood pressure and FPG were significantly higher in the male patients, compared with the controls. The mean values for the HDL-C did not differ significantly between the female patients and the controls; however, the systolic and diastolic blood pressures, FPG, TC, LDL-C, VLDL-C, and TG were significantly higher in the female patients, compared with the controls.

Table 5 lists the correlations of FPG with other parameters that were found to be statistically significant in the male and female type 2 diabetic patients. The FPG showed positive correlations with body fat percentage, waist/hip ratio, and daily sleep duration, and negative correlations with maximal aerobic capacity values (VO$_2$ max [l/min] and VO$_2$ max [ml/kg/min]), right handgrip strength, back-leg strength, total energy expenditure, and daily number of

### Table 1. Characteristics of male patients with type 2 diabetes and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>50.2±6.7</td>
<td>49.1±6.6</td>
<td>0.492</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>29.9±3.1</td>
<td>29.3±3.2</td>
<td>0.879</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>36.8±7.7</td>
<td>33.5±6.4</td>
<td>0.015*</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>50.8±7.5</td>
<td>54.9±7.9</td>
<td>0.026*</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.98±0.05</td>
<td>0.94±0.04</td>
<td>0.001**</td>
</tr>
<tr>
<td>Abdominal circumference (cm)</td>
<td>104.0±8.9</td>
<td>98.9±9.2</td>
<td>0.013*</td>
</tr>
<tr>
<td>VO$_2$ max (l/min)</td>
<td>1.74±0.43</td>
<td>2.36±0.50</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>VO$_2$ max (ml/kg/min)</td>
<td>21.7±5.0</td>
<td>27.9±5.4</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>Right handgrip strength (kg)</td>
<td>37.0±7.2</td>
<td>43.3±6.7</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>Left handgrip strength (kg)</td>
<td>36.3±7.1</td>
<td>40.3±6.6</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>Back-leg strength (kg)</td>
<td>78.3±18.6</td>
<td>89.4±22.8</td>
<td>0.028*</td>
</tr>
<tr>
<td>Trunk flexibility (cm)</td>
<td>-4.7±7.5</td>
<td>-2.4±8.9</td>
<td>0.266</td>
</tr>
<tr>
<td>RMR (kcal/day)</td>
<td>1341.8±376.0</td>
<td>1276.6±371.4</td>
<td>0.153</td>
</tr>
<tr>
<td>Daily number of steps</td>
<td>7423.5±3857.7</td>
<td>9924.9±4597.8</td>
<td>0.018*</td>
</tr>
<tr>
<td>Total energy expenditure (kcal/day)</td>
<td>2692.8±361.4</td>
<td>2968.3±422.8</td>
<td>0.011*</td>
</tr>
<tr>
<td>Daily sleep duration (hour)</td>
<td>6.8±1.7</td>
<td>6.1±1.0</td>
<td>0.034*</td>
</tr>
</tbody>
</table>

**BMI = body mass index; VO$_2$ max = maximal aerobic capacity; RMR = resting metabolic rate; *p < 0.05; **p < 0.01.**

### Table 2. Characteristics of female patients with type 2 diabetes and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>51.5±7.7</td>
<td>50.4±7.8</td>
<td>0.350</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>32.2±5.0</td>
<td>32.0±5.9</td>
<td>0.877</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>50.8±7.7</td>
<td>45.9±8.4</td>
<td>0.026*</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>37.4±6.4</td>
<td>39.6±5.9</td>
<td>0.125</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.86±0.07</td>
<td>0.77±0.06</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>Abdominal circumference (cm)</td>
<td>108.9±11.2</td>
<td>98.5±12.5</td>
<td>0.001**</td>
</tr>
<tr>
<td>VO$_2$ max (l/min)</td>
<td>1.9±0.3</td>
<td>2.1±0.2</td>
<td>0.001**</td>
</tr>
<tr>
<td>VO$_2$ max (ml/kg/min)</td>
<td>19.9±5.9</td>
<td>24.1±6.1</td>
<td>0.002**</td>
</tr>
<tr>
<td>Right handgrip strength (kg)</td>
<td>21.3±4.8</td>
<td>24.9±6.1</td>
<td>0.005**</td>
</tr>
<tr>
<td>Left handgrip strength (kg)</td>
<td>20.7±4.0</td>
<td>23.5±5.3</td>
<td>0.012*</td>
</tr>
<tr>
<td>Back-leg strength (kg)</td>
<td>40.4±8.6</td>
<td>48.3±10.2</td>
<td>0.007**</td>
</tr>
<tr>
<td>Trunk flexibility (cm)</td>
<td>4.4±6.6</td>
<td>6.7±6.2</td>
<td>0.170</td>
</tr>
<tr>
<td>RMR (kcal/day)</td>
<td>1073.5±274.0</td>
<td>1095.7±221.9</td>
<td>0.308</td>
</tr>
<tr>
<td>Daily number of steps</td>
<td>6691.1±2151.4</td>
<td>7904.6±2519.2</td>
<td>0.032*</td>
</tr>
<tr>
<td>Total energy expenditure (kcal/day)</td>
<td>2204.2±312.0</td>
<td>2378.3±391.2</td>
<td>0.040*</td>
</tr>
<tr>
<td>Daily sleep duration (hour)</td>
<td>6.8±0.8</td>
<td>6.5±1.3</td>
<td>0.139</td>
</tr>
</tbody>
</table>

**BMI = body mass index; VO$_2$ max = maximal aerobic capacity; RMR = resting metabolic rate; *p < 0.05; **p < 0.01.**
steps in male patients. However, the FPG showed positive correlations with systolic and diastolic blood pressures; and negative correlations with the maximal aerobic capacity value (VO\textsubscript{max} [l/min]), right handgrip strength, back-leg strength, and daily number of steps in the female patients.

**DISCUSSION**

In this cross-sectional study, health-related physical fitness and daily energy expenditure parameters were investigated together and comprehensively in newly diagnosed (not treated) type 2 diabetic patients in both genders. We found that the VO\textsubscript{max}, muscle strength, daily physical activity, total energy expenditure, obesity, and body fat distribution were impaired, and firstly found that the RMR showed that the training program resulted in improved glucose tolerance, metabolic control, and management of illness. Parallel to the literature, we found that the daily step number, VO\textsubscript{max}, and total energy expenditure values were lower in male and female patients than in the controls (tables 1 and 2). The daily sleep duration was significantly higher in male patients, compared with the controls (table 1). In addition, the FPG value was negatively correlated with the daily number of steps and VO\textsubscript{max} values in the patients, in both genders, and negatively correlated with the total energy expenditure value in male patients (table 1). The VO\textsubscript{max} is considered to be the gold standard for the evaluation of cardiorespiratory fitness and/or physical functional capacity, and it is most often affected by moderate to vigorous activities, such as sports activities.

Previous cross-sectional studies have indicated that individuals with type 2 diabetes have a higher RMR. Conversely, we found no significant difference with regard to the RMR between the patients and controls in either gender (tables 1 and 2). The RMR is the

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**Table 3. Blood pressure, fasting plasma glucose and lipid profile of male patients with type 2 diabetes and healthy controls**

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 40)</th>
<th>Controls (n = 40)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>126.8±15.1</td>
<td>116.6±9.7</td>
<td>0.001**</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>81.1±9.3</td>
<td>78.9±8.1</td>
<td>0.263</td>
</tr>
<tr>
<td>FPG (mg/dl)</td>
<td>219.3±119.5</td>
<td>93.0±8.6</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>202.5±39.3</td>
<td>198.1±35.0</td>
<td>0.615</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>39.9±13.5</td>
<td>43.2±9.5</td>
<td>0.228</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>137.3±40.9</td>
<td>133.7±31.8</td>
<td>0.668</td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>41.4±24.9</td>
<td>31.3±18.1</td>
<td>0.059</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>208.8±119.6</td>
<td>158.6±90.1</td>
<td>0.053</td>
</tr>
</tbody>
</table>

SBP = systolic blood pressure; DBP = diastolic blood pressure; FPG = fasting plasma glucose; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol = VLDL-C = very low-density lipoprotein cholesterol; TG = triglycerides; *p < 0.05; **p < 0.01.

