Abdominal pain and melaena; what is your diagnosis?

Lung ultrasound for the internist

Hepcidin in chronic kidney disease

The standardised mortality ratio in acute leukaemia

Hypovitaminosis D in patients with low-energy fractures

Cholesteryl Ester Storage disease
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Department of Internal Medicine
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The Netherlands
Tel.: +31 (0)10-703 39 54
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EDITORIAL

Ultrasound for internists: changing bedside examination

J. Alsmaw, F.H. Bosch

1Department of Internal Medicine, Erasmus UMC, Rotterdam, the Netherlands, 2Department of Internal Medicine, Rijnstate Hospital, Arnhem, the Netherlands, *corresponding author: tel.: +31 (0)10-7040704, email: j.alsma@erasmusmc.nl

In this issue of the journal, Touw et al. describe the principles of bedside lung ultrasound in a narrative review. Lung ultrasound allows for rapid, non-invasive and bedside patient assessment and the authors believe that lung ultrasound will be the most used imaging technique in dyspnoeic patients in the near future, thereby possibly replacing the stethoscope almost two centuries after its invention by Laennec at the Hôpital Necker in Paris in 1816.

The rise of bedside ultrasound is the result of several developments. The costs of portable ultrasound equipment have decreased, the handling of the equipment has become easy, the quality of the scans has increased and physicians have come to realise how ultrasound can be of benefit for rapid diagnosis and treatment. Cardiologists, gynaecologists and many others use ultrasound routinely. Compared with other imaging modalities, such as computed tomography scanning, bedside ultrasonography is rapidly deployed and noninvasive, and there is no administration of contrast agents or exposure to radiation.

The scope of bedside ultrasound differs from diagnostic ultrasound, as performed by radiologists. In bedside ultrasound physicians mainly search for dichotomous answers to basic clinical questions raised by the patient’s chief symptoms or differential diagnosis, and for this reason the term binary ultrasound (yes or no) is advocated. In this manner ultrasound increases diagnostic accuracy, shortens the time to definitive therapy and supports the clinician in the decision-making process.

That bedside ultrasonography can be of use for internists in many types of patients and care settings has been shown in previous studies. Jones et al. showed a contribution of ultrasound to medical decision making in critically ill patients with non-traumatic, undifferentiated hypotension. Incorporation of bedside ultrasound led to a more timely and more accurate diagnosis compared with usual care.

Physicians in this study used a seven-step goal-directed protocol to assess the heart for cardiac tamponade, left ventricle function, and right ventricle function and size. Subsequently, the inferior vena cava was assessed to estimate volume status, and the abdomen was examined for the presence of free intraperitoneal fluid or an aneurysm of the abdominal aorta. The average time to complete the ultrasound examination using this protocol was approximately six minutes. This seven-step protocol is comparable with the focused assessment with sonography in trauma (FAST), which is successfully used in trauma patients.

Lung ultrasound can aid physicians in making a rapid diagnosis in patients with acute respiratory failure. In their review, Touw et al. describe the Bedside Lung Ultrasound in Emergency (BLUE) protocol by Lichtenstein et al. Using this BLUE protocol, physicians can rapidly differentiate between pulmonary oedema, COPD, asthma, pulmonary embolism, pneumothorax and pneumonia with sensitivities and specificities ranging from 81 to 100%.

In patients suspected of deep vein thrombosis, Magazzini et al. showed bedside ultrasound by emergency physicians was both accurate and safe, allowing rapid discharge from emergency departments and preventing improper treatment with anticoagulants. Ultrasound also aids physicians in performing invasive procedures, and increases success and lowers the risk for the patient. For example, procedural guidance with ultrasound allows safer drainage in patients with pleural or intra-abdominal fluid, or safe placement of central venous catheters.

Ultrasound is not only of use in the emergency department and the wards; also in the outpatient department the possibilities of ultrasonography for various subspecialties of internal medicine are plenty. Examples include thyroid ultrasonography by endocrinologists, carotid artery intima media thickness measurement by vascular internists, vascular access for dialysis by nephrologists, and musculoskeletal ultrasound by rheumatologists.
Ultrasound is an operator-dependent technology, and therefore it is paramount that the quality of ultrasound examinations is assured. Operators should be appropriately trained and certified, and continuous medical education is required to maintain and increase acquired skills. The European Federation of Societies for Ultrasound in Medicine and Biology defined, in accordance with the view of World Health Organisation, practice of ultrasonography on three levels of competence.

Level 1 ultrasonographers are able to perform common examinations safely and accurately. Level 2 ultrasonographers are able to recognise and correctly diagnose almost all pathology within the relevant organ systems and can perform ultrasound-guided invasive procedures. Level 3 ultrasonographers perform on an advanced level of practice performing specialised ultrasound examinations and advanced ultrasound-guided invasive procedures, next to teaching and research. These levels of competence are supported by various medical societies, for example the Royal College of Radiologists (United Kingdom) and Deutsche Gesellschaft für Ultraschall in der Medizin (DEGUM, Germany).

The ultrasound training programs vary between different countries and specialties. Countries where ultrasonography is part of the routine practice of internists have structured training programs incorporated in residency training. In Germany, ultrasound is usually trained during residency according to DEGUM standards and in Italy, the Italian Society of Internal Medicine organises an ultrasound Summer School for internal medicine residents, allowing them to achieve level 1 and 2 competence during their residency. Implementation of ultrasonography in undergraduate training increases understanding of ultrasound and improves traditional physical examination skills. In countries without structural ultrasonography training for residents, physicians mostly acquire ultrasound skills by following one of the many ultrasonography courses. For example, Touw et al. describe in their review a two-day Intensive Care Ultrasound (ICARUS) curriculum, followed by a practical and theoretical exam, and the program is open for medical specialists and residents in the final stages of their training. As findings during ultrasound are in some ways comparable to findings during physical examination, we propose that internists describe the interpretation of the images as part of the physical examination. However, to assure maximum quality and minimise the risks for patients that accompany the use of ultrasonography (e.g. false-positive or false-negative findings), it is important that each hospital has a proper governance system for all physicians using ultrasound. This governance system should oversee training, certification, education and supervision. For such a governance system to work optimally, we advocate that hospital organisations facilitate the systematic recording of scans that are acquired at the bedside, in the same way that scans performed by the radiologist and other experts are recorded in the (electronic) patients medical record. This not only allows for structural supervision of level 1 and 2 ultrasonographers, thereby assuring maximum quality of care, but it also facilitates ultrasonographers in keeping a logbook with the number of performed examinations, which is required for continuous education. At the hospital level, it gives insight into the number of physicians using bedside ultrasound.

In conclusion, the bedside availability of ultrasound potentially makes ultrasonography the stethoscope of the 21st century. For optimal use, physicians should undergo structural ultrasound training in medical residency training programs, or during undergraduate training, followed by continuous medical education. Internists should describe the results of their ultrasound examination in the medical record as part of the physical examination. Hospital organisations should facilitate governance by making structurally recording of scans possible.

REFERENCES

Lung ultrasound: routine practice for the next generation of internists

H.R.W. Touw1,2, P.R. Tuinman1,3,4, H.P.M.M. Gelissen1, E. Lust1, P.W.G. Elbers1,3,4

Departments of 1Intensive Care Medicine, 2Anesthesiology, 3Research VUmc Intensive Care (REVIVE), 4Institute for Cardiovascular Research (ICaR-VU), VU University Medical Center, Amsterdam, the Netherlands, *corresponding author: tel.: +31(0)20-4443697, fax: +31(0)20-4443901, email: p.tuinman@vumc.nl

ABSTRACT

Background: The lung is at the crossroads of ventilation and circulation and can provide a wealth of diagnostic information. In the past, lung ultrasound (LUS) was considered impossible. However, the interplay between air, fluid and pleurae creates distinctive artefacts. Combinations of these artefacts can help differentiate between various pathological processes, including pulmonary oedema, pneumonia, pulmonary embolism, obstructive airway disease and pneumothorax. LUS, when used by experienced physicians, is superior to chest X-ray and comparable to computed tomography for establishing a diagnosis in acutely dyspnoeic patients. LUS allows for rapid, non-invasive and bedside patient assessment. It is therefore unfortunate that unlike many other medical specialists in the Netherlands, internists have not yet incorporated LUS into their daily practice.

Objectives: This review aims to be the starting point for internists wanting to acquire competence in LUS.

Review content: This narrative review describes the principles of ultrasound equipment, LUS artefacts, gives practical guidance to perform LUS and provides a road map towards LUS competence. Furthermore, it presents a decision tree to differentiate between causes of acute dyspnoea.

Authors conclusions: LUS is a promising diagnostic technique that can be of great help for the internist. It can be applied directly at the bedside and can also be used to follow up on disease progression and therapy. It is our belief that it will replace the stethoscope and that it will be the most used imaging technique in the near future, especially in dyspnoeic patients.

KEYWORDS

Dyspnoea, internist, lung ultrasound, medical training

INTRODUCTION

The lungs can provide a wealth of diagnostic information, as they represent the crossroads of respiration and circulation. However, physical examination of the lungs remains a significant challenge. This is largely due to the poor acoustical performance of the stethoscope. Even seasoned physicians may fail to become experts at this 19th century technique. For example, for detecting congestive heart failure or pneumonia, auscultation has a low sensitivity. Clinical decision-making entered another era when the chest X-ray was introduced, followed by computed tomography (CT). Due to its superior sensitivity, the CT scan is now the gold standard in the diagnosis of community acquired pneumonia. But CT scanning requires transportation of a potentially critically ill patient, which is not without risk. In addition CT scanning is associated with significant costs, radiation and contrast burden, which should not be taken lightly. Lung ultrasonography (LUS) is the answer to these limitations. LUS enables physicians to differentiate between causes of acute dyspnoea within minutes at the bedside, and as such is particularly helpful in the emergency department and on the ward. LUS is not only superior to the physical examination and chest X-ray, but even comparable to CT for many diagnoses. Pneumonia, pulmonary oedema, pulmonary embolism, asthma, chronic obstructive pulmonary disease and pneumothorax can be assessed with sensitivity and specificity ranging from 90 to 100% (table 1).

LUS yields important diagnostic information within minutes and is able to answer important binary questions. The examination can easily be repeated to evaluate the progress of the disease or the effect of initiated therapy. And importantly, LUS avoids radiation, transport and excessive cost. We have extensive experience with LUS, as it is an essential component of our intensive
Touw et al. Lung ultrasound for the internist.

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Table 1. Performance of ultrasound compared with computer tomography scan as gold standard

<table>
<thead>
<tr>
<th>Condition</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleural effusion</td>
<td>94</td>
<td>97</td>
</tr>
<tr>
<td>Alveolar consolidation</td>
<td>90</td>
<td>98</td>
</tr>
<tr>
<td>Interstitial syndrome</td>
<td>93</td>
<td>93</td>
</tr>
<tr>
<td>Complete pneumothorax</td>
<td>100</td>
<td>95</td>
</tr>
<tr>
<td>Occult pneumothorax</td>
<td>79</td>
<td>100</td>
</tr>
</tbody>
</table>

DEVELOPMENT OF LUS

In medicine, ultrasound frequencies between 1 and 15 MHz are used, to allow for non-invasive visualisation of tissue structures. Ultrasound physics have been extensively reviewed elsewhere. In short, ultrasound beams travel through tissues until they are reflected when acoustic impedance of the adjacent tissue is different. Ultrasound cannot penetrate bone and is fully reflected by air. Therefore, the lung was considered unsuitable for ultrasound in the past. However, LUS creates artefacts through the interplay of air, lung tissue, pleurae, fluid and bone. Through reflection, scatter and absorption of ultrasound beams, physiological and pathological processes generate distinctive combinations of artefacts and these speak a language of their own. This was studied in detail by Lichtenstein and colleges who named many of these artefacts and showed their reproducibility. In fact, virtually all acute life-threatening respiratory disorders abut the pleural line, generating artefacts that unveil the great potential of LUS.

MACHINE AND PROBE SELECTION

For LUS, virtually any ultrasound machine will do, even legacy equipment. Handy features include a supporting trolley, fast start-up time and a small width, allowing bedside use. Most importantly, as LUS relies on artefacts, it should be possible to suppress all software artefact reduction, such as harmonics, filters and other image optimisation. Higher beam frequency yields higher resolution, but less maximum depth. In practice, linear vascular probes, cardiac phased array probes, or even abdominal probes may be used.

SYSTEMATIC SCANNING

LUS is a trade-off between diagnostic speed and extensive scanning. A reasonable approach is to scan at least three different zones of the lung: anterior, anteromedial and posterior. We recommend the approach suggested by Lichtenstein in his BLUE protocol. Figure 1 shows how to find these points. The anterior points are called upper BLUE points and the anteromedial points lower BLUE points, after the protocol. The posterior points are called the ‘posterolateral alveolar and/or pleural syndrome point’, or PLAPS point. In addition, the protocol includes optional identification of the ‘lung point’ and scanning for lower extremity venous thrombosis to aid diagnosis. Table 2 shows the proposed compulsory and optional views for LUS. As discussed in detail below, these views enable discrimination between acute causes of dyspnoea. LUS does not require any cardiac ultrasound imaging, as a cardiac cause of dyspnoea can be diagnosed from lung imaging only.
There are many LUS artefacts, but for daily practice only a few of these need to be remembered. It is essential to first identify the pleural line, as most pathology abuts it. To do so, the probe should be positioned perpendicular to the ribs. A rib is easily recognised as a shadow, caused by absorption of ultrasound by bone tissue. The pleural line is horizontal and hyperechoic, situated slightly beneath the two ribs. This yields a characteristic image called the ‘bat sign’ (figure 2a). Under normal circumstances, the parietal pleura and visceral pleura are seen as one line.

Lung sliding
Although visualised as one, respiration causes visceral pleura to slide relative to the parietal pleura. This causes a subtle sparkling to-and-fro movement artefact called lung sliding. Lung sliding indicates that both pleura are adjacent, ruling out pneumothorax at that point. Lung sliding is so characteristic that it takes only a few seconds to recognise. Lung sliding can be confirmed using the M-mode. This setting essentially repeats one line of the screen over time. In the presence of lung sliding, the ‘seashore phenomenon’ occurs (figure 2b). If lung sliding is absent, the ‘stratosphere sign’ appears (figure 2c). Of note, abolished lung sliding is far from specific: pneumothorax is the classic example, but a motionless pleural line can also be caused by inflammatory or chronic adherences, fibrosis and atelectasis.