**Table 4. Blood pressure, fasting plasma glucose and lipid profile of female patients with type 2 diabetes and healthy controls**

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 40)</th>
<th>Controls (n = 40)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>127.4±13.9</td>
<td>116.5±13.3</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>81.1±9.0</td>
<td>76.8±8.0</td>
<td>0.019</td>
</tr>
<tr>
<td>FPG (mg/dl)</td>
<td>183.1±86.5</td>
<td>89.6±9.1</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>206.3±40.7</td>
<td>184.4±34.2</td>
<td>0.022</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>48.9±13.7</td>
<td>53.3±14.2</td>
<td>0.225</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>138.6±37.2</td>
<td>117.1±31.2</td>
<td>0.008**</td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>29.7±17.5</td>
<td>20.9±10.8</td>
<td>0.011</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>152.7±89.6</td>
<td>106.5±54.0</td>
<td>0.009**</td>
</tr>
</tbody>
</table>

SBP = systolic blood pressure; DBP = diastolic blood pressure; FPG = fasting plasma glucose; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol = VLDL-C = very low-density lipoprotein cholesterol; TG = triglycerides; *p < 0.05; **p < 0.01.
component of energy expenditure that explains the largest proportion (70-80%) of an individual’s total daily energy expenditure.60 Fontvieille et al.61 suggested that increased basal and sleeping metabolic rates, resulting in increased 24-hour sedentary energy expenditure, may play a role in the weight loss so often observed in type 2 diabetic patients, in addition to the energy loss from glycosuria. The increased RMR in individuals with type 2 diabetes may be a late abnormality, secondary to their metabolic deterioration. Our results revealed that because of reduced physical activity, total energy expenditure was found to be lower in the patients when compared with the controls (tables 1 and 2). We believe that impaired total energy expenditure can be ameliorated by both daily physical activity and aerobic exercise training in newly diagnosed type 2 diabetic patients. The glucose balance is significantly influenced by the physical fitness level, which is likely to be an important element in the pathogenesis of type 2 diabetes, as well as in the treatment of that disease.62 Ozdirenc et al.67 evaluated body composition, cardiopulmonary, musculoskeletal and motor fitness in 30 patients with type 2 diabetes, and 30 healthy non-diabetic controls matched for BMI and age. They found that the body fat percentage was higher, and VO2 max and handgrip strength were lower in the diabetic patients when compared with the control group. In some prospective studies, strength training was proven to be effective with clinical improvements in the glycaemic control and muscle mass.68-70 Nevertheless, Zois et al.71 found that a combined 16-week strength and aerobic training program could induce positive adaptations in the lipid profile, glycaemic control, insulin resistance, cardiovascular function, and physical fitness in post-menopausal women with type 2 diabetes. In this study, we evaluated right and left handgrip strength and back-leg strength as samples of whole body strength. We found that both the handgrip and back-leg strength values were significantly lower in male and female patients than in the healthy controls (tables 1 and 2). The daily sleep duration was significantly higher, whereas the lean body mass value was significantly lower in male patients compared with the controls (table 1). Further, the FPG value was negatively correlated with the right handgrip strength and back-leg strength values in the patients, in both genders (table 3).

The skeletal muscle mass is a large part of the lean body mass,72 and we believe that impaired aerobic exercise capacity and whole body strength might lead to decreased muscle mass, especially in male type 2 diabetic patients. Both aerobic and resistance training are important for individuals with diabetes and, ideally, a program that combines the two types of training should be undertaken to achieve maximal glycaemic and other benefits.73 The epidemic of overweight and obesity has caused a dramatic increase in the number of individuals with metabolic abnormalities and premature cardiovascular disease.74 Daniele et al.74 found that sedentary diabetic patients had higher waist circumference, waist-to-hip ratios, more depressive symptoms, and worse health-related quality of life. Furthermore, Kim et al.75 suggested that the BMI and central obesity were good predictors of type 2 diabetes risk in Koreans. Kaizu et al.76 divided a total of 4,870 Japanese type 2 diabetic patients into eight groups according to their leisure-time physical activity. They found that leisure-time physical activity was dose-dependently associated with BMI and waist circumference. In a prospective study with respect to blood pressure, it was found that systolic and diastolic blood pressures, as well as heart rate, were significantly decreased by aerobic exercise (three times per week for three years) in patients with type 2 diabetes.77 Similarly, we found that the body fat percentage, waist/hip ratio, abdominal circumference, and systolic blood pressure values were significantly higher in

### Table 5. The correlations of fasting plasma glucose in male and female patients with type 2 diabetes

<table>
<thead>
<tr>
<th></th>
<th>Male patients</th>
<th>Female patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r value</td>
<td>p value</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>0.243</td>
<td>0.016</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.436</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VO2 max (l/min)</td>
<td>-0.482</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VO2 max (ml/kg/min)</td>
<td>-0.434</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Right handgrip strength (kg)</td>
<td>-0.259</td>
<td>0.025</td>
</tr>
<tr>
<td>Left handgrip strength (kg)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Back-leg strength (kg)</td>
<td>-0.231</td>
<td>0.046</td>
</tr>
<tr>
<td>Total energy expenditure (kcal/day)</td>
<td>-0.238</td>
<td>0.026</td>
</tr>
<tr>
<td>Daily number of steps</td>
<td>-0.289</td>
<td>0.012</td>
</tr>
<tr>
<td>Daily sleep duration (hour)</td>
<td>0.236</td>
<td>0.043</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = Not significant; VO2 max = maximal aerobic capacity; SBP = systolic blood pressure; DBP = diastolic blood pressure.
patients, as compared with the controls, in both genders (tables 1 and 2). Additionally, the lean body mass value was significantly lower in male patients, compared with the controls, whereas the diastolic blood pressure value was significantly higher in female patients, compared with the controls (tables 1 and 4).

The FPG value was positively correlated with the body fat percentage and waist/hip ratio in male patients; and positively correlated with the systolic and diastolic blood pressures in female patients (table 5). Although the blood pressure values were found to be significantly different between the patients and controls, the blood pressures were in the normal margin in both groups. The lipid profile was found to be deteriorated in female patients, but no correlation was found between the FPG value and lipid profile parameters (tables 3 and 4). Nevertheless, multiple factors, such as nutritional status, content of food, genetic and environmental factors, and level of physical activity, may change the lipid profile. We believe that physical inactivity might contribute to obesity and central obesity in patients with type 2 diabetes. Therefore, lifestyle changes which promote adherence to habitual physical activity must be included in the management of newly diagnosed type 2 diabetes. These early lifestyle changes may provide numerous benefits, such as the prevention of obesity, metabolic syndrome, and cardiovascular risks through type 2 diabetes.

The lack of a validity study using the Astrand exercise test in type 2 diabetic patients was a limitation of this study. Future studies should investigate the validity of this exercise test in patients with type 2 diabetes.

In conclusion, this study revealed that daily physical activity, total energy expenditure, aerobic exercise capacity, muscle strength, obesity, and body fat distribution were impaired in male and female patients with type 2 diabetes, when compared with the healthy controls. In addition, lean body mass was decreased in male patients, compared with the controls, whereas the lipid profile was deteriorated in female patients, as compared with the controls. We found that resting metabolic rate and trunk flexibility values were not significantly different between the patients and the controls in either gender. We suggest that using exercise intervention especially comprised of strength training and aerobic activities, including not only daily slow activities but also moderate to vigorous activities, as a lifestyle modification in newly diagnosed type 2 diabetic patients, might be helpful for the development of more successful illness management strategies, including early intervention.

DISCLOSURES

The authors declare no conflicts of interest.

REFERENCES


ORIGINAL ARTICLE

Immunosuppressive therapy in patients with IgA nephropathy


*Department of Internal Medicine, Isala Clinics, Zwolle, the Netherlands, #Department of Nephrology, Radboud University Medical Centre, Nijmegen, the Netherlands, †Department of Nephrology, Leiden University Medical Centre, Leiden, the Netherlands, *corresponding author: tel.: +31(0)38-4245544, fax: +31(0)38-4243001, email: hi.peters@isala.nl

ABSTRACT

Background: There is limited evidence to support cytotoxic therapy in patients with IgA nephropathy and renal insufficiency. We studied the effect of cytotoxic therapy in patients with IgA nephropathy and renal insufficiency, and evaluated possible predictors of response.

Methods: Retrospective analysis of patients with IgA nephropathy who received immunosuppressive therapy. The primary outcome measure was progression of renal disease, defined as an increase in serum creatinine levels of ≥ 50% or development of end-stage renal disease.

Results: From 1996 to 2008, 19 patients with biopsy-proven IgA nephropathy were treated with cytotoxic agents and prednisone because of renal insufficiency and/or severe proteinuria. Characteristics of patients at the start of therapy: age 42 ± 11 years, serum creatinine 208 (96-490) μmol/l, estimated glomerular filtration rate (eGFR) 33 (12-65) ml/min/1.73 m², and protein-creatinine ratio 3.8 (0.6-18.2) g/10 mmol. Follow-up after initiation of therapy was 35 (7-133) months. Ten patients had progressive renal disease, whereas eGFR was stable in nine. Serum creatinine levels and proteinuria at the start of treatment were not significantly different between responders and non-responders. Proteinuria response at six months after start of therapy proved a good predictor: proteinuria decreased by ≥ 50% to levels below 1 g/day in 8/9 responders. In contrast, proteinuria decreased by more than 50% and reached levels < 1 g/day in only 3/10 non-responders (p < 0.01).

Conclusion: Prolonged immunosuppressive therapy with cytotoxic agents and prednisone may benefit a subgroup of patients with progressive IgA nephropathy. A reduction of proteinuria ≥ 50% to levels below 1 g/day within six months predicts a favourable long-term response.

KEYWORDS

IgA nephropathy, immunosuppression, therapy, end-stage renal disease, progression

INTRODUCTION

IgA nephropathy is the most common glomerular disease worldwide. Its natural course is highly variable and far from benign in many patients. Overall, approximately 25% of patients experience a lasting remission, whereas 30% of patients develop end-stage renal disease (ESRD) within 20 years. Risk factors associated with disease progression include heavy proteinuria, impaired baseline renal function and renal morphological lesions such as the presence of segmental sclerosis and the degree of tubular atrophy/interstitial fibrosis.