A-lines
An A-line is the main horizontal artefact and a repetition of the pleural line. If ultrasound beams encounter a tissue-air

**Table 2.** The standard lung ultrasound (LUS) views, including the optional lung point identification and venous analysis. At our institute, these LUS views are embedded in the Intensive Care Ultrasound Protocol (ICARUS), which consists of 22 compulsory views and includes echocardiography.

<table>
<thead>
<tr>
<th>LUS views as embedded in the ICARUS protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Upper BLUE point, left</td>
</tr>
<tr>
<td>2. Upper BLUE point, left, M-mode</td>
</tr>
<tr>
<td>3. Lower BLUE point, left</td>
</tr>
<tr>
<td>4. Lower BLUE point, left, M-mode</td>
</tr>
<tr>
<td>5. Upper BLUE point, right</td>
</tr>
<tr>
<td>6. Upper BLUE point, right, M-mode</td>
</tr>
<tr>
<td>7. Lower BLUE point, right</td>
</tr>
<tr>
<td>8. Lower BLUE point, right, M-mode</td>
</tr>
<tr>
<td>9. PLAPS point – left</td>
</tr>
<tr>
<td>10. PLAPS point – right</td>
</tr>
<tr>
<td>11. Lung point (optional)</td>
</tr>
<tr>
<td>12. Venous analysis (optional)</td>
</tr>
</tbody>
</table>

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**Figure 2a.** Normal LUS image with the bat sign and A-lines

**Figure 2b.** Normal LUS with normal M-mode scanning (seashore phenomenon)

**Figure 2c.** M-mode scanning in pneumothorax (stratosphere sign)
interface, they are reflected back to the probe. However, the probe itself reflects them once again. This causes the beams to travel through the soft tissue a second time. This is called reverberation and causes the visualisation of A-lines (figure 2a). The depth at which an A-line is displayed is always equal to the distance of the pleural line to the probe and can be used to differentiate A-lines from other artefacts. It follows that A-lines represent the presence of air at the pleural line. This can be alveolar air and therefore normal lungs yield A-lines. However, it can also be air outside the alveoli, such as the air between the visceral and parietal pleura in pneumothorax.

B-lines
B-lines are the main vertical artefacts and are reminiscent of helicopter search spotlights. They are sometimes referred to as lung rockets or comet tail artefacts. However, strictly speaking, B-lines are a specific subtype of these. Interstitial oedema first appears in the subpleural interlobular septa which are surrounded by alveolar gas. Ultrasound beams entering this small pleural line-fluid-air system are reflected again and again, essentially being trapped. This results in many to-and-fro moments, generating a long vertical hyperechoic artefact, the B-line. Thus, by definition, B-lines arise from the pleural line. They erase A-lines and immediately rule out pneumothorax. B-lines are hyperechoic and narrow, span across the whole ultrasound image without fading and move with lung sliding.

More than two anterior B-lines are pathological and indicate interstitial syndrome. Although semantically unrelated, B-lines correlate with Kerley B-lines on chest X-ray (figure 2d). Any disease affecting the pulmonary interstitium can cause an interstitial syndrome. The commonest cause is pulmonary oedema. The number of B-lines per screen or the distance between B-lines allows assessment of severity. It has been suggested that very dense B-lines, i.e. more than 10 per screen or only 3 mm apart, favour the diagnosis of ARDS.

C-lines, air bronchogram, shred sign and pleural effusions
Alveolar consolidation causes typical ultrasound patterns at the different scanning points. At the anterior points, this yields C-lines, identified by a curvilinear aspect of the pleural line (figure 2e). This is caused by adjacent consolidated tissue. Pulmonary consolidations are fluid disorders. The non-aerated lung tissue is therefore easily traversed by ultrasound beams and creates an image comparable with that of liver tissue (figure 3a and b). Virtually all consolidations touch the pleural line. However, because of gravity, consolidation usually appears first at the PLAPS point.

For similar reasons, pleural effusions also first appear at the PLAPS point (figure 3b). They can be hypoechoic or hyperechoic with particles. Hyperechoic effusions are associated with exsudate, but accuracy is not perfect. Hyperechoic effusions in combination with consolidated lung tissue at the PLAPS point are associated with the diagnosis of pneumonia. Consolidations can be further analysed by looking for air bronchograms or the shred sign (figure 3b). An air bronchogram is caused by reflection of ultrasound beams in the air-filled bronchi surrounded by consolidated tissue. The shred sign appears when the border of aerated lung and consolidated lung is not sharp. Both are suggestive of pneumonia. Furthermore LUS will identify the presence, size, and nature of an effusion and can be used to guide thoracocentesis (figure 3b).

THE DECISION TREE
We propose a decision tree for a structured approach of the dyspnoeic patient using LUS. This is based on the original data from the BLUE study. This landmark investigation related combinations of ultrasound signs to final diagnosis.
in acutely dyspnoeic patients presenting at the emergency department. Our decision tree consists of binary questions. Answering these rapidly narrows the differential diagnosis with impressive accuracy (figure 4).

In addition, table 3 gives a number of combinations of findings that either directly point to a certain diagnosis or directly exclude it. This may be useful in hyperacute settings.

The protocol starts by scanning the anterior BLUE points. The first step in our decision tree is to identify line artefacts. These determine the BLUE profile, depending on the predominance of anterior artefacts. Thus, A and B profiles are distinguished. If one lung shows A-predominance and the other B-predominance, this is called the A/B profile. If any anterior C-line is noted, the C-profile is said to be present, regardless of other artefacts. In case of A or B profiles, the decision tree calls for further analysis based on combinations of lung sliding, findings at the PLAPS point, identification of a lung point and/or venous analysis.

Imaging both PLAPS points again aims to answer a binary question. PLAPS is said to be positive if any sign of alveolar consolidation is seen or if any pleural effusion is seen. If not, PLAPS is said to be negative. Of course, more information can be deducted from the exact findings, but for application of the decision tree, this simple yes or no will suffice.

Identification of the lung point to confirm the presence of pneumothorax becomes necessary if an A-profile is present without lung sliding. Starting at the BLUE point where lung sliding was found to be absent, the operator moves the probe down the chest, while staying at the same intercostal space to search for the lung point. This is the point on the thorax where the visceral pleura is sliding in and out of the ultrasound image, due to respiratory movement (figure 5). This implies that M-mode imaging at the lung point will yield a pattern that alternates between the seashore sign and the stratosphere sign. Interestingly, LUS was shown to be superior to bedside chest radiographs for the detection pneumothorax. In addition, the size of the pneumothorax may be estimated from the distance between the lung point and the sternum, as the pneumothorax extends anteriorly from this point.

Venous analysis is not performed routinely for LUS, but can be of great help in the setting of A-lines with lung sliding. Presence of thrombus in the large veins of the lower extremity strongly suggests pulmonary embolism as the final diagnosis in this situation. Subtle compression manoeuvres starting from the femoral vein can distinguish thrombus from patent veins. Veins can usually be followed up to the popliteal fossa with relative ease. However, venous analysis is not an absolute requirement to narrow down the differential diagnosis.

Using the diagnostic tree is fairly straightforward. For example: the presence of lung sliding, bilateral A-lines but without evidence of PLAPS leads to the diagnosis of COPD/asthma.

**BE Comes AN EARLY ADAPTER**

There are limited data on the efficiency of LUS education. Lichtenstein suggests that short sessions, with a total duration of 90 minutes, focusing on lung sliding (yes/no) and searching for B-lines (yes/no), yield an average accuracy of 95%. Medical students using ultrasound identified abnormalities more accurately than certified specialists who performed physical examination. This stresses that it is not difficult to learn point-of-care LUS.

Recently, an international expert group recommended at least 30 studies to achieve global competency in basic critical care echocardiography and we think the same holds true for LUS. Recently, it was suggested to incorporate ultrasound training in standard undergraduate training.
Figure 4. Schematic overview to guide physicians in using lung ultrasound. A step-by-step approach is provided including typical ultrasound findings. Pie graphs represent the differential diagnosis at each step, including percentages of incidence. Green pie graphs indicate the end of the protocol, as only one likely diagnosis remains. All data based on the original BLUE study. Confirmatory findings for the various diagnosis groups include the following: Cardiogenic pulmonary oedema — cardiac ultrasound for left ventricular systolic and diastolic dysfunction and atrial dimensions, NT-pro BNP; Pulmonary embolism — cardiac ultrasound for right heart dimensions, D-dimer; Pneumonia — shred sign, air bronchogram, infection parameters such as white blood cell count and differential, C-reactive protein and procalcitonin; ARDS — very dense B-lines, abolished lung sliding, shred sign, air bronchogram.

* denotes that the diagnosis of ARDS is also possible for that finding. EMB = pulmonary embolism; PNA = pneumonia; OBS = chronic obstructive pulmonary disease or asthma; EDE = cardiogenic pulmonary oedema; PTX = pneumothorax; LP = lung point; PLAPS = posterolateral alveolar and/or pleural syndrome.
Ultrasound can also be used for other parts of the body, for example the abdomen, but this is beyond the scope of this review.

**OUR EXPERIENCE**

At VU University Medical Center (VUmc), we have developed the Intensive Care Ultrasound (ICARUS) protocol and curriculum. The protocol consists of 22 compulsory views (table 2). Eight views are devoted to LUS. The other views are dedicated to echocardiography. This can provide further evidence for the BLUE diagnosis, for example in pulmonary embolism (enlarged right heart) or cardiogenic pulmonary oedema (decreased ventricular function).

The program is open to fellows in intensive care medicine and anaesthesiology residents. Intensivists, anaesthesiologists, internists and emergency medicine physicians from the Netherlands and abroad can also take part. Following a two day hands-on introductory course, candidates perform the ICARUS protocol on 50 patients in their own hospital. After 5-10 patients, external candidates return to VUmc for hand-on refinement of their technique on VUmc patients. Following a practical and theoretical exam, candidates become ICARUS certified and are able to perform LUS for clinical purposes. Endorsement of the ICARUS certification program by the Netherlands Society for Intensive Care Medicine and other ultrasound courses may facilitate widespread dissemination of ultrasound knowledge amongst physicians, including those specialising in internal medicine, in all hospitals in the Netherlands.

**LIMITATIONS**

Performing a LUS examination and interpreting the acquired images correctly requires formal training. The reliability of LUS is therefore dependant on the experience of the ultrasonographer. It is important to note that point-of-care ultrasound does not replace specialised ultrasound examinations by comprehensively trained physicians, such as an echocardiography by a cardiologist. Also patient-dependent factors such as obesity, the presence of subcutaneous emphysema and wound dressings alters the transmission of ultrasound beams and makes ultrasound a challenge in some patients. Most of the studies validated ultrasound for acute dyspnoeic patients in the emergency department and ultrasound has yet to be validated for other indications. Acquired images should be uploaded to a hospital server so they can be reviewed by more experienced ultrasonographers, discussed with colleagues and used to compare with newer images to evaluate the effect of initiated therapy or progress of the disease over time.

<table>
<thead>
<tr>
<th>Finding</th>
<th>Rules out</th>
<th>Rules in</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/B profile</td>
<td></td>
<td>PNA* (100%)</td>
</tr>
<tr>
<td>C profile</td>
<td></td>
<td>PNA* (90%)</td>
</tr>
<tr>
<td>Lung sliding</td>
<td>PTX (0%)</td>
<td>PNA* (90%)</td>
</tr>
<tr>
<td>B profile</td>
<td>PTX (0%), OBS (3%), PE (0%)</td>
<td></td>
</tr>
<tr>
<td>B profile AND lung sliding</td>
<td>EDE (87%)</td>
<td></td>
</tr>
<tr>
<td>B profile WITHOUT lung sliding</td>
<td>PNA* (100%)</td>
<td></td>
</tr>
</tbody>
</table>

* denotes that the diagnosis of ARDS is also possible for that finding. PNA = pneumonia; EDE = cardiogenic pulmonary oedema; PTX = pneumothorax; OBS = chronic obstructive pulmonary disease or asthma; PE = pulmonary embolism.
CONCLUSION

LUS is a rapid diagnostic bedside tool that is easily accessible for internists and should therefore be considered an extension of physical examination and used in combination with laboratory tests and when needed other imaging techniques. Answering clinical questions with LUS enables immediate therapy for potentially lethal conditions. LUS will give the internist an advantage in clinical care in the near future.

DISCLOSURES

The authors declare no conflicts of interest. Institutional funding only.

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Hepcidin in chronic kidney disease: not an anaemia management tool, but promising as a cardiovascular biomarker


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ABSTRACT

Hepcidin is a key regulator of iron homeostasis and plays a role in the pathogenesis of anaemia of chronic disease. Its levels are increased in patients with chronic kidney disease (CKD) due to diminished renal clearance and an inflammatory state. Increased hepcidin levels in CKD patients are supposed to be responsible for functional iron deficiency in these patients and contribute to renal anaemia and resistance to erythropoiesis-stimulating agents. Therefore, hepcidin was purported to be useful as a management tool guiding treatment of renal anaemia. Furthermore, since hepcidin is associated with iron accumulation in macrophages in the vessel wall inducing oxidative stress and atherosclerosis, it has been speculated that hepcidin might function as a biomarker of cardiovascular disease. In this descriptive review, the merits of hepcidin with respect to its role in the pathophysiology of renal anaemia in CKD patients, its presumptive role as a practical diagnostic tool guiding management of renal anaemia, and its possible usefulness as a prognostic biomarker will be discussed.

KEYWORDS

Anaemia, cardiovascular, chronic kidney disease, dialysis, hepcidin, iron

INTRODUCTION

Hepcidin was first identified in 2001 as a peptide with antimicrobial properties. It was postulated that hepcidin contributes in the defence against extracellular infection by reducing serum iron levels, as iron availability is necessary for bacterial growth and enhances oxidative stress. Furthermore, suppressed hepcidin expression was shown to be related to iron overload disorders, such as haemochromatosis. Subsequently, hepcidin was established as a key regulator of iron homeostasis and involved in the pathogenesis of anaemia of chronic disease. Anaemia is also a well-known complication in patients with chronic kidney disease (CKD). In addition to ‘true’ iron deficiency, many CKD patients have functional iron deficiency, which is characterised by impaired iron release from body stores that are unable to meet the demand for erythropoiesis. Availability of ‘functional’ iron is important to obtain increased haemoglobin levels by treatment with erythropoiesis-stimulating agents (ESA). Because of the above-mentioned sequelae in patients with CKD and end-stage renal disease (ESRD), the relevance of hepcidin for this patient group seems obvious. Indeed, many papers on various aspects of hepcidin in CKD and ESRD patients have been published and the expectations of the clinical utility of measuring hepcidin to guide the treatment of renal anaemia were high.

In this review, several characteristics of hepcidin, such as its function and regulatory pathways, will be discussed, as well as the various measurement assays. Furthermore, the value of hepcidin with respect to its use in clinical decision-making for renal anaemia in CKD patients and its role as a (prognostic) biomarker is described. The present knowledge on the role of hepcidin in this respect will be presented as a descriptive review. A literature search

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was performed in PubMed and Google Scholar with the search terms hepcidin, chronic kidney disease, dialysis (hemodialysis, hemo(dia)filtration and peritoneal dialysis) and cardiovascular (disease). The search was limited to full-text articles published in English. Furthermore, references of the selected articles were screened.

HEPCIDIN: STRUCTURE AND KINETICS

Systemic hepcidin is mainly produced in the liver, but expression by almost all other cells and tissues has been described, such as kidney tubuli, the heart, retina, fat, the lungs and the pancreas as well as in monocytes, neutrophils and macrophages.\(^{10}\) Hepcidin is a hairpin-shaped molecule stabilised by four disulphide bridges.\(^{11-13}\) It is mainly present in its bioactive form hepcidin-25, a 25 amino acid peptide of 2.8 kD. Hepcidin-20, -22 and -24 are isoforms with no or unknown biological function.\(^{14}\) Under physiological conditions, these isoforms are present in the urine, but virtually absent in the blood.\(^{15-17}\) However, in patients with CKD, and especially in those on dialysis, both serum levels of hepcidin-25 and its isoforms are elevated.\(^{13,16-18}\)

Hepcidin-25 can circulate freely, or it can be bound to \(\alpha\)-macroglobulin and to a lesser extent to albumin.\(^{19-22}\) The extent to which hepcidin is protein bound is not clear and estimates of the freely circulating fraction vary from 11 to 98%.\(^{23,24}\) Protein-bound hepcidin might be biologically more active than unbound hepcidin, as was shown in mice.\(^{25}\) However, this could be explained by the fact that clearance of protein-bound hepcidin is diminished, thereby increasing its half-life and also its activity.

Clearance of hepcidin is assumed to occur via cellular degradation at its sites of action, and via excretion with the urine. In healthy individuals, the fractional excretion of hepcidin is negligible (never exceeding 3-5%),\(^{26,27}\) implicating that under normal conditions, hepcidin is either almost completely reabsorbed by the tubuli,\(^{28,29}\) and/or degraded in kidney tubules, and/or not freely filtered by the glomerulus (due to its protein-bound character).

Furthermore, in dialysis patients, it has been shown that hepcidin levels are influenced by mutations and polymorphisms in genes encoding proteins that are involved in the expression of hepcidin. Examples of these proteins are the haemochromatosis protein (HFE) and matriptase-2 (encoded by the TMPRSS6 gene), resulting in lower and higher hepcidin levels, respectively.\(^{30,31}\)

In the general population, the associations between polymorphisms in HFE and TMPRSS6 with hepcidin are less clear.\(^{32}\)

HEPCIDIN: FUNCTION AND REGULATION

Hepcidin is able to express its regulatory function in iron homeostasis by binding to ferroportin on the membranes of iron-exporting cells, such as hepatocytes, macrophages and enterocytes (figure 1).\(^{33-35}\) Ferroportin is a transmembrane efflux channel that transfers cellular iron to the plasma. The binding of hepcidin to ferroportin induces endocytosis and lysosomal degradation of ferroportin, thereby decreasing the delivery of iron to plasma and diminishing iron availability for erythropoiesis.\(^{36,37}\) Furthermore, it has been reported that hepcidin can bind iron directly.\(^{38,39}\) However, the proportion of hepcidin which binds iron, its binding capacity and the relevance of this process in vivo are unknown.

Hepcidin expression can be modulated through several mechanisms (figure 1). Hepatic hepcidin production is increased by one of two main regulatory signals: (1) Elevated body iron availability. This results in inhibition of further iron adsorption from the gastrointestinal tract. Hepcidin expression can be both regulated by stored iron and by circulating iron (reviewed by Meynard et al.)\(^{40}\) but many aspects of this regulatory pathway are still not completely clear. Hepatic iron accumulation leads to increased expression of bone morphogenic protein-6 (BMP-6) in the liver. This results in nuclear translocation of SMAD proteins and subsequent activation of hepcidin transcription. Haemojuvelin (HJV) is a major co-factor for BMP and matriptase-2 is an important inhibitor of hepcidin expression in response to iron deficiency, possibly by cleaving HJV.\(^{41}\) Concerning hepcidin regulation by circulating iron, much is still unclear. It is likely that transferrin-bound iron is bound by transferrin receptor (TTR) 1 and 2, and that HFE is involved, which possibly activates the SMAD pathway. However, the exact links between TTR 1 and 2, HFE and the SMAD pathway are unknown.\(^{42,43}\)

(2) Inflammation. This is mainly mediated via IL-6 and the IL-6/Janus kinase 2 (JAK2)-signal transducer and activator of transcription 3 (STAT3) pathway.\(^{44-46}\) Recent data in mice suggest that the BMP receptor, which is also involved in regulation of hepcidin by hepatic iron stores (see above), is required for induction of hepcidin expression by IL-6.\(^{47}\) Hepcidin production is inhibited by low circulating iron levels and low iron stores (see above) and two main other regulatory signals:

(1) Increased erythropoietic activity, such as after ESA administration. The exact underlying mechanism is largely unclear, but it is most probably mediated by molecules released by erythroid precursors, such as growth differentiation factor 15, twisted gastrulation protein homologue 1 and erythropherrone.\(^{48}\)
Van der Weerd et al. Hepcidin in chronic kidney disease.

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HEPCIDIN: MEASUREMENT TECHNIQUES

Measurement of hepcidin in human sera has been challenged by the fact that its structure with both hydrophobic and hydrophilic regions results in adsorption to surfaces (e.g. to the plastic of the blood tubes). Moreover, the creation of antibodies for immunochemical assays is complicated due to the small size of hepcidin, the compact structure caused by the disulphide bridges and its highly conserved sequence among species which impedes an immune response in host species. Finally, under physiological circumstances, hepcidin concentrations increase during the day. In dialysis patients, this circadian rhythm has been reported to be virtually absent. Currently, two types of hepcidin quantification methods have been studied in patients with CKD, namely:

(1) *Hypoxia*, mediated by the hypoxia-inducible factor (HIF), resulting in enhanced expression of matriptase-2 (encoded by the *TMPRSS6* gene). This protein cleaves HJV and thereby inhibits the BMP-6 signalling pathway.

(2) *Inflammation*, mediated via IL-6 and the JAK-2/STAT-3 pathway and for which activation of the BMP receptor may be required, and by the iron status. Liver iron stores influence the expression of BMP-6 which is produced in the liver and leads to SMAD activation, ultimately leading to activation of hepcidin transcription. HJV is a major co-factor for BMP and matriptase-2 is an inhibitor of hepcidin expression in response to iron deficiency. The regulation via circulating iron is not completely clear yet. It is known that TFR 1 and 2 and HFE are involved, which may activate hepcidin transcription directly or via the SMAD pathway. Hepcidin expression is diminished by hypoxia, mediated by the HIF, resulting in enhanced expression of matriptase-2, which cleaves HJV and thereby inhibits the BMP-6 signalling pathway. Furthermore, hepcidin is inhibited by *erythropoiesis*, although the regulatory mechanism is largely unclear. Potential mediators of bone marrow signalling are growth differentiation factor-15, twisted gastrulation-1 and erythroferrone. Finally, the binding of hepcidin to ferroportin induces the endocytosis and lysosomal degradation of ferroportin, thereby decreasing the delivery of iron to plasma and diminishing iron availability for erythropoiesis.

TFR = transferrin receptor; BMP = bone morphogenic protein; HIF = hypoxia-inducible factor; HJV = haemojuvelin; GDF15 = growth differentiation factor-15; TWSG1 = twisted gastrulation-1; ERFE = erythroferrone.

**Figure 1. Schematic representation of the (patho)physiology of the expression of hepcidin and its effects on functional iron availability**

**Legend**

- *Hypoxia* mediated by the hypoxia-inducible factor (HIF), resulting in enhanced expression of matriptase 2 (encoded by the *TMPRSS6* gene).
- *Inflammation* mediated via IL-6 and the JAK-2/STAT-3 pathway.
- *Iron availability* stored iron.
- *Hepcidin* expression is enhanced by inflammation, mediated via IL-6 and the JAK-2/STAT-3 pathway, and by the iron status. Liver iron stores influence the expression of BMP-6 which is produced in the liver and leads to SMAD activation, ultimately leading to activation of hepcidin transcription. HJV is a major co-factor for BMP and matriptase-2 is an inhibitor of hepcidin expression in response to iron deficiency. The regulation via circulating iron is not completely clear yet. It is known that TFR 1 and 2 and HFE are involved, which may activate hepcidin transcription directly or via the SMAD pathway. Hepcidin expression is diminished by hypoxia, mediated by the HIF, resulting in enhanced expression of matriptase-2, which cleaves HJV and thereby inhibits the BMP-6 signalling pathway. Furthermore, hepcidin is inhibited by *erythropoiesis*, although the regulatory mechanism is largely unclear. Potential mediators of bone marrow signalling are growth differentiation factor-15, twisted gastrulation-1 and erythroferrone. Finally, the binding of hepcidin to ferroportin induces the endocytosis and lysosomal degradation of ferroportin, thereby decreasing the delivery of iron to plasma and diminishing iron availability for erythropoiesis.

**TFR** = transferrin receptor; **BMP** = bone morphogenic protein; **HIF** = hypoxia-inducible factor; **HJV** = haemojuvelin; **GDF15** = growth differentiation factor-15; **TWSG1** = twisted gastrulation-1; **ERFE** = erythroferrone.
immunochemical assays, including radioimmunoassays and ELISA, of which some are commercially available. Most of these techniques measure both biologically active and inactive isoforms; and (2) mass spectrometry, which is more expensive and technically demanding, but able to detect each isoform separately.\textsuperscript{10,13-15,41} Thus far, the gold standard hepcidin assay has not yet been defined. Besides, several questions remain.

First, there is a considerable inter-assay variability and measured values can vary by a factor 10.\textsuperscript{10,43-45} This precludes the definition of universal reference intervals and clinical decision limits. Nevertheless, within- and between-sample variation is fairly similar for most assays and hepcidin levels obtained are mutually correlated.\textsuperscript{44,45} However, some commercial assays do not correlate with many other assays and do not provide physiologically meaningful results; these include the prohepcidin kits.

Second, with most immunoassays, it is unclear if total hepcidin (sum of hepcidin-20, -22, -24 and -25) is measured, or whether bio-active hepcidin-25 is specifically quantified (with specific hepcidin-25 antibodies or two-site ELISA).\textsuperscript{46} Third, in general, it is not yet fully established whether protein-bound or free hepcidin is measured. Since protein-bound hepcidin has been reported to be more biologically active,\textsuperscript{44} it cannot be completely ruled out that this might be of importance.

Initiatives have been undertaken to standardise hepcidin results worldwide and to enable the definition of reference values and clinical decision limits for different patient categories, such as CKD patients.\textsuperscript{44,45} Since reliable calibrators are still lacking, algorithms have been constructed enabling different laboratories to calculate a HEPcidin CONsensus (HEPCON) value using their own hepcidin results, measured with both mass spectrometric and immunochemical assays.\textsuperscript{43} This is an important step towards harmonisation of different assays, which may facilitate the interpretation and comparison of different studies.

HEPCIDIN AND CKD

It has been well established that hepcidin levels are increased in non-dialysis CKD patients as well as in dialysis patients, possibly due to increased production (driven by inflammation and elevated stored body iron levels) or reduced clearance.\textsuperscript{9,17,47} In one study from Italy, however, hepcidin levels measured with mass spectrometry were similar in 199 haemodialysis patients and 188 age- and sex-matched controls.\textsuperscript{45} This could be explained by careful matching and inclusion of haemodialysis patients, namely those who received relatively little iron supplementation and were not iron-loaded.\textsuperscript{46} These data need confirmation.

Hepcidin and its correlation with eGFR

Research on the relation between hepcidin and the estimated glomerular filtration rate (eGFR) has shown conflicting results. In a study in CKD patients, total hepcidin (sum of all isoforms) measured with an ELISA was associated with eGFR.\textsuperscript{9} In other studies in this patient category, in which hepcidin-25 was measured with mass spectrometry, this association was not present.\textsuperscript{17,49-50}

Previously, it has been suggested that the relation between eGFR and hepcidin might be due to the measurement of inactive isoforms in non-specific assays.\textsuperscript{7} However, the bioactive form of hepcidin measured with a hepcidin-25 specific radioimmunoassay was associated with eGFR.\textsuperscript{44} Furthermore, in a cohort of over 400 chronic haemodialysis patients, hepcidin-25 levels assessed by mass spectrometry were correlated with the eGFR measured by an interdialytic 24-hour urine collection in univariate and multivariate models.\textsuperscript{51} In a study in 199 non-dialysis CKD patients, it was shown that hepcidin levels increase as eGFR decreases, although those patients who were markedly iron deficient still had low levels of hepcidin at low eGFR values. These data suggest that hepcidin still reflects iron status at low eGFR levels.\textsuperscript{47} In conclusion, hepcidin levels correlate inversely with eGFR, but this relation is blunted by iron deficiency in patients with low eGFR.

Removal of hepcidin with renal replacement techniques

Hepcidin can be removed from the blood by both haemodialysis and peritoneal dialysis.\textsuperscript{16,73-76} Hepcidin reduction was similar after treatment with different dialysers\textsuperscript{57,58} and reduction tended to be superior with haemodiafiltration treatment.\textsuperscript{59} After a haemodialysis treatment, hepcidin was present both in the ultrafiltrate and bound to the dialysate membrane.\textsuperscript{57} However, sustained lowering of hepcidin levels with extracorporeal renal replacement therapy does not occur, since after an initial decrease in hepcidin levels, post-dialysis levels were already back to pre-dialysis values as soon as one hour after the end of a dialysis session.\textsuperscript{72,74}

Correlations between hepcidin parameters of inflammation and iron status

It is well known that anaemia, iron deficiency and inflammation are highly prevalent in CKD patients. As this might all be associated with (or explained by) elevated hepcidin levels, many studies in CKD patients correlating hepcidin with various clinical parameters have been performed (table 1).