Many patients with IgA nephropathy can be effectively treated with angiotensin-converting enzyme inhibitors (ACEi) or angiotensin II receptor blockers (ARB) (The Kidney Disease: Improving Global Outcomes (KDIGO) Guideline 2012). There is evidence to support the use of corticosteroids in patients with persistent proteinuria and minimal renal impairment. It is debated whether patients with IgA nephropathy and renal insufficiency benefit from more aggressive treatment consisting of corticosteroids combined with alkylating agents. The evidence is derived from small studies, mostly conducted in a period with less defined blood pressure targets and variable use of ACEi/ARB as opposed to the current guidelines. More specifically, evidence favouring the use of cyclophosphamide in patients with non-crescentic IgA nephropathy and severe renal insufficiency is derived from a single small controlled trial. During
the last decade we have used cytotoxic therapy including cyclophosphamide in patients with advanced or severe IgA nephropathy. In the present study, we aimed to determine the response to immunosuppressive therapy in patients with progressive IgA nephropathy. Specifically, we sought to identify possible predictors of response.

**METHODS**

**Patients**

We retrospectively analysed data of adults with biopsy-proven IgA nephropathy who were referred for therapeutic advice to the Radboud University Medical Centre and the Leiden University Medical Centre and were subsequently treated with cytotoxic agents because of progressive IgA nephropathy. Patients with evidence of systemic disease, such as systemic lupus erythematosus, chronic liver disease and Henoch-Schönlein purpura or a follow-up of less than 12 months were excluded. Relevant clinical and biochemical data were retrieved from patient records. Baseline was defined as the date of first presentation. During follow-up quantitative analysis of proteinuria was assessed by the ratio of urinary protein to urinary creatinine in the majority of patients. This parameter is closely correlated with 24-hour urinary protein excretion. This study was carried out in the Netherlands in accordance with the applicable rules concerning the review of research ethics committees and informed consent.

**Treatment protocol**

Standard immunosuppressive therapy for IgA nephropathy consisted of prednisone 40 mg per day with monthly tapering for a maximum period of 24 months plus cyclophosphamide (1.5 mg/kg) during the first three months orally followed by azathioprine (1.5 mg/kg) for 18 months (derived from study treatment protocol by Ballardie et al.). During prednisone therapy, concomitant treatment with H$_2$-blockers or proton pump inhibitors was used. Co-trimoxazole was prescribed to prevent *Pneumocystic jiroveci* infections during the administration of cyclophosphamide. Young women were treated with azathioprine and not with cyclophosphamide because the latter is associated with infertility. Severe leukopenia, infections or active malignancy were regarded as stringent reasons to discontinue immunosuppressive treatment.

**Assessment of response**

The primary outcome measure was progression of renal disease defined as an increase in serum creatinine levels of ≥ 50% or development of ESRD (serum creatinine ≥ 500 μmol/l, dialysis or renal transplantation). Proteinuria responses were evaluated in terms of a reduction in proteinuria of 50% or a persistent urine protein-creatinine ratio < 1 g/10 mmol.

**Calculations**

The glomerular filtration rate was estimated (eGFR) by the simplified Modification of Diet in Renal Disease (MDRD) formula.$^9$ Mean arterial pressure was calculated as the diastolic pressure plus one-third of the pulse pressure.

**Statistical analysis**

Data values are expressed as mean ± standard deviation or median (range) as appropriate. Parameters between groups were compared using the Mann-Whitney test for non-parametric continuous data, the independent t-test for parametric data, and the chi-square test for categorical data. A p-value of less than 0.05 was considered to be statistically significant. Statistical analysis was performed using SPSS for windows software, version 20 (IBM SPSS).

**RESULTS**

**Baseline characteristics**

Between 1996 and 2008, 19 patients received immunosuppressive therapy because of progressive IgA nephropathy. Standard immunosuppressive treatment consisting of prednisone plus cyclophosphamide during the first three months orally followed by a cytotoxic agent was prescribed in 11 out of 19 patients. Five patients received prednisone plus cyclophosphamide without subsequent alternative agent. This was due to side effects (leucopenia, infection) or failure of improvement of already severely impaired renal function ($n = 5$). Three patients were started on azathioprine plus prednisone instead of cyclophosphamide plus prednisone. Clinical characteristics at presentation are depicted in table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>39±11 years</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>208 μmol/l</td>
</tr>
<tr>
<td>eGFR</td>
<td>55 ml/min/1.73 m$^2$</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>4.5 g/10 mmol</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>(128±100) mmHg</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>(70±15) mmHg</td>
</tr>
</tbody>
</table>

At presentation, mean age was 39±11 years, median serum creatinine was 128 μmol/l, a median eGFR of 55 ml/min/1.73 m$^2$, and median proteinuria was 4.5 g/10 mmol creatinine. All patients but one were treated with ACEI or ARB prior to the initiation of immunosuppressive therapy. Three patients had previously been treated with prednisone monotherapy. In the majority of patients therapy was started because of an increase in serum creatinine of 15% or more in the previous 12 months (n = 12), or because of a serum creatinine > 135 μmol/l and proteinuria > 1 gram per day (n = 6). In one patient therapy was initiated because of severe impairment of renal function at the time of presentation. The median time between baseline and start of therapy was 19 months. At start of therapy, patients were 42±11 years and had a median serum creatinine of 208 μmol/l, a median eGFR of 33 ml/min/1.73 m$^2$, and a median urine protein-creatinine ratio of 3.8 g/10 mmol (table 1).

Peters et al. Immunosuppressive therapy in patients with IgA nephropathy.
Clinical outcome
Follow-up after initiation of therapy was 35 (7-133) months. In ten patients renal disease was progressive despite the initiation of immunosuppressive therapy: six developed ESRD at a median follow-up of 24 (range 7-46) months and four experienced doubling of serum creatinine after 17 (range 12-81) months. Renal function remained stable or improved in nine patients.

Serum creatinine levels and proteinuria at the start of treatment were lower in responders (183 (120-381) versus 224 (96-490) μmol/l, p=0.23 and 2.2 (0.6-9.7) versus 4.1 (0.7-18.2) g/10 mmol creatinine, p = 0.31 respectively), but these differences were not statistically significant (table 2). There were no differences in other characteristics at baseline between responders and non-responders (table 2). Blood pressures were well controlled during treatment. Of note, there were no differences in mean arterial pressure between responders and non-responders (table 2). In eight out of nine responders proteinuria decreased by ≥ 50% within six months and attained levels below 1 g/day in eight patients (figure 1A). In contrast, in non-responders proteinuria decreased by more than 50% and reached values < 1g/day in only three out of ten patients (p < 0.01, figure 1B).

Treatment duration was 19 (range 3-81) months. Treatment duration was less than 12 months in two of the responders, while four of the non-responders received treatment for less than 12 months (table 3). Following the discontinuation of therapy, we observed no relapses of proteinuria.

Six patients had a serum creatinine level ≥ 250 μmol/l (the point of no return) prior to the start of therapy. In this small subgroup renal function remained stable after treatment in two patients during a follow-up of more than eight years. The remaining four patients developed ESRD within 21-46 months after initiation of treatment.

We evaluated the possible predictive value of pathological characteristics. In many patients the interval between kidney biopsy and start of immunosuppressive therapy was > 12 months (n = 10). Only nine biopsies were classified using the Oxford MEST scores (median eGFR 20 (range 15-42) ml/min/1.73 m², median proteinuria 4.2 g/10 mmol creatinine (range 0.7-18) at start of therapy), in six of these patients time between renal biopsy and start of therapy was < 4 months, in the other three patients the interval between renal biopsy and start of therapy was > 20 months. Four out of these nine patients were non-responders to therapy. When comparing non-responders and responders, non-responders seemed to exhibit more mesangial hypercellularity and more tubulointerstitial fibrosis, variables associated with progression of renal disease. Surprisingly, non-responders also seemed to have more endocapillary proliferation, a variable that was not associated with a decline in renal function in recent validation studies of the Oxford score.

Adverse effects of treatment
Adverse events were reported in 11 patients (58%) and included anaemia (n = 3), leucopenia (n = 4).

### Table 1. Clinical characteristics of patients receiving cytotoxic therapy

<table>
<thead>
<tr>
<th>Presentation</th>
<th>Start of therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>19</td>
</tr>
<tr>
<td>Male /female</td>
<td>14/5</td>
</tr>
<tr>
<td>Age (years)</td>
<td>39±11</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>100±10</td>
</tr>
<tr>
<td>UP/UCr (g/10 mmol creatinine)</td>
<td>4.5 (0.9-18.2)</td>
</tr>
<tr>
<td>Serum creatinine (μmol/l)</td>
<td>128 (69-285)</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73m²)</td>
<td>55 (22.87)</td>
</tr>
<tr>
<td>Use of ACEi or ARB (%)</td>
<td>89</td>
</tr>
<tr>
<td>Change in serum creatinine (%)</td>
<td>-</td>
</tr>
<tr>
<td>Change in UP/UCr (%)</td>
<td>-</td>
</tr>
<tr>
<td>Time from baseline to start of therapy (months)</td>
<td>-</td>
</tr>
<tr>
<td>Follow-up after start of therapy (months)</td>
<td>35 (7-133)</td>
</tr>
</tbody>
</table>

Data expressed as median (range) or mean ± SD; MAP= mean arterial pressure; UP = urinary protein; UCr = urinary creatinine; eGFR = estimated glomerular filtration rate; ACEi = angiotensin-converting enzyme inhibitors; ARB = angiotensin II receptor blockers.
thrombocytopenia (n = 1), infection (n = 4) or liver toxicity (n = 1). In two patients this led to discontinuation of immunosuppressive therapy. None of the patients developed malignancy during follow-up.

**DISCUSSION**

This study suggests that immunosuppressive therapy may result in stabilisation of renal function in approximately 50% of patients with IgA nephropathy at high risk for progression of renal disease. More importantly, a decrease in proteinuria of 50% or more within six months predicted a sustained response to therapy.

Over two decades, beneficial effects of corticosteroids have been reported by various studies in patients with no or mild renal impairment.\(^13,22-25\) However, in patients with advanced IgA nephropathy, administration of corticosteroids did not improve renal function.\(^26\) A few randomised controlled trials, enrolling a small number of patients, have been conducted to investigate the efficacy of

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**Table 2. Clinical characteristics of non-responders versus responders at start of treatment with cytotoxic agents and mean arterial pressures during follow-up**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Progression of renal disease</th>
<th>Stable renal function</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (male/female)</td>
<td>7/3</td>
<td>7/2</td>
<td>0.70</td>
</tr>
<tr>
<td>Age (years)</td>
<td>44±14</td>
<td>40±7</td>
<td>0.42</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>104±11</td>
<td>104±10</td>
<td>0.96</td>
</tr>
<tr>
<td>UP/UCr (g/10 mmol creatinine)</td>
<td>4.1 (0.7-18.2)</td>
<td>2.2 (0.6-9.7)</td>
<td>0.31</td>
</tr>
<tr>
<td>Serum creatinine (μmol/L)</td>
<td>224 (96-490)</td>
<td>183 (120-381)</td>
<td>0.23</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73m²)</td>
<td>26 (12-65)</td>
<td>36 (17-46)</td>
<td>0.21</td>
</tr>
<tr>
<td>Duration of treatment (months)</td>
<td>16 (13-81)</td>
<td>22 (12-63)</td>
<td>0.50</td>
</tr>
<tr>
<td>Duration of follow-up after treatment (months)</td>
<td>26 (6-81)</td>
<td>82 (28-133)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>50% reduction in proteinuria ≤ 6 months after start of therapy (n)</td>
<td>3(^a)</td>
<td>8</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Persisting proteinuria &lt; 1g/10mmol creatinine (n)</td>
<td>3(^a)</td>
<td>8</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

**Table 3. Overview of treatment schedule in responders and non-responders**

<table>
<thead>
<tr>
<th>Treatment schedule</th>
<th>Non-responders (n = 10)</th>
<th>Responders (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard course, i.e. prednisone plus cyclophosphamide for 3 months, followed by azathioprine; (^1)</td>
<td>n = 6 (54%)</td>
<td>n = 5 (46%)</td>
</tr>
<tr>
<td>Shortened course, i.e. cyclophosphamide and prednisone without subsequent conversion to azathioprine(^1)</td>
<td>n = 2 (40%)</td>
<td>n = 3 (60%)</td>
</tr>
<tr>
<td>Alternative, i.e. prednisone and azathioprine instead of cyclophosphamide(^1)</td>
<td>n = 2 (67%)</td>
<td>n = 1 (33%)</td>
</tr>
</tbody>
</table>

\(^1\) 4 patients were treated for a total of 2.5-3 months with cyclophosphamide and prednisone. 1 patient was treated for a longer period thereafter with prednisone monotherapy, \(^*\) duration of treatment at least 9 months.
cytotoxic agents combined with prednisone.\textsuperscript{17-20,27} Only the study performed by Ballardie et al. showed that prednisone plus cytotoxic agents were effective in preserving renal function in patients with moderate, progressive disease.\textsuperscript{18} Thus, the optimal management of patients with advanced IgA nephropathy remains controversial.

Clearly, this small, retrospective and uncontrolled study cannot provide compelling evidence for benefit of corticosteroids and cyclophosphamide or azathioprine in high-risk IgA nephropathy patients. Yet, our data support the results reported by Ballardie; based on observational data on known major risk factors, at least 70-90% of this study population characterised by heavy, persistent proteinuria, (median 3.1 g/d) and severe impairment of renal function (median serum creatinine 208 μmol/l) at the start of treatment with immunosuppressive drugs, would be expected to progress to ESRD.\textsuperscript{18,19} However, after immunosuppressive therapy progression of disease was halted and renal function remained stable in approximately 50% of patients. Admittedly, responders to therapy had slightly better renal function and less heavy proteinuria at the start of therapy. However, clinical characteristics of the responders still predict a dismal outcome. Of note, there were no differences in blood pressures between the responder and non-responder groups (table 2).

Thus, immunosuppressive combination therapy may benefit IgA nephropathy patients with a poor prognosis. Unfortunately, side effects are frequently observed, duration of treatment is long and 50% of patients are exposed to cytotoxic agents and prednisone to no avail. Therefore, immunosuppressive therapy seems to benefit only a subset of high-risk patients with IgA nephropathy. Currently it is impossible to identify this subgroup by means of clinical or pathological characteristics. Considering this, our finding that a 50% decrease in proteinuria within six months after the start of therapy is indicative of a sustained response to immunosuppressive therapy is highly relevant. In patients lacking a substantial decrease in proteinuria after six months of therapy, discontinuation of treatment should be strongly considered, in our opinion.

Our study did not allow conclusions regarding the role of the histological classification to predict response to immunosuppressive therapy because the numbers were too small. Of note, this question was addressed in a recent European study intended to validate the Oxford classification.\textsuperscript{19} In this study, the predictive value of the pathology score was no longer present in the patients who received immunosuppressive therapy.

Recently, it has been recognised that patients with IgA nephropathy have increased serum levels of galactose-deficient IgA1 and that circulating autoantibodies recognising this galactose-deficient IgA1 as an autoantigen or the levels of the autoantigen itself allow prediction of disease progression.\textsuperscript{28,31} Perhaps these and other promising biomarkers will allow identification of patients responsive to immunosuppressive therapy in the near future.

The results of this retrospective study differ to some extent from the data reported by Ballardie et al. A substantially larger number of patients suffered from therapy-related side effects in our study compared with the frequency reported by Ballardie and others (58% vs 7-12%).\textsuperscript{18,32} Although these side effects were mild and transient in the majority of cases, two patients withdrew from treatment. Second, several studies including Ballardie’s reported that patients with a serum creatinine level ≥ 250 μmol/l inescapably develop ESRD despite therapy.\textsuperscript{18,33,34} We observed a sustained stabilisation of renal function in two out of six patients beyond this point of no return after therapy. Thus, even in patients with severe renal...
impairment, immunosuppressive therapy may be of benefit. In conclusion, the optimal management of IgA nephropathy remains controversial and randomised controlled trials are much needed. Currently, a large randomised prospective controlled trial (STOP IgAN trial) is being conducted in order to determine whether immunosuppressive therapy is able to prevent progression of renal disease. The KDIGO Clinical Practice Guideline for Glomerulonephritis suggests corticosteroid therapy in patients with persistent proteinuria > 1 g/d despite 3-6 months of optimised supportive care (including ACEi/ARB) and eGFR > 50 ml/min. Combined immunosuppressive therapy is not advised unless there is crescentic IgA nephropathy with rapidly deteriorating renal function. In our opinion, immunosuppressive therapy can certainly be considered in IgA nephropathy patients with a decline in renal function and persistent proteinuria > 1 g/d. Immunosuppressive therapy should only be continued beyond six months in those patients demonstrating a significant (> 50%) decrease in proteinuria.

DISCLOSURES

The authors declare no conflicts of interest.

REFERENCES

CASE REPORT

Myelopathy in systemic lupus erythematosus: a case report and a review of the literature

L. Hamming*, R. van der Meulen, A. Vergouwen, C. Siegert

Departments of Internal Medicine and Psychiatry, Sint Lucas Andreas Hospital, Amsterdam, the Netherlands, *corresponding author: email: l.hamming@vumc.nl

ABSTRACT

Myelopathy, a severe condition characterised by paraparesis, sensory deficits and sphincter dysfunction, is one of the neuropsychiatric manifestations that have been described in patients with systemic lupus erythematosus (SLE). SLE-associated myelopathy may confront clinicians with a challenging decision-making process due to the broad differential diagnosis, the lack of disease-specific findings, and the urgency to initiate immunosuppressive therapy early in the course of the disease to favourably affect outcome.

KEYWORDS

Myelopathy, systemic lupus erythematosus, neuropsychiatric lupus erythematosus

INTRODUCTION

Neuropsychiatric manifestations, ranging from headache or mild cognitive confusion to seizure disorder or myelopathy, affect up to 80% of patients with systemic lupus erythematosus (SLE) and are associated with considerable morbidity and mortality. Myelopathy, one of the neuropsychiatric SLE syndromes (NPSLE) that have been described by the American College of Rheumatology (ACR) (table 1), is a rare but clinically important condition, due to the dramatic presentation with rapidly evolving paraparesis, sensory deficits and sphincter dysfunction. The clinical decision-making process in these patients can be challenging as a result of the broad differential diagnosis, and the urgency to initiate treatment early in the course of the disease to improve prognosis. This case report is aimed at the recognition of myelopathy in SLE patients and outlines the most important clinical challenges in these patients.

CASE REPORT

A 29-year-old female of Creole descent with a history of non-inflammatory arthralgias in the ankles and wrists (2009) and a transient psychosis during the third trimester of her first pregnancy, for which she was briefly treated with haloperidol (2011), developed paraparesis and urine incontinence two months after her first childbirth. Medical history was otherwise unremarkable. On physical examination, grade II paresis of the lower extremities (according to the Medical Research Council scale) was found with normal sensory functions, diminished quadriceps and gastrocnemius reflexes and absent Babinski’s sign. Laboratory results showed an elevated erythrocyte sedimentation rate of 119 mm/h (reference range 1-20 mm/h), leukopenia of 3.5 x 10⁹/l (4.0-10.0 x 10⁹/l) and normocytic anaemia of 5.1 mmol/l (7.5-10.0 mmol/l). Apart from a positive IgG Coombs test, there were no signs of active haemolysis, based on a normal

What was known on this topic?