Virtually all studies on hepcidin, including those in dialysis patients, have observed a strong association with C-reactive protein (CRP)\textsuperscript{73,74,69,66} or IL-6\textsuperscript{79} in small groups of chronic haemodialysis patients.
In a study in over 400 chronic haemodialysis patients, it was demonstrated that the relation between hepcidin-25 and ferritin was present in all strata of inflammation (as measured by high-sensitivity CRP), whereas the relation between hepcidin-25 and high-sensitivity CRP was only present when ferritin was not markedly elevated (< 530 ng/ml; figure 2). This finding suggests a stronger relation between hepcidin-25 and ferritin than between hepcidin-25 and specific markers of inflammation.

HEPCIDIN: A TOOL FOR CLINICAL DECISION-MAKING IN RENAL ANAEMIA?

Based on early insights into hepcidin regulation and function, it has been speculated that measurement of hepcidin could be applied in clinical assessment of anaemia in CKD patients, including those on dialysis. In CKD patients, anaemia in the presence of low hepcidin indicates absolute iron deficiency and warrants iron

**Table 1. (Clinical) determinants of hepcidin in CKD patients (dialysis and non-dialysis)**

<table>
<thead>
<tr>
<th>Author &amp; year</th>
<th>Method</th>
<th>Study population</th>
<th>Associations with hepcidin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ashby 2009&lt;sup&gt;a&lt;/sup&gt;</td>
<td>RIA</td>
<td>44 CKD, 94 HD</td>
<td>Ferritin, eGFR, not with IL-6, ESA dose (inverse)</td>
</tr>
<tr>
<td>Chand 2013&lt;sup&gt;b&lt;/sup&gt;</td>
<td>MS</td>
<td>129 CKD</td>
<td>Ferritin; hsCRP and eGFR (univariate)</td>
</tr>
<tr>
<td>Costa 2009&lt;sup&gt;b&lt;/sup&gt;</td>
<td>MS</td>
<td>33 HD</td>
<td>CRP, Hb, ferritin, transferrin, IL-6 (univariate)</td>
</tr>
<tr>
<td>Kato 2008&lt;sup&gt;c&lt;/sup&gt;</td>
<td>MS</td>
<td>15 HD</td>
<td>Ferritin, not with CRP</td>
</tr>
<tr>
<td>Kuragano 2010&lt;sup&gt;d&lt;/sup&gt;</td>
<td>MS</td>
<td>198 HD</td>
<td>All patients: transferrin and ferritin; in patients with CRP &gt;0.3 mg/ml: ferritin and IL-6</td>
</tr>
<tr>
<td>Maruyama 2012&lt;sup&gt;e&lt;/sup&gt;</td>
<td>IA (ELISA)</td>
<td>117 CKD</td>
<td>Sex, oral iron, Hb, transferrin saturation (TSAT), ferritin and hsCRP; not eGFR or 8-OHdG</td>
</tr>
<tr>
<td>Mercadel 2014&lt;sup&gt;f&lt;/sup&gt;</td>
<td>IA</td>
<td>199 CKD</td>
<td>eGFR, oral iron therapy, BMI ≥ 30 kg/m², albumin, EPO</td>
</tr>
<tr>
<td>Pelusi 2013&lt;sup&gt;g&lt;/sup&gt;</td>
<td>MS</td>
<td>199 HD</td>
<td>Ferritin, CRP, absence of HFE/TMPRSS6 mutation</td>
</tr>
<tr>
<td>Peters 2010&lt;sup&gt;h&lt;/sup&gt;</td>
<td>MS</td>
<td>83 CKD, 48 HD</td>
<td>Ferritin, not with eGFR (in CKD)</td>
</tr>
<tr>
<td>Van der Putten 2010&lt;sup&gt;i&lt;/sup&gt;</td>
<td>MS</td>
<td>33 CKD and heart failure</td>
<td>Hb, ferritin, TSAT, not with eGFR, hsCRP, IL-6 and sTfR (univariate)</td>
</tr>
<tr>
<td>Uehata 2012&lt;sup&gt;j&lt;/sup&gt;</td>
<td>MS</td>
<td>505 CKD</td>
<td>Ferritin, Hb, ESA use, not with eGFR</td>
</tr>
<tr>
<td>Samouilidou 2014&lt;sup&gt;k&lt;/sup&gt;</td>
<td>IA (ELISA)</td>
<td>30 HD, 30 PD</td>
<td>IL-6 and triglycerides</td>
</tr>
<tr>
<td>Tomosugi 2006&lt;sup&gt;l&lt;/sup&gt;</td>
<td>MS</td>
<td>40 HD</td>
<td>Ferritin, IL-6 (univariate)</td>
</tr>
<tr>
<td>Troutt 2013&lt;sup&gt;m&lt;/sup&gt;</td>
<td>IA (ELISA)</td>
<td>103 CKD</td>
<td>Ferritin, transferrin, eGFR (univariate)</td>
</tr>
<tr>
<td>Valenti 2009&lt;sup&gt;n&lt;/sup&gt;</td>
<td>MS</td>
<td>65 HD</td>
<td>Ferritin, absence of HFE mutation</td>
</tr>
<tr>
<td>Van der Weerd 2012&lt;sup&gt;o&lt;/sup&gt;</td>
<td>MS</td>
<td>405 HD</td>
<td>Ferritin, hsCRP, reticulocytes, sTfR, eGFR, not with IL-6</td>
</tr>
<tr>
<td>Weiss 2009&lt;sup&gt;o&lt;/sup&gt;</td>
<td>MS</td>
<td>20 HD</td>
<td>Reticulocytes, iron, ferritin, TSAT, not with CRP and IL-6 (univariate)</td>
</tr>
<tr>
<td>Zaritsky 2009&lt;sup&gt;p&lt;/sup&gt;</td>
<td>IA (ELISA)</td>
<td>48 paediatric CKD, 32 adult CKD, 26 paediatric PD</td>
<td>Paediatric CKD: ferritin; Adult CKD: ferritin, sTfR, eGFR (inverse)</td>
</tr>
<tr>
<td>Zaritsky 2010&lt;sup&gt;q&lt;/sup&gt;</td>
<td>IA (ELISA)</td>
<td>30 paediatric HD, 33 adult HD</td>
<td>Ferritin, TSAT, hsCRP</td>
</tr>
</tbody>
</table>

Associations are results of multivariate analyses, unless otherwise specified. IA = immunochemical assay; RIA = radio-immuno assay; MS = mass spectrometry; CKD = chronic kidney disease (not on dialysis); HD = haemodialysis; PD = peritoneal dialysis; eGFR = estimated glomerular filtration rate; hsCRP = high sensitivity C-reactive protein; Hb = haemoglobin; TSAT = transferrin saturation; sTfR = soluble transferrin receptor; EPO = erythropoietin; ESA = erythropoiesis stimulating agents; 8-OHdG = 8-hydroxy-2’-deoxyguanosine (a marker of DNA oxidative injury).
supplementation. In contrast, in anaemia with high hepcidin levels, one of two situations may be present: (1) high hepcidin levels may reflect sufficient iron stores and ESA therapy (with or without additional maintenance iron supplementation) is indicated; (2) high hepcidin levels may indicate a situation in which its expression could not be inhibited, e.g. due to inflammation. As a result, iron cannot be mobilised, erythropoiesis is impaired and ESA resistance is present. In this situation, anti-hepcidin agents might be an appropriate therapy (with or without additional ESA therapy).

Hepcidin and ESA therapy

It has been suggested that hepcidin might contribute to ESA resistance, since animal data have shown that overexpression of hepcidin impaired the response to even very high ESA doses. In humans, however, data on the relation between hepcidin levels, ESA treatment and ESA resistance are inconsistent. In haemodialysis patients and in patients with the cardio-renal syndrome, hepcidin levels decreased after ESA administration. In cross-sectional data of almost 100 haemodialysis patients on ESA maintenance therapy, hepcidin levels were lower in patients receiving higher ESA doses, regardless of the haemoglobin level that was reached. However, in another cross-sectional study in over 400 haemodialysis patients, hepcidin levels were not associated with either the dose of ESA or intravenous iron. Furthermore, two small studies revealed that hepcidin-25 levels were similar in ESA-responsive and ESA-resistant haemodialysis patients, suggesting that the assessment of hepcidin is not helpful in deciding which individual patient may benefit from ESA therapy. Nevertheless, in patients with the cardio-renal syndrome, ESA responders showed higher hepcidin-25 levels than non-responders, suggesting that hepcidin might rather be a marker of ESA responsiveness than associated with ESA resistance. Of note, in all the above-mentioned studies, bioactive hepcidin was measured (either with mass spectrometry or with a specific radioimmunoassay).

Hepcidin and iron therapy

Concerning the utility of hepcidin in assessing iron stores and managing iron supplementation therapy, overall, negative results have been reported. In a study in 56 patients with iron deficiency, hepcidin was lower in patients with low ferritin than in patients with normal ferritin, and the difference was larger in patients with low iron stores than in those with normal iron stores.

Figure 2. Cross-sectional relations between ferritin, hsCRP and hepcidin-25 in haemodialysis patients (from Van der Weerd et al. with permission)

In a cohort of 405 chronic haemodialysis patients, the relation between hepcidin-25 (measured with mass spectrometry) and ferritin was present in all strata of inflammation (as measured by high-sensitivity CRP), whereas the relation between hepcidin-25 and inflammation was only present when ferritin was low. This finding suggests a stronger relation between hepcidin-25 and ferritin than between hepcidin-25 and specific markers of inflammation under these conditions.

In theory, measurement of hepcidin may theoretically differentiate between the management options for renal anaemia in individual CKD patients according to a flowchart as presented in figure 3.
chronic haemodialysis patients, neither hepcidin-20 nor hepcidin-25 could predict an increase in haemoglobin levels after administration of intravenous iron.\textsuperscript{54} Notably, in this study, ROC curve analysis showed that also ferritin failed to accurately predict a response, whereas the percentage of hypochromic red blood cells was the only biomarker independently associated with iron responsiveness. Furthermore, in a small group of haemodialysis patients, hepcidin-25 levels were similar before and after the administration of intravenous iron.\textsuperscript{55} In another study in 129 consecutive non-dialysis CKD patients, hepcidin-25 was able to predict a haemoglobin response after intravenous iron administration.\textsuperscript{56} However, ferritin and transferrin saturation had similar predictive utility. Again, in all the mentioned studies, hepcidin was measured with mass spectrometry.

**Limitations of available evidence**

In conclusion, thus far, conflicting and even forthright negative data are available to answer the question whether measurement of hepcidin may be a suitable and unique tool for clinical decision-making in ESA and iron management in CKD patients. In assessing iron stores, hepcidin did not appear to be superior over ferritin.\textsuperscript{66} An important aspect further hampering the use of hepcidin as a management tool is that in stable haemodialysis patients, intra-individual hepcidin levels, measured with both ELISA and mass spectrometry, varied widely in a relative short period of time, likely dependent on fluctuations in the inflammatory state.\textsuperscript{66a,67} These studies implicate that short-term measurements of serum hepcidin in individual (dialysis) patients might not be appropriate to guide clinical decisions regarding ESA or iron management. Moreover, at this point it should be noted that comparing the available studies is complex and hazardous as a result of the large variety in experimental circumstances. Available studies differ with respect to patients’ iron stores, levels of inflammation, stages of renal failure, dialysis regimens, doses of iron supplementation and ESA. Besides, studies use different hepcidin assays and there is a variable lag time between ESA and iron administration and blood sampling. Furthermore, most available studies included a limited number of patients precluding multivariate statistics.\textsuperscript{68}

The above-mentioned aspects make systematic reviews on the usefulness of hepcidin measurements in anaemia management virtually impossible. Therefore, prospective clinical studies investigating the utility of a hypothetical treatment algorithm as shown in figure 3 are warranted.

**HEPCIDIN: A BIOMARKER FOR CARDIOVASCULAR DISEASE?**

In a study in 335 non-dialysis CKD patients, it was shown that hepcidin-25, measured with mass spectrometry, predicted the progression of renal anaemia both in iron replete and deplete patients, during a median follow-up period of 3.6 years.\textsuperscript{69} This observation demonstrates the concept of hepcidin as a biomarker, in this specific case for renal anaemia. Recent evidence also shows a new conceptual role for hepcidin as a biomarker for cardiovascular disease. The involvement of hepcidin in the development of atherosclerosis and cardiovascular disease is supported by several (pre-)clinical studies, which will be briefly reported below.

**The role of hepcidin in atherosclerotic disease**

Several experimental animal studies provided evidence for the involvement of hepcidin in atherosclerotic processes. In mice, suppression of hepcidin resulted in reduced intracellular iron content in macrophages, resulting in an augmented efflux capacity of cholesterol.\textsuperscript{70} Furthermore, these animals exhibited diminished foam cell formation and less atherosclerosis. In an atherosclerotic mice model, hepcidin promoted plaque destabilisation by inducing inflammatory cytokine release, intracellular lipid accumulation, oxidative stress and apoptosis of macrophages with iron retention.\textsuperscript{71} In an experiment in humans, intracellular iron content in monocytes derived from atherosclerotic plaques was increased in the presence of hepcidin, which resulted in enhanced reactive oxygen substances preventing cholesterol efflux from these cells.\textsuperscript{72} In clinical and epidemiological studies, associations have been found between hepcidin and markers of vascular stiffness, atherosclerosis and cardiovascular disease. In 143 patients with non-alcoholic fatty liver disease, hepcidin-25 levels were associated with the presence of carotid plaques.\textsuperscript{73} In a similar group of 130 patients with non-alcoholic fatty liver disease, monocyte chemo-attractant protein-1, a chemokine that plays a crucial role in both the initiation and progression of atherosclerosis, was correlated with hepcidin-25 and an independent predictor of the presence of atherosclerotic plaques.\textsuperscript{74} In 60 patients with rheumatoid arthritis, hepcidin was correlated with coronary artery atherosclerosis, measured by a coronary calcium score, even after multivariate adjustment.\textsuperscript{75} Finally, in a Dutch population-based cohort of 766 post-menopausal women, hepcidin was associated with the presence of plaques in the carotid artery, adjusted for eGFR, inflammation and traditional cardiovascular risk factors.\textsuperscript{76} Recently, the role of hepcidin as a cardiovascular marker gained interest in the high-risk CKD population.\textsuperscript{77} Two studies, one in 168 chronic haemodialysis patients and
Van der Weerd et al. Hepcidin in chronic kidney disease.