Myelopathy is one of 19 neuropsychiatric syndromes that have been described in patients with systemic lupus erythematosus. Immunosuppressive therapy prescribed early in the course of the disease may favourably affect its outcome.

What does this add?

This case illustrates the clinical challenges in a patient diagnosed with SLE-associated myelopathy, due to the broad differential diagnosis of myelopathy and the lack of disease-specific findings, and may therefore contribute to current clinical practice.
Serological testing for human immunodeficiency virus and cytomegalovirus was negative. Electromyography and motor evoked potential were both normal, thereby ruling out peripheral nerve disease, plexopathy and neuromuscular junction disease as an explanation for the patient’s paraparesis and urine incontinence. At this point, we excluded compressive causes, demyelination, and infectious diseases as an explanation for the patient’s myelopathy. The diagnostic process focused on inflammatory disorders, supported by the patient’s history of arthralgias and concomitant leukopenia, anaemia and elevated ESR. The differential diagnosis of inflammatory myelopathy was further subdivided into SLE, sarcoidosis and antiphospholipid antibodies syndrome. A more detailed history revealed no other autoimmune phenomena. We performed additional laboratory testing, which revealed the presence of antinuclear antibody (ANA), anti-double-stranded DNA (anti-dsDNA) with a titre of 15 kU/l, anti-ribonucleoprotein and anti-smooth muscle antibodies. Complement levels were elevated. A recent study has demonstrated that complement levels and anti-dsDNA are unreliable markers for NPSLE: only one-third of patients with NPSLE had low complement levels and positive anti-dsDNA during a symptomatic phase. Diagnostic work-up for antiphospholipid syndrome was negative (absent lupus anticoagulant, anti-cardiolipin antibodies and anti-β2 glycoprotein I antibodies). Sarcoidosis was deemed unlikely due to an unremarkable chest X-ray (no hilar lymphadenopathy or interstitial abnormalities) and the absence of skin or eye abnormalities. Subsequently, the diagnosis SLE (psychosis, leukopenia, positive ANA and anti-ds DNA) with SLE-associated myelopathy was made. Intravenous treatment with methylprednisone (1000 mg for three days) and cyclophosphamide (1500 mg, 750 mg/m²) was started when she developed bilateral Babinski’s signs, after which her symptoms quickly improved.

DISCUSSION

Myelopathy, affecting 1-2% of SLE patients, can be divided into two subtypes. Firstly, transverse myelopathy, which is more prevalent in SLE, with involvement across one level of the spinal cord. Secondly longitudinal myelopathy in which more than four levels of the spinal cord are affected, either continuously or separately. The most common presentation of patients with transverse myelopathy consists of paresthesias (usually at level T3-T8), which can be accompanied by paraparesis and/or sphincter dysfunction.

Table 1. Nineteen NPSLE syndromes, divided in two groups: syndromes of the central nervous system (central NPSLE) and syndromes of the peripheral nervous system (peripheral NPSLE)²

<table>
<thead>
<tr>
<th>Central NPSLE</th>
<th>Peripheral NPSLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aseptic meningitis</td>
<td>Guillain Barré syndrome</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>Autonomic neuropathy</td>
</tr>
<tr>
<td>Demyelinating syndrome</td>
<td>Mononeuropathy</td>
</tr>
<tr>
<td>Headache</td>
<td>Myasthenia gravis</td>
</tr>
<tr>
<td>Movement disorder</td>
<td>Cranial neuropathy</td>
</tr>
<tr>
<td>Myelopathy</td>
<td>Plexopathy</td>
</tr>
<tr>
<td>Seizure disorders</td>
<td>Polyneuropathy</td>
</tr>
<tr>
<td>Acute confusional state</td>
<td></td>
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<tr>
<td>Anxiety disorder</td>
<td></td>
</tr>
<tr>
<td>Cognitive dysfunction</td>
<td></td>
</tr>
<tr>
<td>Mood disorder</td>
<td></td>
</tr>
<tr>
<td>Psychosis</td>
<td></td>
</tr>
</tbody>
</table>

Bilirubin of 8 µmol/l (reference range < 17 µmol/l), normal reticulocytes of 38 x 10⁹/l (25-120 x 10⁹/l) and a high haptoglobin of 2.8 g/l (0.3-2.0 g/l). Other laboratory results, including electrolytes and vitamin B₁₂, were normal. Myelopathy was suspected, and a broad differential diagnosis was formulated, including compressive causes (e.g. trauma or neoplasms) and non-compressive causes (e.g. demyelination, infections, inflammatory or vascular disorders, toxic or metabolic abnormalities and paraneoplastic syndromes).

Additionally, CT and MRI imaging of the brain and spine were performed, which were both normal. Cerebrospinal fluid (CSF) examination was not suggestive of infections of the nervous system, with a normal opening pressure, mild leukocytosis (9.0 x 10⁹/l; reference range < 5.0 x 10⁹/l), elevated protein levels (2.8 g/l; 0.2-0.5 g/l), low glucose (2.3 mmol/l; 2.8-4.0 mmol/l) and elevated IgG (79 g/l; 0.0-34.0 g/l) with an increased IgG index (0.68; 0.30-0.60) and oligoclonal IgG band. CSF cultures were negative.

Table 1.

<table>
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<td>Mood disorder</td>
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<tr>
<td>Psychosis</td>
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</tbody>
</table>

Figure 1.

A. Longitudinal myelopathy in SLE
B. Transverse myelopathy in SLE

DISCUSSION

Myelopathy, affecting 1-2% of SLE patients, can be divided into two subtypes. Firstly, transverse myelopathy, which is more prevalent in SLE, with involvement across one level of the spinal cord. Secondly longitudinal myelopathy in which more than four levels of the spinal cord are affected, either continuously or separately. The most common presentation of patients with transverse myelopathy consists of paresthesias (usually at level T3-T8), which can be accompanied by paraparesis and/or sphincter dysfunction. A small subgroup of patients may have normal sensory functions.
In patients suspected of SLE-associated myelopathy, MRI is the preferred neuro-imaging technique and may detect T2-weighted hyperintense lesions, the most common finding in NPSLE. In 14-93% of these patients abnormal lesions are found. The meaning of these lesions is uncertain, although they may be indicative of microinfarcts, focal ischaemia, demyelination, or gliosis. Importantly, they correlate poorly with clinical manifestations and are aspecific for NPSLE. Similar lesions can be found in non-SLE patients and in SLE patients without neuropsychiatric manifestations.

CSF examination and cultures should be performed in order to exclude infectious diseases. In 50-80% of NPSLE patients mild, non-specific abnormalities (e.g. pleocytosis, elevated protein levels and decreased glucose levels) are found. An elevated IgG index and oligoclonal bands, indicative of an immunological response, may be found in patients with SLE but are also found in other diseases, such as multiple sclerosis and sarcoidosis. As soon as infectious causes are excluded or SLE-associated myelopathy is suspected, treatment with immunosuppressive drugs should be initiated. Preferably, initial treatment should consist of intravenous methylprednisone for three consecutive days followed by intravenous cyclophosphamide ($750 \text{ mg/m}^2$). This regimen should be followed by monthly infusions of cyclophosphamide for 6-12 months, combined with oral prednisolone 1 mg/kg for three months, which should be tapered afterwards according to disease activity. In NPSLE patients with refractory symptoms, treatment with rituximab (anti-CD20 monoclonal antibody) can be considered. Next to immunosuppressive therapy, anticoagulation has been suggested as a therapeutic option for SLE patients with aPL-positive myelopathy. However, a systemic review failed to show any additional effect of anticoagulation on the neurological outcome of 70 patients with aPL-positive myelopathy. Anticoagulant therapy has not yet been evaluated in a randomised, placebo-controlled setting. Our patient was aPL-negative and therefore not treated with anticoagulant therapy. She showed clinical improvement after the start of immunosuppressive therapy but still had a grade IV paresis in the lower extremities after discharge. For four consecutive months, during which she received monthly cyclophosphamide infusions, her disease remained in remission. Due to side effects, cyclophosphamide was changed to oral azathioprine, which successfully maintained the remission. The overall outcome of patients with SLE-associated myelopathy seems reasonably favourable. Complete recovery occurs in up to 50% of the cases, whereas 21-31% of the patients show no clinical improvement or deterioration after immunosuppressive therapy. In conclusion, the diagnostic work-up in patients suspected of SLE-associated myelopathy, consisting of MRI and CSF examination, is vital in order to rule out the presence of other underlying diseases. Thereafter, aggressive immunosuppressive therapy with methylprednisone and cyclophosphamide should be initiated early in the course of the disease to favourably affect outcome. Clinical trials should be initiated to evaluate the current treatment regime, including the role of anticoagulant therapy, for SLE-associated myelopathy.

DISCLOSURES
The authors declare no conflicts of interest.

REFERENCES
CASE REPORT

Horner’s syndrome in a patient presenting with chest pain

S.H.W. van Bree*, M.D.R. van Bree, G.A. Somsen

1Department of Internal Medicine, Academic Medical Centre, Amsterdam, the Netherlands, 2Department of Sports Medicine, Isala Clinics, Zwolle, the Netherlands, 3Cardiology Centres of the Netherlands; location Lelystad, the Netherlands; *corresponding author: tel.: +31(0)020-5665575, email: s.h.vanbree@amc.uva.nl

ABSTRACT

An altered mental status and peripheral nerve dysfunction are alarming signs in a patient presenting with chest pain. If complicated by acute myocardial infarction, this raises the suspicion of aortic dissection and warrants immediate CT angiography. We report a dramatic case of chest pain in a 79-year-old man with somnolence and Horner’s syndrome, subsequently complicated by myocardial infarction. Autopsy demonstrated a type A aortic dissection involving the carotid arteries and the right coronary artery.

KEYWORDS

Aortic dissection, CT scan, chest pain, Horner’s syndrome

INTRODUCTION

The differential diagnosis of chest pain is extremely diverse. In acute chest pain accompanied by somnolence and neurological deficits, one should be aware of serious diseases such as pulmonary embolism, acute myocardial infarction, severe aortic valve stenosis, left-sided endocarditis with embolisation, or aortic dissection. In this context we discuss a dramatic case of chest pain in a 79-year-old man with somnolence and Horner’s syndrome.

CASE REPORT

A 79-year-old man was presented in the emergency department because of an acute violent and sharp pain located retrosternally and in the throat. Patient was known with hypertension, a coronary stent and a dilated aortic root (diameter 53 mm; stable for years) with a tricuspid aortic valve. The patient was somnolent with a significant difference in blood pressure in the two upper extremities (right 85/40 vs. left 110/60 mmHg) and cold extremities with normal peripheral oxygen saturation. Physical examination of the heart, lungs and arteries revealed no abnormalities. The right pupil was constricted with drooping of the eyelid. An electrocardiogram showed no abnormalities except sinus bradycardia. Blood tests showed a normal C-reactive protein, but a prolonged international

What was known on this topic?
Aortic dissection has a high mortality and great diversity in clinical presentation. The early detection and diagnosis therefore has been proven to be difficult in clinical practice. If untreated the mortality rate is 50% in the first 48 hours. A CT scan is the first choice for patients with a high suspicion. Emergency surgery is indicated for type A aortic dissection. A type B dissection can be treated medically unless there is a complication or haemodynamic instability.

What does this case add?
Acute myocardial infarction with neurological deficits should raise the suspicion of a type A aortic dissection. This case report illustrates the diverse clinical manifestations of aortic dissection secondary to the various organs involved and highlights the importance of rapid diagnosis. If an aortic dissection is suspected an immediate CT angiography needs to be performed.
normalised ratio (INR) (2.7) with thrombocytopenia (113 x 10^9/l) and a normocytic anaemia (haemoglobin 6.1 mmol/l). The chest X-ray showed no abnormalities. The most likely diagnosis was aortic dissection with neurological symptoms fitting Horner’s syndrome. This was supported by the difference in blood pressure between the left and the right arm together with the spontaneously prolonged INR, thrombocytopenia and anaemia. However, in the meantime the chest pain increased. A new electrocardiogram showed signs of acute inferior myocardial infarction and the patient deteriorated haemodynamically. CT angiography demonstrated aortic dissection from the aortic valve to the renal arteries, extending to the right coronary artery, the common carotid artery and the right and left subclavian artery with a minimal antegrade flow in the ascending aorta (figure 1a). Taking into account the age of the patient, the complexity of the dissection and the high mortality from operation in a haemodynamically poor initial situation it was decided, in collaboration with the family, to refrain from surgery. The patient died three hours after presentation. Autopsy confirmed the diagnosis of type A aortic dissection. The defect in the arterial wall was situated in an atherosclerotic plaque in the ascending aorta and extended to the descending anterior branch (figure 1b), the right coronary artery, the right common carotid artery (until the bifurcation) (figure 1c), right and left subclavian artery, and along the aorta (until 8 cm above the level of the renal arteries origin).

**DISCUSSION**

This case is illustrative of the differential diagnostic thinking in a patient with acute chest pain with neurological symptoms. The two most commonly involved organ systems in the process of aortic dissection are the cardiovascular and neurological system. Alarming findings with this presentation were the experienced subjective somnolence in combination with haemodynamic instability. This may fit insufficient cerebral perfusion, which is a sign of (impending) shock. It is important to quickly identify the cause. In this case there were several signals that pointed to aortic dissection. The nature of the pain (acute, violent and sharp) was in line with the classic presentation of aortic dissection. The spontaneously prolonged INR and thrombocytopenia indicate diffuse intravascular clotting as a result of bleeding in the aortic wall. In addition, the clinical diagnosis is supported by the difference in blood pressure between the arms (> 20 mmHg). Striking in this case were the miosis and ptosis of the right eye. This is a result of compression of the sympathetic fibres of either the preganglionic neuron (dissection of the subclavian artery), or of the postganglionic neuron (dissection of the internal carotid artery). In this case the cause was most likely preganglionic as the dissection extended to just after the origin of the right subclavian artery. Other possible neurological manifestations of an aortic dissection are a cerebrovascular accident, syncope, vocal cord paralysis and hoarseness. Cerebral ischaemia/stroke is the most common neurological manifestation associated with aortic dissection and has been reported to affect 5-10% of patients. Most patients with aortic dissections who present with stroke also reveal a history of chest pain. Besides stroke, the altered cerebral perfusion may cause symptoms of transient cerebral hypoperfusion ranging from altered mental status to syncope. The subacute inferior myocardial infarction was the result of compression of the right coronary artery by extension of the ascending aortic dissection 2 cm beyond the origin of this artery.

Dissection of the aorta has a low prevalence and high mortality. A dissection is created by a tear in the intima resulting in a progressive ‘false’ lumen in the wall of the vessel. Extension of the dissection can lead to a diverse clinical spectrum. The clinical manifestations depend on which of the various branches of the aorta,
the pericardium, the coronaries and/or the aortic valve are involved (table 1). The early detection and diagnosis, therefore, has been proven to be difficult in clinical practice. In 25-50% of patients with an aortic dissection the wrong diagnosis is made. The diagnosis of aortic dissection begins with clinical suspicion, which is the most crucial step in diagnosing this catastrophic disease. The mortality rate for patients with untreated proximal aortic dissections has been reported to increase by 1-3% per hour after presentation and is approximately 50% during the first 48 hours after the initial presentation. Rapid diagnosis and treatment may result in a one-year survival of 90%.

A CT scan is the first choice for patients with a high suspicion. In acutely ill patients, delays in imaging may adversely affect patient care. Emergency surgery is indicated for type A aortic dissection. A type B dissection can be medically treated unless there is a complication or haemodynamic instability.

In conclusion, neurological symptoms resulting from cerebral hypoperfusion and peripheral nerve dysfunction are alarming symptoms in a patient with chest pain. As soon as an aortic dissection is suspected immediate CT angiography is warranted, as early recognition of an aortic dissection type A is of great importance in view of the high mortality.

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DISCLOSURES

The authors declare no conflicts of interest.


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CASE REPORT

Melioidosis and renal failure in a Dutch man after a trip to Gambia

F. Morelli*, L. Smeets, M. Hobijn, H. Boom

Department of Internal Medicine, Reinier de Graaf Gasthuis, Delft, the Netherlands, *corresponding author: tel.: +31(0)6-16799876, email F.Morelli@rdgg.nl

ABSTRACT

Melioidosis is due to Burkholderia pseudomallei and is known to be endemic in South-East Asia, while epidemiology of disease in Sub-Saharan Africa is still unclear. Prompt recognition of infection is crucial for adequate antibiotic treatment. Infection can lead to visceral abscesses and awareness of this complication is important for proper management.

KEYWORDS

Acute kidney injury, melioidosis, prostatic abscess

CASE REPORT

A 63-year old Caucasian man presented to the Emergency Department with fever and renal failure two weeks after a recreational trip to Gambia, Western Africa. Together with his family he stayed at a resort on the seaside, and had a brief visit to an inland rural area where he walked barefoot in the riverbed. He complained of diarrhoea, cramping abdominal pain, stranguria and vesical tenesmus. Recently, he had been referred to a nephrologist because of isolated proteinuria. Physical examination revealed fever and tachypnoea, but no other signs of acute distress or peculiar findings. Laboratory results showed leucocytosis (20 x 10⁹/l, neutrophils 18.3 x 10⁹/l), slightly elevated C-reactive protein (20 x 10⁹/l), normocytic anaemia, and increased serum creatinine (707 μmol/l). Urinary dipstick was positive for protein, blood, leukocyte esterase and nitrite. The patient was admitted to the hospital with suspected urosepsis and acute kidney injury. Cefuroxime, a second-generation cephalosporin, and fluid resuscitation were initiated. Urine and blood cultures grew colonies of Burkholderia pseudomallei, a rod-shaped bacterium which is the causative organism of melioidosis. Consequently, the antimicrobial therapy was changed to ceftazidime. Further radiological investigation revealed a prostatic abscess (figure 1) and finally transurethral drainage was performed. Culture of resected prostatic material also grew B. pseudomallei. The patient slowly recovered. A two-week course of ceftazidime was followed by oral treatment with trimethoprim-sulfamethoxazole and doxycycline, for three months. Unfortunately, his kidney function did not improve and haemodialysis was required. Kidney biopsy revealed end-stage renal disease. The term melioidosis refers to a clinically variable disease, ranging from local skin infection to fatal septicaemia, caused by infection with B. pseudomallei. This highly virulent, Gram-negative, environmental bacillus can infect humans after exposure to contaminated soil or water. Melioidosis is endemic in Southeast Asia and Northern Australia, with Northeast Thailand having the highest incidence (up to 50 cases per 100,000 people). Case clusters have been reported in Central America and the...
Melioidosis recently emerged in Brazil as a result of improved awareness and diagnostic procedures. Very little is known about the distribution of this disease in Africa. During the past 30 years, a total of 11 cases of human melioidosis from Sub-Saharan Africa have been described in the literature.