Hepcidin and atherosclerotic disease: pathophysiological concept

As mentioned before, hepcidin is predominantly synthesised in the liver, but low levels of expression in other cells, including macrophages, are present, enabling local fine-tuning of systemic iron regulation. Furthermore, two studies investigated the relation between hepcidin and arterial stiffness (measured with brachial-ankle pulse wave velocity and flow-mediated dilatation, respectively) and hepcidin. In another in 56 patients on peritoneal dialysis, showed an association between arterial stiffness (measured with brachial-ankle pulse wave velocity and flow-mediated dilatation, respectively) and hepcidin. Furthermore, two studies investigated the relation between hepcidin and left ventricular mass index (LVMi). One study in 146 CKD patients not on dialysis showed that lower hepcidin levels were associated with higher LVMi, possibly due to the concomitant iron deficiency resulting in an anaemic state. In a study in 327 chronic haemodialysis patients, no association between hepcidin and LVMi was observed. Finally, in a cohort of 405 chronic haemodialysis patients with a median follow-up of three years, hepcidin-25 levels were associated with the incidence of cardiovascular events, even after stepwise adjustments of clinical and anaemia-related parameters, including inflammation. Of note, the association between hepcidin-25 and all-cause mortality was attenuated after adjustment for inflammatory markers. Although these associations certainly do not prove causality, this study adds evidence to the hypothesis that hepcidin may be directly or indirectly involved in the pathogenesis of cardiovascular disease and mortality in patients on dialysis and therefore may function as a biomarker.

**Figure 4. Schematic representation of the hypothesis that elevated hepcidin levels may result in iron retention in macrophages, inducing oxidative stress and contributing to atherosclerotic vascular disease**

Increased circulating hepcidin levels result in iron retention in macrophages in the vascular wall and in atherosclerotic plaques. As a result, intracellular oxidative stress is stimulated which may contribute to atherosclerosis and cardiovascular disease.
atherosclerotic plaque size was not increased. The authors explain their findings by suggesting that in macrophages, antioxidant defence strategies may be very efficient.66

In CKD patients, several additional remarks with regard to the ‘iron hypothesis’ of cardiovascular disease need to be considered. First, since the cardiovascular risk profile of CKD patients seems to differ from the general population,67 the question arises whether the atherogenic effect of iron accumulation and oxidative stress in the vascular wall, as has been observed in non-CKD patients, is relevant in this specific high-risk CKD patient group as well. Second, it is not known whether iron sequestration in macrophages plays the same role in atherosclerotic plaques in the arterial intima as in calcified plaques in the arterial media, as the latter is a characteristic of cardiovascular disease in dialysis patients.68,69 Finally, the relation between hepcidin and oxidative stress in CKD patients has not yet been unequivocally demonstrated.69 Nevertheless, the available experimental and epidemiological evidence of the complex interplay between hepcidin, iron accumulation in macrophages in the vascular wall and cardiovascular disease is hypothesis generating for further research on the potential usefulness for hepcidin as a cardiovascular biomarker, especially in the vulnerable CKD population.

CONCLUSIONS AND REMAINING QUESTIONS

In this review, currently available pre-clinical and clinical data have been summarised in order to weigh the merits of hepcidin in three areas of interest.

1. Hepcidin and the pathophysiology of renal anaemia in CKD patients. The identification of hepcidin provided relevant information regarding the understanding of the concept of functional iron deficiency in dialysis and non-dialysis CKD patients, and it revealed a possible mechanism for the presence of ESA resistance despite extensive intravenous iron loading. In addition, knowledge on the clinical characteristics of hepcidin, such as its inflammation-driven regulatory pathway and its renal clearance profile, made it a relevant peptide in CKD patients. However, several features restrict its clinical use at the moment: pre-analytical handling of hepcidin is critical, the accessibility of the most accurate and precise assay for hepcidin-25 is limited and the clinical relevance of specifically measuring hepcidin-25 instead of total hepcidin by more accessible ELISA assays is unclear. Available studies are difficult to compare since absolute levels obtained by various assays that are used worldwide differ substantially. This precludes the assessment of universal clinical decision limits. Furthermore, other important characteristics of hepcidin, such as its protein binding, have not yet been completely elucidated.

2. Hepcidin as a diagnostic tool and for guiding clinical decisions in CKD patients. Hepcidin levels in dialysis and non-dialysis CKD patients are elevated, due to decreased (renal) clearance and/or enhanced production, e.g. by inflammation and iron loading. Its levels are highly correlated with ferritin in virtually all studies, and therefore the question arises what the advantage is of measuring hepcidin as compared with measuring ferritin, which is easier and less expensive. Inflammation is an important factor in the expression of hepcidin and most studies show an association between hepcidin and inflammatory markers such as CRP and IL-6. Studies on the role of hepcidin in distinguishing between a situation of ESA responsiveness or ESA resistance have shown negative or inconsistent results. Similarly, iron responsiveness could not be predicted by hepcidin levels. Furthermore, the high intra-patient variability makes hepcidin unsuitable as an instrument to guide treatment in individual patients. Therefore, it can be concluded that currently, hepcidin cannot be considered a valuable clinical tool in diagnosing and treating renal anaemia.

3. Hepcidin as a prognostic biomarker for cardiovascular disease. Several experimental, clinical and epidemiological studies in animals and humans showed an association between hepcidin and atherosclerotic disease and clinical events, possibly mediated via iron sequestration in macrophages in the vascular wall. These observations point towards a role for hepcidin as a biomarker for cardiovascular disease, which is also shown in several clinical reports in dialysis patients.

Future perspectives

Finally, although beyond the scope of this review, the presence of numerous anti-hepcidin compound initiatives that are currently under way and use hepcidin as a target of therapy, should be acknowledged.64-66 Although no clinical studies of anti-hepcidin therapy in patients with CKD have been published yet, the first results of an anti-hepcidin antibody tested in humans are promising: administration of this antibody in humans was able to block inflammation-induced reduction in serum iron.64 Whether, in the future, measuring hepcidin could be useful to establish an indication for anti-hepcidin therapy, or whether it could be helpful in monitoring anti-hepcidin therapy, needs to be determined. Taken together, hepcidin has contributed to our understanding of the pathophysiology of iron misdistribution and anaemia in CKD, but it has not fulfilled its promise as a diagnostic and management tool thus far. Its role as a biomarker of cardiovascular disease is promising and needs confirmation.
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Van der Weerd et al. Heparicin in chronic kidney disease.
ABSTRACT

Background: The standardised mortality ratio (SMR) is a quality indicator used to measure quality of care in the Netherlands. It is subject to much criticism, which was the reason to study the value of the SMR as a quality indicator for the treatment of acute leukaemia.

Methods: A retrospective analysis was performed in patients with acute leukaemia admitted to a Santeon hospital during the period 2005-2009. SMR values were calculated and compared with the overall survival (OS).

Results: During the study period, 455 unique patients were admitted with acute leukaemia. SMR calculation was based on 992 admissions. SMR analysis yielded a high mortality ratio in hospital 1, 2, 3 and 4 in comparison with the national average (100), significant for hospital 1 and 4 (180 [CI 95% 126-257] and 187 [CI 95% 134-261], respectively). OS analysis also showed a significantly different outcome between hospitals. However, using OS as outcome parameter, hospital 2 and 6 showed the lowest performance as compared with hospital 1 and 4 using SMR as parameter.

After multivariate analysis, age (HR 1.04; CI 95% 1.03-1.05; p < 0.001) and hospital (hospital 5 compared with 6: HR 0.54; CI 95% 0.30-0.98; p = 0.043; hospital 2 compared with 1: HR 1.51; CI 95% 1.02-2.23; p = 0.039) were the only significant variables that influenced OS.

Conclusion: Outcome according to SMR is not equivalent to outcome according to OS. This study shows that the use of the SMR as a quality indicator for the treatment of acute leukaemia does not appear to be justified.

KEYWORDS

Acute leukaemia, quality indicator, standardised mortality ratio (SMR)

INTRODUCTION

For several years there has been a growing interest in measuring performance and quality of health care. Hospital performance can be assessed using various quality indicators. One of the quality indicators used by the Health Care Inspectorate in the Netherlands is the Hospital Standardised Mortality Ratio (HSMR), which is the ratio of observed and expected number of deaths in a specific hospital. In order to compare hospital performance on the basis of mortality rates, these rates have to be adjusted for casemix: differences in characteristics of the patients admitted to those hospitals. The HSMR is composed of 50 diagnosis groups. For each group, it is determined which characteristics (retrieved from the National Medical Registry [LMR]) influence the mortality rate. The standardised mortality ratio (SMR) is the adjusted mortality ratio for the varying diagnosis groups.

The use of the HSMR and SMR as a quality indicator is subject to much criticism. It is stated that the registration and coding of the data by the hospitals is often inconsistent and incomplete, which can influence the HSMR of the respective hospitals. The 50 diagnosis groups on which the HSMR is based cover 80% of the total hospital mortality. As a consequence, 20% of all deaths will be excluded from analysis. Adjustment of the (H)SMR is based on variables registered in the LMR, and therefore insufficient, since factors not registered in the LMR cannot be accounted for.

The HSMR was first published in the Netherlands in 2011. In addition, the SMR for each diagnosis group was calculated. SMR data were not published, but only disclosed within the varying hospitals. Internal disclosure of the SMR of the diagnosis group leukaemia showed a significant difference in SMR among the six Santeon hospitals. In one of the Santeon hospitals, the SMR was used to express the quality of
leukaemia treatment. This was the reason to study the value of the SMR within the diagnosis group leukaemia, specifically in the group of patients with acute leukaemia, and to relate it to a clinically valuable measure of outcome: the five-year overall survival. We wanted to gain insight into the cohort of patients diagnosed with acute leukaemia, the percentage of patients who were actually treated within the respective hospitals, the type of treatment the patients had undergone and for which variables the SMR had been adjusted.

MATERIALS AND METHODS

Patient selection
Data of all patients with acute leukaemia, who were admitted to one of the six Santeon hospitals in the period 2005-2009, were collected retrospectively. Patients were identified using a database generated by Statistics Netherlands (CBS), a database used for the SMR calculation of the diagnosis group leukaemia. For the purpose of our analysis, only patients with the ICD-9 code ‘acute lymphoblastic leukaemia’ (204.0); ‘acute myeloid leukaemia’ (205.0); ‘acute monocytic leukaemia’ (206.0); ‘other specified leukaemia’ (207.0) or ‘leukaemia of unspecified cell type’ (208.0) were selected. For each patient, the diagnosis of acute leukaemia was confirmed. Furthermore, data concerning year and month of diagnosis, risk classification, type of treatment, survival status and date of death were collected using the electronic health record system (EHRS). If, according to registration in the EHRS, the patient was alive, this was confirmed via the general practitioner. If data were missing in EHRS, the medical record was reviewed. Patients with chronic leukaemia, other haematological malignancies and children were excluded.

Statistics
Patient characteristics were described based on frequencies and means. Differences between groups were compared using the Pearson chi-square test (discrete variables) or one-way ANOVA test (continuous variables). Differences with a p-value < 0.05 were considered statistically significant. The overall survival was estimated using the Kaplan-Meier method. Univariate analyses were carried out using the log-rank test. Variables affecting overall survival (OS) (p < 0.1) were included in a multivariate Cox regression analysis. P-values were calculated from the regression models with the Wald test. The analysed variables were: age; urgency of the admission; source (indicating the patient’s residence before admission); comorbidity (Charlson index) and year of discharge. This was in accordance with the SMR analysis for the diagnosis group leukaemia. In addition, the impact of the other HSMR variables (sex, socio-economic status and month of admission) and the impact of centre of treatment was examined.

The SMR of a hospital h for diagnosis d was defined as
\[ \text{SMR}_{dh} = 100 \times \frac{\text{observed mortality}_{dh}}{\text{expected mortality}_{dh}}. \]
The numerator was the observed number of deaths with main diagnosis d in hospital h. The denominator was the expected number of deaths for this type of admission under the assumption that individual mortality probabilities (per admission) do not depend on the hospital, i.e. are equal to mortality probabilities of identical cases in other hospitals. Confidence intervals of the SMRs were calculated. For each diagnosis d, the average SMR across hospitals is equal to 100. A lower limit above 100 referred to a statistically significant high SMR and an upper limit below 100 referred to a statistically low SMR. The SMR of acute leukaemia was compared with the OS of this cohort of patients. The calculations were performed by using SPSS version 19.0 (IBM Corp., Armonk, NY USA).

RESULTS

Patient selection
In the period 2005-2009, 455 unique patients were admitted for treatment of acute leukaemia in the Santeon hospitals. A total of 410 patients met the inclusion criteria, 45 patients were excluded (9.9%). 

Patient characteristics
Patient characteristics are shown in Table 1. The mean age in hospital 6 was significantly higher compared with the other hospitals (p < 0.001). There were significant differences in the risk classification and treatment between hospitals (p < 0.001). In hospital 6, no intensive treatment was performed. In addition, risk classification was lacking in 74.5% of the patients in this hospital. This was probably related to the high mean age and the lack of therapeutic consequences. There was no significant difference in sex and comorbidity between hospitals.

Figure 1. A schematic view of the selection of patients

455 patients suitable for inclusion
45 patients not included:
Other haematological malignancy (n = 8)
Children (n = 24)
Data extraction impossible (n = 13)

Data analysis of 410 patients
Analysis SMR
The calculation of the SMR for the diagnosis group acute leukaemia in the period between 2005-2009 was based on 992 admissions. The data from these admissions were used to calculate the SMR in the cohort of patients who had actually been treated in the respective hospital. Hospital 5 had a low SMR in comparison with the national average, the other hospitals had a high SMR; however, this was only significant for hospitals 1 and 4 (180 [CI 95%: 126-257] and 187 [CI 95%: 134-261]) respectively. Table 2 gives an overview of the SMR values for the diagnosis group of acute leukaemia in the different hospitals.

Analysis overall survival
The analysis of the OS of patients with acute leukaemia per hospital was performed in the cohort of patients who had actually been treated in the respective hospital. Table 3 shows the five-year OS per hospital. The five-year OS was significantly different between the six hospitals, with hospital 2 and 6 showing the lowest survival (p = 0.032). Figure 2 shows the corresponding Kaplan-Meier curves. Furthermore, the impact of cytogenetic risk classification was analysed. Patients in the good-risk group had a better OS than patients in the intermediate- or poor-risk group, as expected (p = 0.013). In the group of patients for whom data regarding risk classification were lacking, the lowest survival was seen. The OS in the different risk groups is shown in figure 3.