Table 1 gives an overview of the cases reported in the international literature. Among these, two come from Western Africa (Gambia and Sierra Leone respectively). Moreover, B. pseudomallei has been isolated from soil in Gabon, and one of these strains was isolated in a patient who died of septic shock.

Lack of sanitary assistance, diagnostic facilities and data collection in rural areas could lead to underestimation of the extension of this disease. Infection results from percutaneous inoculation, inhalation, or, less frequently, ingestion. The incubation period following documented exposure was shown to be 1-21 days in an Australian cohort from Darwin.

In our case, a life-long travel history was taken. Patient had travelled regularly to Gambia in the previous years and, more than 40 years ago, he visited Indonesia once. Though an apparent incubation period of 62 years has been described in one report, the clear correlation between exposure to wet soil in Gambia and onset of symptoms makes reactivation of a latent infection unlikely.

Currie et al. identified specific risk factors for melioidosis in 80% of symptomatic patients: diabetes, heavy alcohol use, chronic pulmonary disease, chronic renal disease, thalassaemia and, less commonly, glucocorticoid therapy and cancer. In our patient, pre-existing chronic kidney disease could hypothetically have resulted in higher susceptibility to the infection. Severity and course of the disease are thought to result from host and environmental factors, which is why clinical manifestations widely range from a mild infection to fulminant septicemia, or a chronic condition that may simulate tuberculosis.

The most common presentation of melioidosis is community-acquired pneumonia, occurring in more than a half of all cases, followed by genitourinary infection (14%), skin infection (13%), bone and joint infection (4%) and neurological involvement (5%). Visceral abscesses are very common and can affect almost any organ.

Melioidosis can potentially cause kidney injury. In a retrospective study including 220 patients with melioidosis, 77 patients with septicemia developed acute kidney injury. In our patient, infection with B. pseudomallei and renal failure coexisted but no causal association could be found.

Mortality rates for melioidosis are approximately 14% in Australia and 40% in Northeast Thailand. A delay in diagnosis can be fatal, since this microorganism is inherently resistant to most empirical antibiotic regimens (among others first-generation and second-generation cephalosporins, and aminoglycosides). Unfortunately, classic biochemical identification is difficult and often time-consuming. In our case, the Maldi-TOF mass spectrometry (BioTyper, Bruker, Germany) initially yielded an incorrect identification due to an incomplete database. The diagnosis was established by 16rDNA sequence analysis. Subsequent analysis in the Maldi-TOF with an updated database also yielded a B. pseudomallei identification.

The treatment of melioidosis consists of at least 10-14 days of ceftazidime, meropenem, or imipenem administered intravenously, followed by oral eradication therapy, usually with trimethoprim-sulfamethoxazole for three to six months.
With this case and brief review of the literature, we underline some important epidemiological and clinical aspects of melioidosis. Already endemic in Southeast Asia, this disease is emerging in other tropical and sub-tropical regions, and also present in Gambia. Prompt diagnosis and adequate therapy is crucial, especially in at-risk patients where infection can be aggressive. Depending on clinical picture, imaging techniques are needed in order to spot visceral abscesses and eventually perform drainage.

DISCLOSURES

The authors declare no conflicts of interest.

REFERENCES

A 29-year-old woman with multiple pulmonary masses

P.A. Rootjes1*, H. Gelderblom2, M.P. Hendriks

1Department of Internal Medicine, Medical Centre Alkmaar, Alkmaar, the Netherlands, 2Department of Medical Oncology, Leiden University Medical Centre, Leiden, the Netherlands, *corresponding author: tel.: +31(0)6-1352426, email: p.a.rootjes@mca.nl

CASE REPORT

A 29-year-old woman with an unremarkable medical history was referred to our emergency department with complaints of shortness of breath and right-sided chest pain on inspiration since three weeks and three kilograms of weight loss in the last six weeks. She did not smoke and used neither drugs nor medication. Physical examination revealed a fixed elastic swelling with a diameter of 10 cm of the right thigh. There were no enlarged lymph nodes and examination of the lungs and the breasts revealed no abnormalities. Pulse oximetry showed an saturation of 99% on room air. Laboratory results revealed: haemoglobin 6.9 mmol/l, leucocytes 10.8 x 10⁹/l, erythrocyte sedimentation rate 97 mm/h lactate dehydrogenase 259 U/l, gamma-glutamyltransferase 176 U/l and normal transaminases. A chest X-ray demonstrated multiple large round masses in both lungs (figure 1A and 1B).

WHAT IS YOUR DIAGNOSIS?

See page 300 for the answer to this photo quiz.
DIAGNOSIS

A biopsy of the swelling of the right thigh was taken and demonstrated synovial sarcoma (figure 2A, B and C). For additional staging a CT scan was performed, showing multiple bone and pulmonary ‘cannonball’ metastases. The latter are typically seen in choriocarcinoma, sarcomas, melanoma and testicular cancers.

Synovial sarcoma is a soft tissue sarcoma which originates from primitive mesenchymal tissue on the ending of large joints; however it can occur in any part of the body. Due to its local invasiveness and propensity to metastasise, synovial sarcoma has a high mortality rate. It most often affects adolescents and young adults with a peak incidence in the third decade of life. In 2013, 805 sarcomas were diagnosed in the Netherlands with an incidence of about 4.16 per 100,000 patient-years (European standardised rate). The diagnosis of synovial sarcoma is made by histological biopsy and is characterised by the presence of the SS18/SSX fusion gene. Before a patient undergoes a biopsy, in a non-metastatic setting, local imaging by means of MRI needs to be done. Subsequently additional staging should be performed by CT scan.

Treatment depends on the extent of disease. For local disease, surgical resection with neo-adjuvant or adjuvant radiotherapy is the treatment of choice. For locally advanced disease, neo-adjuvant chemotherapy can be considered. Patients with metastasised soft tissue sarcoma should preferably be treated in a trial-related manner. Standard first-line treatment includes doxorubicin (or ifosfamide when doxorubicin is contraindicated) monotherapy with an approximate response rate of 14-30% and a median survival of one year. Combination therapy with doxorubicin and ifosfamide does increase response rates by about 10% and should be used in symptomatic patients up to 60 years of age when a response is the goal of treatment. However, this intensive combination does not increase overall survival and has more side effects. Nowadays, further line-treatment options include trabectedin and pazopanib.

Figure 2B and 2C. Immunohistochemistry of biopsy taken from swelling of the right thigh showing epithelial membrane antigen (EMA) positivity in figure 2B and transducer-like enhancer of split 1 (TLE1) positivity in figure 2C which are suggestive of synovial sarcoma. The diagnosis was confirmed using molecular diagnostics revealing the characteristic t(X;18)
Our patient was referred to an academic centre, specialised in the treatment of soft tissue malignancies. She immediately started on palliative combination chemotherapy (six cycles of doxorubicin and ifosfamide). After finishing these six cycles of chemotherapy a CT scan of the chest and abdomen was performed, showing a partial response with a decrease in tumour size of the pulmonary metastases as well as the tumour of the right thigh. The largest pulmonary metastasis located in the apical part of the right lung (figure 1A and B) decreased in size from 116 x 40 mm to 50 x 40 mm. The tumour of the right thigh decreased from 100 mm to 56 mm. The response lasted for six months. A year later, she now is stable on trabectedin therapy.

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PHOTO QUIZ

A 7-year-old boy with fever, rash and coughing

S.A.S. van der Bent¹*, V.E.J. van Ham-Borawitz², E.D de Kleijn², S.W.I. Reeder¹, R. Laeijendecker¹

Departments of ¹Dermatology, ²Paediatrics, Albert Schweitzer Hospital, Dordrecht, the Netherlands,
*corresponding author: tel.: +31(0)78-6541111, fax: +31(0)78-654 2179, email: s.bent@vumc.nl

CASE REPORT

A 7-year-old boy visited our emergency department after referral by his general practitioner because of coughing, fever and malaise for two weeks. During the last two days the patient had also developed an asymptomatic generalised rash. There was no dyspnoea or any other relevant symptoms. His medical history included asthma. No medication was used.

On physical examination, the extremities and trunk showed ‘target’ or ‘bulls-eye’ lesions (figures 1 and 2). In some of the lesions, central erosions and clear vesicles were observed. The affected body surface was less than 10%. Further inspection revealed haemorrhagic crusting of the lips and erosions of the buccal mucosa (figure 3), a bilateral conjunctivitis and genital erythema. Cervical painful enlarged lymph nodes were palpated bilaterally. On auscultation, crackling sounds and rhonchi were heard during inspiration. Photographs were taken with permission of the patient and his parents.

WHAT IS YOUR DIAGNOSIS?

See page 303 for the answer to this photo quiz.