Univariate analysis
Table 4 shows that after the univariate analysis variables: age (p < 0.001); urgency of admission (p < 0.001); comorbidity (p = 0.002) and hospital (p < 0.001) showed an effect on OS.

Multivariate analysis
Table 5 shows the results after multivariate analysis. Reviewing the variables used in the analysis of HSMR as well as SMR, age (per increase of one year; HR 1.04; CI 95% 1.03-1.05; p < 0.001) and hospital (p = 0.032) were the only significant variables that had an impact on the OS.

DISCUSSION
The objective of this study was to examine the value of the SMR as a quality indicator for the treatment of acute leukaemia. To this end, we compared the SMR with overall survival of acute leukaemia. We show a discrepancy between measurement of performance according to SMR as

### Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Hospitals</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>104</td>
<td>48</td>
<td>57</td>
<td>115</td>
<td>39</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Sex •♀</td>
<td>50 (48.1)</td>
<td>24 (50.0)</td>
<td>27 (47.4)</td>
<td>50 (43.5)</td>
<td>21 (53.8)</td>
<td>22 (46.8)</td>
<td>ns</td>
</tr>
<tr>
<td>Age, mean in years (range)</td>
<td>58.5 (18-96)</td>
<td>60.3 (22-87)</td>
<td>58.8 (18-88)</td>
<td>58.3 (18-92)</td>
<td>59.0 (28-80)</td>
<td>74.6 (39-90)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diagnosis •AML</td>
<td>91 (87.5)</td>
<td>43 (89.6)</td>
<td>51 (89.5)</td>
<td>95 (82.6)</td>
<td>36 (92.3)</td>
<td>44 (93.6)</td>
<td>ns</td>
</tr>
<tr>
<td>Comorbidity Charlson index •2</td>
<td>95 (91.3)</td>
<td>45 (93.8)</td>
<td>49 (86.0)</td>
<td>105 (91.3)</td>
<td>33 (84.6)</td>
<td>44 (93.6)</td>
<td>ns</td>
</tr>
<tr>
<td>Risk classification •Good</td>
<td>12 (11.5)</td>
<td>2 (4.2)</td>
<td>5 (8.8)</td>
<td>19 (16.5)</td>
<td>4 (10.3)</td>
<td>5 (10.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment •Intensive treatment</td>
<td>85 (87.1)</td>
<td>26 (54.2)</td>
<td>36 (61.2)</td>
<td>89 (77.4)</td>
<td>21 (53.8)</td>
<td>-</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are number of patients (%), unless otherwise indicated; AML = acute myeloid leukaemia; ALL = acute lymphoblastic leukaemia; risk classification according to HOVON or EORTC study in which patient was treated.
Table 2. SMR acute leukaemia by hospital

<table>
<thead>
<tr>
<th>Hospital</th>
<th>SMR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>180 (126-257)</td>
</tr>
<tr>
<td>2</td>
<td>162 (85-261)</td>
</tr>
<tr>
<td>3</td>
<td>137 (76-235)</td>
</tr>
<tr>
<td>4</td>
<td>187 (134-261)</td>
</tr>
<tr>
<td>5</td>
<td>35 (4-120)</td>
</tr>
<tr>
<td>6</td>
<td>117 (63-202)</td>
</tr>
</tbody>
</table>

Table 3. Overall survival (OS) in patients with acute leukaemia by hospital

<table>
<thead>
<tr>
<th>Hospital</th>
<th>5-year OS % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23.4 (13.6-33.2)</td>
</tr>
<tr>
<td>2</td>
<td>13.0 (2.6-23.4)</td>
</tr>
<tr>
<td>3</td>
<td>29.1 (15.9-42.3)</td>
</tr>
<tr>
<td>4</td>
<td>27.7 (18.9-36.5)</td>
</tr>
<tr>
<td>5</td>
<td>40.0 (22.2-57.8)</td>
</tr>
<tr>
<td>6</td>
<td>3.1 (0-9.3)</td>
</tr>
</tbody>
</table>

Table 4. Univariate analysis of overall survival (OS) in patients with acute leukaemia

<table>
<thead>
<tr>
<th>OS (p-values)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Urgency</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Comorbidity</td>
<td>0.002</td>
</tr>
<tr>
<td>Hospital</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

compared with measurement of performance according to overall survival. Qualified by SMR, hospital 1 and 4 had the lowest performance, while qualified by OS hospital 2 and 6 showed the lowest performance. This study shows that the use of the SMR as a quality indicator for the treatment of acute leukaemia does not appear to be justified. Several potential explanations exist for these differences. Firstly, the (H)SMR is a mortality probability per admission, while hospitals may differ in their admission and discharge policy during the treatment of acute leukaemia. This may affect the in-hospital mortality rate. For example, intensive chemotherapy is regularly given during an admission of 4-6 weeks. However, several hospitals have currently adopted intensive treatment procedures in a combined inpatient and
outpatient setting or outpatient setting only. In addition, during the palliative phase of treatment, the availability of terminal care facilities in the neighbourhood may affect the timing of discharge. Secondly, the SMR may be influenced by the performance of high-risk interventions or procedures, since adjustment for such procedures is lacking. In case of acute leukaemia, autologous stem cell transplantation is part of the first-line treatment protocols, which only level B hospitals are allowed to perform in the Netherlands. Our study showed that specifically hospitals accredited to perform autologous stem cell transplantations had a high SMR. This may have been a consequence of insufficient adjustment. Furthermore, the SMR shows the in-hospital mortality, which does not necessarily reflect the performance of a hospital with respect to treatment of acute leukaemia. In-hospital mortality is sometimes an expected and accepted outcome when patients are admitted for palliative care. The ratio of in-hospital (40-65%) and out-hospital mortality is influenced by geographical factors. It also reflects the availability of terminal care facilities in the neighbourhood. One of the main limitations of this study is the retrospective design. Adjustment for casemix was based on data from the LMR. Variation in the coding of data in this registry may lead to inconsistencies and thus negatively influence the reliability of the SMR. Furthermore, it is possible that patients were missed during data collection, for example because they had an incorrect ICD-9 code. In addition, the calculation of the SMR for the subgroup ‘acute leukaemia’ is based on the expected mortality for the diagnosis group ‘leukaemia’. This may have had a slight impact on the SMR for the diagnosis group ‘acute leukaemia’. Finally, the number of patients in the varying hospitals is relatively small, which may have affected the reliability of both the SMR and the survival analysis.

Nevertheless, we conclude that the SMR should not be used as a quality indicator for the treatment of acute leukaemia. Differences in the SMR cannot be solely attributed to differences in the quality of care between hospitals. This study and the existing literature on this subject show that standardisation for the possible variables is insufficient. The most important critique is the use of hospital mortality as a quality indicator for the treatment of a disease. The outcome of acute leukaemia treatment is the result of in-hospital, day-care and outpatient treatment. In the SMR analysis, mortality is restricted to in-hospital mortality, while in-hospital care reflects just part of the treatment for acute leukaemia.

**ACKNOWLEDGEMENTS**

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**DISCLOSURES**

The authors declare no conflicts of interest. No funding or financial support was received.

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**Table 5. Multivariate analysis of overall survival (OS) in patients with acute leukaemia**

<table>
<thead>
<tr>
<th>Hospital (vs. hospital 6)</th>
<th>HR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (continuous)</td>
<td>1.04</td>
<td>1.03-1.05</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>1</td>
<td>0.80</td>
<td>0.51-1.26</td>
<td>ns</td>
</tr>
<tr>
<td>2</td>
<td>1.21</td>
<td>0.73-2.01</td>
<td>ns</td>
</tr>
<tr>
<td>3</td>
<td>0.72</td>
<td>0.44-1.19</td>
<td>ns</td>
</tr>
<tr>
<td>4</td>
<td>0.71</td>
<td>0.45-1.10</td>
<td>ns</td>
</tr>
<tr>
<td>5</td>
<td>0.54</td>
<td>0.30-0.98</td>
<td>0.043</td>
</tr>
<tr>
<td>Hospital (vs. hospital 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.51</td>
<td>1.02-2.33</td>
<td>0.039</td>
</tr>
<tr>
<td>3</td>
<td>0.90</td>
<td>0.61-1.33</td>
<td>ns</td>
</tr>
<tr>
<td>4</td>
<td>0.88</td>
<td>0.64-1.20</td>
<td>ns</td>
</tr>
<tr>
<td>5</td>
<td>0.67</td>
<td>0.40-1.11</td>
<td>ns</td>
</tr>
<tr>
<td>6</td>
<td>1.25</td>
<td>0.80-1.95</td>
<td>ns</td>
</tr>
</tbody>
</table>
Prevalence and correction of severe hypovitaminosis D in patients over 50 years with a low-energy fracture

E-J. ter Borg\textsuperscript{1,}, W. van den Hoeven-van Kasteel\textsuperscript{2,}, J.C. Kelder\textsuperscript{3,}, W.F. Lems\textsuperscript{4}

Departments of \textsuperscript{1}Rheumatology, \textsuperscript{2}Surgery, \textsuperscript{3}Teaching and Research, St. Antonius Hospital, Utrecht/Nieuwegein, the Netherlands, \textsuperscript{4}Department of Rheumatology, VU University, Amsterdam, the Netherlands, \textsuperscript{*}corresponding author: email: borg@antoniusziekenhuis.nl

ABSTRACT

Purpose/Introduction: To examine the increase in serum 25(OH) vitamin D levels after supplementation with 800 IU/day of vitamin D in patients with low vitamin D levels and which factors affected the increase in vitamin D levels.

Methods: The study included patients > 50 years with a low-energy fracture and a vitamin D level < 30 nmol/l. This was a retrospective study and was carried out at a large non-teaching hospital in the Netherlands.

Results: 82 patients were included, mean basal 25(OH) vitamin D level was 21.2 nmol/l. After a mean of 9.8 weeks, the mean increase in vitamin D was 48.5 nmol/l. Only 45.1\% reached the target level of > 50 nmol/l. The increase was correlated with the basal level of vitamin D (p < 0.05), and the time interval between the two vitamin D measurements (p < 0.05) and was inversely related to body weight (p < 0.05), but was not related to age, gender or renal function.

Conclusions: We found that the generally recommended dosage of 800 IU of vitamin D per day resulted in suboptimal serum levels after ten weeks of treatment in more than half of the patients. The increase in vitamin D levels was higher in patients with low body weight and in patients with very low basal vitamin D levels. These data suggest that these patients should initially be treated with higher dosages of vitamin D. If not possible, vitamin D measurements should be performed after at least six months of supplementation with dosage adjustment.

KEYWORDS

25-hydroxyvitamin D levels, vitamin D deficiency, low-energy fracture, osteoporosis

INTRODUCTION

Vitamin D deficiency is common in elderly patients with a fracture caused by a low-energy trauma. Bours et al. found a vitamin D deficiency (< 50 nmol/l) in 64\% of their patients, all with a recent fracture.\textsuperscript{1} Severe vitamin D deficiency is associated with muscle weakness, bone pain, and an increased risk of falls and fractures.\textsuperscript{2} In general, the supply of vitamin D mainly relies on exposure to the sun, body mass index (BMI) and skin colour.\textsuperscript{3} The recent Dutch guidelines on osteoporosis and fracture prevention advise a daily intake of 800 IU cholecalciferol for people over 50 years of age and those suffering from osteoporosis. They additionally recommend a 25-hydroxyvitamin D level target value of at least 50 nmol/l.\textsuperscript{4} However, several authors consider the optimum level to be > 75 nmol/l, since it is considered to be the minimum level to prevent falls.\textsuperscript{5} All in all, considering its impact on preventing falls and fractures, vitamin D supplementation is of great importance.

No consensus has been reached on whether a post-treatment control level should be established when a mild or severe vitamin D deficiency has been diagnosed, or on what the optimum daily dose of vitamin D supplementation should be in clinical practice.\textsuperscript{6} Van den Bergh et al. recently proposed to establish a control 25-hydroxyvitamin D level after three months of supplementation and, if necessary, to adjust the recommended dose of cholecalciferol.\textsuperscript{6} They based their proposal on the finding that, in a low-energy fracture patient population, the optimal level of > 50 nmol/l was often not reached with a daily dose of 800 IU.\textsuperscript{7} A meta-analysis showed that with a 25-hydroxyvitamin D basal level of < 50 nmol/l, vitamin D supplementation with 400 IU/day led to an average increase in vitamin D levels of 12 nmol/l.\textsuperscript{5} So far, there have been few reports
on the effect of a relatively low dose (400-800 IU a day) of oral vitamin D supplementation on the increase of 25-hydroxyvitamin D levels in patients > 50 years with a (low-energy) fracture. The objective of this study was to examine the following research questions:

A: What is the increase of the 25-hydroxyvitamin D level after supplementation with a daily dose of 800 IU of cholecalciferol in patients with a severe vitamin D deficiency (< 30 nmol/l) and a low-energy fracture?

B: What percentage of patients will reach the minimum target value of 50 nmol/l?

C: Which factors affect the increase of the 25-hydroxyvitamin D level? Factors that were expected to influence this increase were body weight, BMI, renal function, gender, age, season and 25-hydroxyvitamin D basal level.

METHODS/PATIENTS

Since 2007, the St. Antonius Hospital screen all patients over 50 years of age with a low-energy fracture (except in fingers, toes and metatarsal bones) at the fracture-osteoporosis outpatient clinic for the presence of osteoporosis/osteopenia, by means of DEXA and X-rays of the lumbar and thoracic vertebrae. All patients are asked to complete a questionnaire on known risk factors for osteoporosis/osteopenia. In addition, relevant laboratory tests are run, including a measurement of the 25-hydroxyvitamin D level. Serum 25-hydroxyvitamin D levels were determined on a high-performance liquid chromatography (HPLC) column with two mobile phases (Chromosystems, Munich, Germany) after a purification step. Data on serum 25-hydroxyvitamin D levels were collected retrospectively. The patients with a serum 25-hydroxyvitamin D level < 30 nmol/l were referred to the rheumatologist to further investigate the cause of their severe vitamin D deficiency. For the purposes of this study, patients were not allowed to take supplements containing any vitamin D < 3 months prior to their first vitamin D measurement. All patients were prescribed a supplement of 800 vitamin D IU/day (12 received 880 IU/day).

For all patients included in this study, a second measurement was performed after a mean period of 9.8 (SD 5.3) weeks. All patients showed a highly variable increase in 25-hydroxyvitamin D level, with an average increase of 48 nmol/l (SD 21; range 8-101).

RESULTS

Between January 2008 and June 2011, 134 patients who met the inclusion criteria for this study and had a 25-hydroxyvitamin D level < 30 nmol/l were seen at the fracture-osteoporosis outpatient clinic. Ninety of these patients had both an evaluation by the rheumatologist and a second 25-hydroxyvitamin D measurement. After exclusion of eight patients (see table 1 for the reasons), 82 patients were included in the final evaluation. An overview of the basal characteristics of the patients is provided in table 2. The baseline 25-hydroxyvitamin D level had an inverse correlation ($r = -0.241, p = 0.0431$) with body weight but, due to missing data on height, not with BMI.

From the 82 patients included, a second 25-hydroxyvitamin D measurement was performed after a mean period of 9.8 (SD 3.3) weeks. All patients showed a highly variable increase in 25-hydroxyvitamin D level, with an average increase of 48 nmol/l (SD 21; range 8-101). Only 37 patients (45.1%) reached a 25-hydroxyvitamin D level of > 50 nmol/l. There was an inverse correlation between the increase in vitamin D levels and body weight ($r = -0.225, p = 0.0431$). We also found an inverse correlation between the individual 25-hydroxyvitamin D basal level and the increase in 25-hydroxyvitamin D levels.

Table 1. Study patient population

<table>
<thead>
<tr>
<th>Patients with a vitamin D level &lt; 30 nmol/l who met inclusion criteria</th>
<th>134</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients seen by rheumatologist + second vitamin D measurement</td>
<td>90</td>
</tr>
<tr>
<td>Exclusion following consultation by rheumatologist:</td>
<td>8</td>
</tr>
<tr>
<td>Reason for exclusion:</td>
<td></td>
</tr>
<tr>
<td>• Doubts about vitamin D intake</td>
<td>1</td>
</tr>
<tr>
<td>• Intolerance for vitamin D</td>
<td>1</td>
</tr>
<tr>
<td>• Crohn's disease</td>
<td>2</td>
</tr>
<tr>
<td>• Primary hyperparathyroidism</td>
<td>2</td>
</tr>
<tr>
<td>• eGFR &lt;40 ml/min</td>
<td>1</td>
</tr>
<tr>
<td>• missing data</td>
<td>1</td>
</tr>
<tr>
<td>Final study population</td>
<td>82</td>
</tr>
</tbody>
</table>

DATA ANALYSIS

Standard descriptive statistical methods were used. To determine the association between two continuous variables, a linear regression was calculated with the correlation coefficient ($r$) and p-value for the beta of the independent variable. A student’s T-test was used to calculate the association between a continuous variable and a binomial variable, and for a multiple category variable, the ANOVA test for an ‘overall’ p-value was used; to further explore the associations we computed Tukey multiple comparisons paired p-values and a p-value for linear trend.
Ter Borg et al. Hypovitaminosis in patients over 50 years with low-energy fracture.

When comparing different subgroups by their basal vitamin D level, the group that had the lowest basal values (0-10 nmol/l, mean increase 73 [SD 28]) clearly showed a stronger increase than both the middle (11-20 nmol/l, mean increase 48 [SD 22]) and the highest (21-30 nmol/l, mean increase 46 [SD 19]) groups [p for trend = 0.0298, figure 2]. The degree to which the level increased was not related to gender, BMI (missing data), age, season (April to October versus November to March) or renal function (data not shown).

Statistical analysis showed a significant positive correlation between the increase in 25-hydroxyvitamin D level and the number of days that passed between the first and second measurement (r = 0.246, p = 0.0260). Even after three months of vitamin D supplementation, a plateau phase was still not reached (figure 3).

**DISCUSSION**

In our mostly Caucasian and female population with a mean age of 68.2 years and severe vitamin D deficiency (average value 21.2 nmol/l), after supplementation with 800 IU/day of vitamin D, we observed a mean increase of 48.5 nmol/l after an average of 9.8 weeks. These results are in concordance with those found by Chel et al., who reported a mean increase of 34.9 nmol/l after two months and 44.9 nmol/l after four months after supplementation with 600 IU/day of vitamin D in nursing home patients (mean age 84 years). In patients with various rheumatic diseases and a mean age of 68 years, vitamin D levels increased from 30.8 to about 60 nmol/l after at least six months of treatment with vitamin D 800-1000 IU a day.

After supplementation with 800 IU/day, Lips et al. observed a larger increase in the 25-hydroxyvitamin D level (namely from 23.7 nmol/l to 80 nmol/l) after three months in elderly patients (> 80 years) living in nursing homes or old peoples’ homes, who likely had a better compliance. Gallagher et al. recently reported in a placebo-controlled study with healthy post-menopausal women (mean basal vitamin D level 38.2 nmol/l) that after three months of supplementation with 800 IU vitamin D a day, a vitamin

<table>
<thead>
<tr>
<th>Table 2. Patient basal characteristics (n = 82)</th>
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<tbody>
<tr>
<td>Patient characteristics</td>
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<tr>
<td>Males n (%)</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
</tr>
<tr>
<td>Caucasians n (%)</td>
</tr>
<tr>
<td>BMI* (kg/m²)</td>
</tr>
<tr>
<td>25-OH vitamin D level (nmol/l)</td>
</tr>
<tr>
<td>Calcium (normal values 2.10-2.50 mmol/l; unadjusted)</td>
</tr>
<tr>
<td>Creatinine (normal values 62-106 µmol/l)</td>
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<tr>
<td>Alkaline phosphatase (normal values 0-120 U/l)</td>
</tr>
</tbody>
</table>

# except for gender and race; * missing data for 36 patients

(r = -0.235, p = 0.0337; figure 1). When comparing different subgroups by their basal vitamin D level, the group that had the lowest basal values (0-10 nmol/l, mean increase 73 [SD 28]) clearly showed a stronger increase than both the middle (11-20 nmol/l, mean increase 48 [SD 22]) and the highest (21-30 nmol/l, mean increase 46 [SD 19]) groups [p for trend = 0.0298, figure 2]. The degree to which the level increased was not related to gender, BMI (missing data), age, season (April to October versus November to March) or renal function (data not shown).

Statistical analysis showed a significant positive correlation between the increase in 25-hydroxyvitamin D level and the number of days that passed between the first and second measurement (r = 0.246, p = 0.0260). Even after three months of vitamin D supplementation, a plateau phase was still not reached (figure 3).
D level of > 50 nmol/l was reached in 97.5% of the cases.\(^5\) These results obviously cannot be translated to clinical practice in fracture patients who are for the most part older and have lower basal vitamin D levels. In daily practice, it is a clinically relevant question to ask whether the generally recommended dosage of 800 IU vitamin D per day\(^2\) is sufficient for elderly patients with a recent fracture and a severe vitamin D deficiency.

We are not aware of any other reports on the increase of vitamin D levels after supplementation in elderly fracture patients with very low basal vitamin D levels. In our study, the patients with the lowest 25-hydroxyvitamin D basal levels showed the highest increase after supplementation. This is in concordance with results previously reported in the literature on this subject.\(^5\) In the present study, no plateau phase was reached after three months of supplementation. Vieth \etal\ reported that healthy volunteers (mean age 41 years) taking 1000 IU/day of vitamin D reached a plateau phase of vitamin D after three months, with vitamin D levels increasing from 40.7 to 68.7 nmol/l.\(^6,8\) However, in a study with elderly subjects, Lips \etal\ reported on a plateau phase after 6-9 months of supplementation.\(^9\) A control measurement of vitamin D should therefore be conducted after at least three months of supplementation\(^6\) or, in our opinion, perhaps preferably after six months.

Fewer than half (45.1%) of our patients reached the generally advised 25-hydroxyvitamin D target value of 50 nmol/l (after ten weeks). We do realise that this threshold of 50 nmol/l is arbitrary and that some advocate higher target levels such as 75 nmol/l.\(^7\) As expected, a level of > 75 nmol/l was reached in only a minority of our patients (12.2%). We do agree that the follow-up time was probably too short to reach a new plateau level of vitamin D.

Despite that, it seems likely that the generally used and recommended dosage of vitamin D of 800 IU/day is too low and that treatment should perhaps consist of a higher, possibly loading, dose,\(^6,8\) especially in cases of severe deficiency and obesity.\(^3\) Van Groningen \etal\ found an increase of vitamin D from 20.5 to 74.8 nmol/l after eight weeks following a loading dose of vitamin D (total dosage 100,00-200,000 IU) in vitamin D-deficient adults. The target levels of vitamin D of 50 and 75 nmol/l were reached in 76% and 48%, respectively.\(^10\) These figures are obviously higher than in our study. Of course, it may also be considered to adjust the dosage of vitamin D suppletion according to the basal vitamin D levels.

We identified an inverse correlation between body weight and basal vitamin D levels. Indeed, it is already known that obese subjects have lower basal vitamin D levels because they have a larger distribution volume.\(^11\) It has been demonstrated in recent literature that also the increase in the 25-hydroxyvitamin D level negatively correlates with body weight and/or BMI.\(^11\) Indeed, in our only slightly obese patients (BMI 25.4), we found a negative correlation between body weight and the increase in vitamin D levels.

We did not find such a correlation between this increase and BMI due to a large quantity of missing data on height. The most important limitations of our study are its retrospective format and the follow-up time < 100 days, which is too short to reach a new plateau level of vitamin D. In addition, there was no information on dietary intake of vitamin D and compliance of intake of vitamin D medication. The study’s strengths are: it reports on a clinically relevant question in daily practice and that it was investigator driven without any financial support.

**CONCLUSIONS**

In conclusion, we have shown that after a dose of 800 IU/day of vitamin D, only 45.1% of the elderly fracture patients with a severe vitamin D deficiency reached the advised 25-hydroxyvitamin D level of > 50 nmol/l after an average of ten weeks. The increase in vitamin D level had an inverse correlation with basal vitamin D levels and body weight, and was expectedly associated with the duration of supplementation. Based on our data and data from the literature, we propose taking a second...
measurement of the vitamin D level after at least six months of supplementation, with dosage adjustment.

**DISCLOSURES**

The authors declare no conflicts of interest. No funding or financial support was received.

**REFERENCES**

ABSTRACT

Cholesteryl ester storage disease (CESD) is a rare autosomal recessive disease caused by mutations in LIPA. Here we describe two different clinical presentations of this disease: one case with a clear phenotype of familial hypercholesterolaemia and one case with hepatosplenomegaly from childhood onwards. These two cases exemplify the diversity of clinical phenotypes of patients with CESD. Knowledge on the phenotypic variability of the disease is of clinical relevance in light of enzyme replacement therapy (sebelipase alpha) for patients with mutations in LIPA, which is currently under development.

KEYWORDS

Cholesteryl ester storage disease, lysosomal acid lipase, familial hypercholesterolaemia, low-density lipoprotein cholesterol

INTRODUCTION

Cholesteryl ester storage disease (CESD), or lysosomal acid lipase deficiency (MIM #613497), is an autosomal recessive disease caused by mutations in the LIPA gene, encoding lysosomal acid lipase (LAL). LAL hydrolyses cholesteryl esters and triglycerides in lysosomes and a deficiency of this enzyme leads to intracellular accumulation of cholesteryl esters and triglycerides in hepatocytes, adrenal glands, intestine and cells of the macrophage-monocyte system. As a consequence, patients suffering from LAL deficiency are in general characterised by hepatomegaly, splenomegaly, diarrhoea and mixed hyperlipidaemia. Depending on the residual enzyme activity, LAL deficiency either results in the very severe and lethal Wolman disease (< 12 months of life) or the late-onset form, called CESD. CESD often remains undiagnosed until severe symptoms (i.e. liver failure and/or atherosclerotic disease) occur in adulthood. To date, ~140 CESD patients have been reported in literature while the prevalence of the disease has been estimated to vary between 1:40,000 and 1:400,000 individuals. The discrepancy in the reported and estimated number of patients might be caused by a large phenotypical variation of the disease and unawareness among medical professionals. We here present two family cases with substantially different clinical presentations of CESD, illustrating that this disease might be easily missed or remain undiagnosed.

What was known on this topic?
Cholesteryl ester storage disease is a rare lysosomal storage disease caused by mutations in the LIPA gene.

What does this add?
We show that the variability of phenotypes of patients with CESD is very large and illustrate that in patients with hypercholesterolaemia of unknown origin or unexplained hepatosplenomegaly, LIPA mutations could be present.

SPECIAL REPORT

Hypercholesterolaemia and hepatosplenomegaly: Two manifestations of cholesteryl ester storage disease

B. Sjouke1, J.W.J. van der Stappen2, J.E.M. Groener1, A. Pepping3, R.A. Wevers4, A. Gouw2, L.D. Dikkeschei2, S. Mijnhout3, G.K. Hovingh1, M.A. Alleman2

1Department of Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands, 2Department of Internal Medicine and Clinical Chemistry, Isala Clinics, Zwolle, the Netherlands, 3Department of Clinical Genetics Leiden University Medical Center, Leiden, the Netherlands, 4Department of Laboratory Medicine, Radboud University Medical Center, Nijmegen, the Netherlands, 5corresponding author: email:b.sjouke@amc.uva.nl
CASE 1

A 23-year-old female was diagnosed with a clinical phenotype of primary hypercholesterolaemia during a routine cholesterol screening at work (total cholesterol 13.1 mmol/l; low-density lipoprotein-cholesterol (LDL-C) 10.6 mmol/l; high-density lipoprotein-cholesterol (HDL-C) 1.75 mmol/l; triglycerides 1.69 mmol/l). She did not have any symptoms and physical examination was normal except for a systolic cardiac murmur (grade IV/VI) due to a supravalvular aortic stenosis. Subsequent laboratory analysis only revealed mild increases in alanine aminotransferase (ALAT) levels in the proband (56 U/l; reference: 0-45 U/l). Family screening showed that both of her siblings were also affected while lipid levels were normal in their parents. However, no mutation was identified in one of the well-annotated genes for either autosomal dominant or recessive hypercholesterolaemia, (LDLR, APOB, PCSK9 and LDLRAP). In order to find a mutation in an unknown gene as cause of the hypercholesterolaemic phenotype, we sequenced all protein coding regions of the genome (exome sequencing). Unexpectedly, homozygosity for the exon 8 splice junction (E8SJ = c.G934A) mutation in LIPA was found. The patients were therefore diagnosed with CESD which was biochemically confirmed by a residual LAL activity of 8% in the proband. Both siblings of the proband were also found to be homozygous for the E8SJ mutation and all three patients were treated with statin therapy resulting in an impressive decrease in LDL-C levels by 73 to 88% in the proband and her siblings. We performed magnetic resonance spectroscopy in order to determine the consequences of the LIPA mutations on cholesteryl ester storage accumulation in the liver. Both the proband and her two siblings turned out to have substantial hepatic cholesteryl ester accumulation, but hepatosplenomegaly was not observed. This case was previously published by Stitziel and co-workers.