Figure 1. Photograph of the patient showing typical target lesions on the back

Figure 2. Photograph of the patient showing typical target lesions on the palm of the hand

Figure 3. Erosions and haemorrhagic crusting of the lips
DIAGNOSIS

Serum mycoplasma IgM and IgG were positive, indicating an acute infection. Herpes simplex and varicella zoster virus PCR were negative. Other investigations including blood culture were non-contributory. Chest X-ray showed a peribronchial pneumonia pattern. Based on these findings and the clinical presentation, the diagnosis erythema multiforme (EM) major was made, most likely the result of infection with *Mycoplasma pneumoniae*. The patient was admitted to the department of paediatrics and treated with azithromycin 10 mg/kg/day for three days for mycoplasma infection. Further treatment consisted of adequate analgesia and administration of fluid to prevent dehydration.

EM is an acute, immune-mediated skin condition. It is characterised by the distinctive ‘target’ lesions: concentric rings with an outer red ring, an inner pale ring and a red centre. Moreover vesicles, bullae and oedematous, erythematous papules can be observed. The target lesions are usually asymptomatic and occur in a symmetrical acral distribution. In up to 70% of the patients the oral mucosa is affected. The ocular and genital mucosa can also be involved. EM is typically seen in adults between the ages of 20 and 40. The incidence is estimated to be less than 1% per year. EM is usually self-limiting and treatment is focused on symptomatic relief. The underlying cause needs to be treated.

However, EM can be caused by numerous potential triggering agents and the exact pathogenesis is still unknown. It is considered to be an immunologically mediated hypersensitivity reaction, most commonly induced by infection. EM has also been associated with malignancy, autoimmune diseases and medication such as antibiotics and anticonvulsants. The most common infectious cause of EM is herpes simplex virus. One of the other less frequently reported causes is *Mycoplasma pneumoniae*. Infection with *Mycoplasma pneumoniae* can result in respiratory disorders. The clinical features are fever, tachypnoea, coughing and malaise. Besides pulmonary manifestations, cutaneous disorders can occur in up to 25% of the patients. The differential diagnosis of EM may include Stevens-Johnson syndrome (SJS). Controversy exists whether EM and SJS are distinct entities or whether they represent a spectrum of the same disease. Furthermore the latest research suggests that *Mycoplasma pneumoniae*-induced rash and mucositis is a syndrome distinct from SJS and EM because of its considered distinct morphology and disease course.

CONCLUSION

If target lesions are observed, *Mycoplasma pneumoniae* should be included in the differential diagnosis as a possible causative agent.

DISCLOSURES

The authors declare no conflict of interest.

REFERENCES

Dear Editor,

Methanol intoxication often runs a deathly course. In most cases the intoxication is due to oral ingestion of this substance, but other routes of administration (intravenously, transdermal) have been described as well. Recently, we saw a patient who survived with allegedly lethal blood levels of methanol after chronic air-borne exposure to this compound. The patient, a previously healthy 28-year old male, attended the emergency department with blurring of vision, somnolence, severe headache and nausea. On physical examination we saw a tachypnoeic, haemodynamically stable patient with a maximal EMV score. Arterial blood gas analysis showed a high anion gap metabolic acidosis (pH 7.10, bicarbonate 5 mmol/l) but the lactate level was normal. Because of the vision disturbances in combination with a high-anion gap acidosis we suspected methanol intoxication, which was confirmed by an elevated serum methanol of 1146 mg/l. The patient admitted that he had worked at a badly ventilated amphetamine laboratory for 12 hours, one and a half days before seeking medical assistance. He had not worn a gas mask or protective clothing. He was immediately admitted to the intensive care unit where we started ethanol intravenously and, because the methanol blood concentration was higher than 200 mg/l, haemodialysis. During dialysis his sight deteriorated to complete blindness. The ophthalmologist was consulted and saw vital optic nerves. After dialysis the vision of the patient partially recovered. The next day the patient could be discharged. He still had minimal visual symptoms, which gradually disappeared over time. Recent data suggest that it is not so much the methanol level but rather the pH that determines outcome. In our patient the methanol level was in the lethal range but the pH was still above 7.0 which is taken as the lowest tolerable value. We suspect that desensitisation to methanol by frequent exposure, suggesting a chronic intoxication, explains why a patient can survive a severe intoxication with this ethanol-like gas. Possibly, this desensitisation is related to genetic make-up. Because of the popularity of recreational drug abuse and the production of these drugs, we should remain alert to other such cases of airborne methanol intoxication.
LETTER TO THE EDITOR

Lung ultrasound: the need of an adequate training for the next generation of internists

F.M. Trovato*, G. Musumeci

Departments of 1Clinical and Experimental Medicine, Internal Medicine Division, 2Biomedical and Biotechnological Sciences, Human Anatomy and Histology Section, School of Medicine, University of Catania, Catania, Italy, *corresponding author: tel.: +390953781550, fax: +390953781549, email: trovatofrancesca@gmail.com

Dear Editor,
We read with interest the review by Touw et al.1 and the related editorial,2 which warmly warrant the use of ultrasound for internists. We would respectfully remark that an adequate comprehensive training is needed to teach and learn the uses and limitations of ultrasound.3

An optimal ultrasound examination of a dyspnoeic patient should be done in sitting position, since it is unlikely that a dyspnoeic patient can lie in a supine position. Moreover, differently from what was suggested,1 the pleural surface is most accessible from the back with longitudinal and transversal intercostal and paravertebral scans.3 The lung ultrasound (LUS) artifacts arise from the difference in acoustic impedance in the pleural spaces and have been classified as simple reverberation (horizontal A-line), ‘comet-tail’ and ‘ring-down’ (vertical B-line) artifacts, but some confusion of these terms is apparent,4 (that ‘comet tail’ is considered to be a synonym of B-line).4 Although Touw et al.1 suggested suppressing all software artefact reduction and image optimisation and prefer the high frequency and high resolution linear probe, which reduces the number of artifacts, most of the images shown in the paper1 are taken by a sector probe, useful to scan between the ribs, but with poor near-field resolution to evaluate pleural line and useless for LUS. It is unrealistic to suppose that LUS allows us to distinguish easily between pulmonary oedema, COPD, asthma, pulmonary embolism, pneumothorax and pneumonia with sensitivities and specificities ranging from 81 to 100%, since even large lung consolidations, easily detectable by X-ray, can only be evaluated by ultrasound if no air is obstructing the beam’s passage and their nature is not identifiable by the sole ultrasound imaging, since cancer, atelectasis and pneumonia have similar aspect.3 Moreover alveolar consolidations, in contrast to pleural effusion, do not appear first on a postero-basal scan; only aspiration pneumonia of mechanically ventilated patients arises in this way.5 Regarding diffuse lung disease, the statement ‘LUS does not require any cardiac ultrasound imaging, as a cardiac cause of dyspnoea can be diagnosed from lung imaging only’1 is quite hazardous. The certainty that more than two anterior B-lines are pathological and indicate interstitial syndrome and thus pulmonary oedema, and that the number of B-lines per screen or the distance between B-lines allows assessment of severity, is quite odd due to the variability related to different probes and setting, particularly in a moving dyspnoeic patient.

The protocol proposed is not a good way to spread the use of ultrasound in the daily clinical practice, since formal training incorporating ultrasound in adequate curricula is crucial for physicians,1 avoiding simplistic numeric rules, since medicine is not arithmetic.

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Response to the letter of Trovato et al.

H.R.W. Touw\textsuperscript{1,4}, P.R. Tuinman\textsuperscript{1,2,3}, H.P.M.M. Gelissen\textsuperscript{1}, E. Lust\textsuperscript{1}, P.W.G. Elbers\textsuperscript{1,2,3}

\textsuperscript{1}Department of Intensive Care Medicine, \textsuperscript{2}Research VUmc Intensive Care (REVIVE), \textsuperscript{3}Institute for Cardiovascular Research (ICaR-VU), \textsuperscript{4}Department of Anesthesiology, VU University Medical Center, Amsterdam, the Netherlands, \textsuperscript{*}corresponding author: p.tuinman@vumc.nl

Dear Editor,

We would like to thank Trovato and Musumeci for their letter. We share their opinion that comprehensive training is required for lung ultrasound (LUS). This is exactly why we designed our Intensive Care Ultrasound (ICARUS) curriculum as described in our paper.\textsuperscript{1} Alisma \textit{et al.} even recommend this program in their editorial.\textsuperscript{2}

ICARUS includes basic cardiac ultrasound but this was beyond the scope of our review. However, LUS can differentiate between most causes of dyspnoea,\textsuperscript{1,3} although Bataille \textit{et al.} described significant advantages of an integrative cardiopulmonary ultrasound approach.\textsuperscript{4}

We have chosen to follow the BLUE protocol, with its impressive sensitivity and specificity. Of course it should be remembered that this study was performed in dyspnoeic patients in the emergency room. However, the physiological principles of ultrasound artifacts are universal. Lung consolidations may arise at any point, but touch the pleural surface in 98\% of cases. Of course, this implies that LUS sensitivity will depend on the extent of scanning. However, most cases (90\%) include findings at the PLAPS point,\textsuperscript{5} which is part of the BLUE protocol.

When choosing the optimal probe, bedside trade-offs need to be made between form factor, ergonomics, scanning depth and resolution. For speed and simplicity we tend to use only one probe (1-5 Mhz sector array), generating the obvious artifacts seen in our figures. Its shape allows satisfactory scanning of the intercostal spaces and facilitates cardiac imaging as well. Of course, the vascular probe (10+ Mhz, linear array) and the abdominal probe (1-5 Mhz, curved array) are also useful.\textsuperscript{1} Answering clinical questions with LUS enables immediate therapy for potentially lethal conditions. We therefore continue to feel that LUS should be standard practice.