CASE 2

A 34-year-old male of Dutch/Indonesian descent was admitted to the hospital because of upper abdominal pain, diarrhoea and intestinal blood loss. His past medical history was relevant for hepatosplenomegaly of unknown origin since childhood, for which no follow-up had taken place. A surgical biopsy obtained when he was 3 years old remained inconclusive. Hepatosplenomegaly without signs of chronic liver failure was confirmed upon physical examination. Routine laboratory tests for primary liver disease were all normal except for increased ALAT levels (86 U/l while treated with a statin). Hypercholesterolaemia (total cholesterol 9.0 mmol/l, total cholesterol/HDL ratio 16.3) was also present. Stool weights were normal with normal fat content. Varices at the rectosigmoid junction were found upon colonoscopy, and gastroscopy performed at a later stage confirmed the diagnosis of portal hypertension (grade I). Liver biopsy (figure 1A-C) showed periportal and bridging fibrosis, without signs of cirrhosis. The hepatocytes were enlarged and contained non-uniform microvesicular. Foamy macrophages were seen both in the portal tracts and in the parenchyma. Bone marrow cytology showed few, but clearly distinguishable, ‘sea-blue histiocytes’ and vacuolated macrophages, indicators of, among others, a lysosomal storage disease (figure 1D). In line, chitotriosidase levels were also increased (325 nmol/ml/h; reference < 200 nmol/ml/h), indicating macrophage accumulation. Microscopy findings were consistent with the diagnosis of Niemann-Pick disease (type B or C) but the finding of normal sphingomyelinase activity in leucocytes and the negative filipin staining of cultured fibroblasts refuted these diagnoses. These results, combined with reduced cholesterol esterification, did not, however, exclude CESD. Therefore, LAL activity

Figure 1.

A. Liver needle biopsy. Portal tract. White arrows indicate an enlarged portal tract containing macrophages. Black arrows indicate hepatocytes in the surrounding parenchyma with non-uniform microvesicular fat droplets. (Periodic acid Schiff after diastase digestion, 20X)
B. CD68 staining highlights the CD68 positive macrophages in a portal tract (brown colour, immunostaining with anti CD68 antibody 40X)
C. Liver needle biopsy showing an enlarged portal tract with bridging fibrosis (indicated by arrow) (Masson Trichrome 10x)
D. Bone marrow cytology of the CESD index patient. Arrow indicates a sea-blue histiocyte (Wright’s stain 400X)
was subsequently measured in fibroblast cultures and was found to be strongly decreased (0.2 nmol/h per mg fibroblast protein in the proband versus 8-15 nmol/h per mg fibroblast protein in control cultures). Since mutations in LIPA are known to cause reduced LAL activity and, as a consequence CESD, we used targeted DNA diagnostics (RT-PCR) of the LIPA gene to confirm the diagnosis. This revealed compound heterozygosity for the E8SJ mutation and the T1107G mutation in exon 10 in LIPA.

Two brothers were also diagnosed with CESD (figure 2). Neither of them had sought medical attention for CESD-related symptoms and might have remained undiagnosed if the proband had not been identified as a CESD patient. The portal hypertension in the proband was successfully treated with propanolol. The diarrhoea ceased after treatment with fibres (psyllium). After confirmation of the diagnosis of CESD, treatment was started with a statin. Serum cholesterol and total cholesterol/HDL ratios decreased twofold.

**DISCUSSION**

We here describe two family cases of CESD. Case 1 presented with a typical phenotype of familial hypercholesterolaemia. In case 2, the combination of bone marrow cytology (‘sea-blue histiocytes’ and vacuolated macrophages), liver histology (microvesicular steatosis, bridging fibrosis and lipid laden macrophages), and a combined hyperlipidaemia finally led to the diagnosis of CESD. These cases illustrate the variability of phenotypes of patients with CESD and emphasise that in patients with hypercholesterolaemia of unknown origin or unexplained hepatosplenomegaly, LIPA mutations could be present. Moreover, our two cases clearly show the differential diagnostic pathways that can ultimately lead to the identification of a uniform molecular pathological substrate of a disease. It is of note that heterozygosity for LIPA mutations is not associated with clinical signs and symptoms. The E8SJ mutation is the most frequently found mutation in CESD patients, leading to skipping of exon 8 and resulting in only a fraction of the normal hLAL enzyme levels. The T1107G mutation in exon 10 of LIPA causes a premature stop codon that consequently results in the synthesis of a truncated LAL protein. Together with the exon 8 mutation this results in a nearly complete abolishment of hLAL activity in the compound heterozygotes. To the best of our knowledge, this mutation has not been previously described. Since elevated LDL-C and triglyceride levels as well as decreased HDL-C levels are associated with development of cardiovascular disease (CVD), CESD patients are likely to be at increased CVD risk. The low number of patients known thus far, however, preclude us from drawing firm conclusions in this regard. Although no specific therapy for CESD is available to date, current treatment of CESD patients comprises treatment of dyslipidaemia with statins, given the established beneficial effect on both lipids and CVD risk. The effect of statin therapy on the extent of hepatic cholesteryl-ester accumulation is, however, unknown.

Sebelipase alpha (SBC-102; Synageva BioPharma Corporation, Lexington, Massachusetts, USA) is a recombinant human LAL enzyme that is currently in the clinical stage of development and that has been tested in nine CESD patients to date. In this population, sebelipase alpha has shown to significantly reduce ALAT and aspartate aminotransferase (ASAT) levels by 52% (44 U/l) and 36% (20 U/l), respectively after 12 weeks (4 weekly infusions followed by 4 bi-weekly infusions of 0.35 mg/kg – 3 mg/kg). These decreases were shown to sustain for up to 52 weeks of treatment in seven patients, where ALAT levels were decreased by 58% (49 U/l) and ASAT levels were decreased by 48% (43 U/l), respectively after 12 weeks (4 weekly infusions followed by 4 bi-weekly infusions of 0.35 mg/kg). Besides the reductions in transaminase levels, sebelipase alpha has also shown to significantly reduce lipid and lipoprotein levels. After 52 weeks of treatment, total cholesterol levels decreased by 1.84 ± 0.80 mmol/l (p = 0.016), LDL-C decreased by 1.89 ± 0.80 mmol/l (p = 0.016) and triglyceride levels decreased by 0.81 ± 0.41 mmol/l.
mmol/l \( (p = 0.047) \). Infusion reactions were only observed in one patient, who was finally successfully re-challenged.\(^1\)

Sebelipase alpha is currently being tested in a placebo-controlled phase III trial (ARISE; NCT 01757184). The non-specific phenotypic characteristics of CESD and the (partial) overlap of phenotypic features with other cardiovascular, liver and metabolic diseases illustrate the difficulty in diagnosing patients with CESD and suggest that patients with this disease might be missed or remain undiagnosed. This is supported by the large discrepancy between the number of reported CESD cases in the literature and the estimated prevalence of this disease.\(^3,10\)

The large variability of CESD phenotypes, as illustrated in this case report, shows that more insight into the natural clinical course of homozygosity/compound heterozygosity for \( \text{LIPA} \) is warranted, since this will influence the identification of patients who will have benefit from future enzyme replacement therapy.

**ACKNOWLEDGEMENTS**

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**DISCLOSURES**

Dr. Hovingh received lecture fees from Pfizer, Sanofi, Regeneron, Synagava, Amgen, Roche, and Genzyme. Dr. Alleman received lecture fees from Orphan Europe. The other authors declare that they have no conflicts of interest.

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Sjouke et al. Hypercholesterolaemia and hepatosplenomegaly as manifestations of CESD.
ABSTRACT

We describe a case of a 44-year-old woman with a borderline personality disorder and chronic gamma-butyrolactone (GBL) use who presented with progressive dyspnoea and an altered mental status. A high anion gap metabolic acidosis and acute lung injury was diagnosed. We hypothesise this was caused by GBL. In this case report we describe the diagnostic process and possible pathophysiological mechanisms that may have led to this life-threatening condition.

KEYWORDS

Gamma butyrolactone, gamma hydroxybutyrate, intoxication, pneumonitis

INTRODUCTION

Gamma-butyrolactone (GBL) (figure 1), a prodrug of gamma hydroxybutyrate (GHB), is increasingly being used as a recreational drug as it is cheap, easily available and has relaxant and sexual effects. However, it is a highly addictive drug and understanding toxicity is essential for the management of intoxicated patients. We report a patient with chronic GBL abuse who presented with a high anion gap metabolic acidosis and acute lung injury (ALI), a clinical syndrome that has not been described before. We will discuss the diagnostic process and possible pathophysiological mechanisms that may have led to this life-threatening condition.

CASE REPORT

A 44-year-old woman with a borderline personality disorder presented to the emergency department with progressive dyspnoea for three days. She had no other relevant medical history. She reported no recent cold, fever, cardiac symptoms nor use of alcohol or illicit drugs. Her medication included benzodiazepines and
10 mg of oxycontin™. She denied nausea or vomiting and there were no signs of aspiration. On examination, she appeared agitated. Her blood pressure was 130/80 mmHg, pulse rate 130/min and temperature 36.6°C. The respiratory rate was 32 breaths/min and the oxygen saturation was 89% on ambient air. The Glasgow-Coma score was 15 and she had normal pupils. Laboratory parameters showed leucocytosis (23.8 x 10⁹/l), an elevated C-reactive protein, (176 mg/l), and a high anion gap metabolic acidosis (pH 7.29; pCO₂ 4.7 kPa; HCO₃ 17 mmol/l; pO₂ 7.5 kPa; sodium 142 mmol/l, chloride 106 mmol/l, anion gap 19). Lactate was 1.0 mmol/l and urine screening only showed a trace of acetone. A chest X-ray showed bilateral alveolar consolidations (figure 2a). She was admitted to hospital and treatment with benzylpenicillin and ciprofloxacin was started as community acquired pneumonia was considered. To further investigate the high anion gap metabolic acidosis, we reinforced the patient to evaluate whether she might have taken any drugs. Subsequently, she confessed smoking cannabis a couple of times a week and ingesting GBL every two hours in the last 3 months. Per ingestion she mixed 2 ml of GBL with water. Toxicological analysis of the urine was performed which showed traces of benzodiazepines and a positive screening for cannabis and opioids; screening for cocaine, methadone and XTC was negative. To further evaluate the abnormalities seen on the chest X-ray, computed tomography of the thorax revealed bilateral extensive ground-glass opacities (figure 2b). The differential diagnosis consisted of Pneumocystis pneumonia due to HIV-infection, viral pneumonia, aspiration pneumonia or a chemical pneumonitis due to substance abuse. She tested negative for HIV. A bronchoalveolar lavage was performed to rule out infectious causes. Adenovirus, respiratory syncytial virus, influenza A and B virus, and parainfluenza virus 1, 2 and 3 tested negative, staining for Pneumocystis jirovecii was negative as was auramine staining; and (myco) bacterial cultures did not reveal respiratory pathogens. Pathological examination of the bronchoalveolar lavage showed acute inflammation with neutrophils; no malignant cells were seen. Repeated...
serology for Chlamydia pneumoniae, Chlamyphila psittaci, and Mycoplasma pneumoniae was negative. Blood cultures remained negative. By exclusion of infectious causes of pneumonia our differential diagnosis was chemical pneumonitis or immune-mediated pneumonia caused by GBL/GHB. Because of respiratory failure she required mechanical ventilation. With the hypothesis of ALI due to GBL, treatment with high-dose prednisolone was started. Subsequently, she recovered and mechanical ventilation was discontinued after three days. She needed high doses of midazolam (up to 25 mg/hour) to suppress her withdrawal symptoms. The serum level of GHB was 440 mg/l (reference < 10 mg/l). With a molecular weight of gamma hydroxybutyrate of 103 this concentration of GHB contributes 4.2 mmol/l of anions, explaining the high anion gap. Eventually, she was discharged from hospital nine days after admission. A chest CT scan performed four weeks after discharge showed normalised lung parenchyma.

DISCUSSION

We present a 44-year-old woman with agitation, a high anion gap metabolic acidosis, GBL abuse and progressive respiratory insufficiency that most likely represented a form of ALI or ARDS. Infectious causes were excluded and aspiration pneumonia was believed to be unlikely based on the presentation and subsequent course.

GBL is a precursor of GHB. It is a hygroscopic solvent which is used as stain remover and paint stripper. It is increasingly being used as a recreational drug. Unlike GHB, GBL is still legal in the Netherlands. It can easily be obtained via internet. GBL is converted into GHB by mixing it with sodium hydroxide, but once ingested it is also rapidly metabolised into GHB. GHB itself rapidly dissociates to the anion and a hydrogen ion and thus high GHB levels will lead to a high anion gap metabolic acidosis.

GHB works as an agonist on the GABAb receptor and the GHB receptor. Initially, dopamine release is inhibited (causing sedation) and paradoxically dopamine release is increased later on (leading to agitation). Recently, it became evident that GHB also has high-affinity for the GABAA receptor in the central nervous system but whether the effect is agonistic or antagonistic remains elusive. It is believed that binding to the GABAA receptors might have paradoxical effects. This is of interest as recent experimental studies demonstrated a role of the GABAA receptor in the pathogenesis of ALI by its involvement in alveolar-fluid homeostasis. In this respect, blocking of the GABAA receptors in the lung is associated with alveolar inflammation and pulmonary oedema. We thus hypothesise that our patient might have developed ALI due to a systemic toxic effect of GHB on alveoli by binding on the GABAA receptor. The hygrosopic nature of GBL might have contributed as small amounts of GBL will be inhaled during ingestion. As our patient ingested GBL very frequently over a long period of time, unintended GBL inhalation might have led to a direct toxic effect on the alveoli.

To our knowledge, this is the first case of ALI after GBL ingestion. A few possible cases have been described previously because pulmonary oedema was a key feature on post-mortem findings. These findings support our hypothesis.

In summary, this case alerts clinicians that the combination of an unexplained high anion gap metabolic acidosis and diffuse alveolar infiltrates should prompt a diagnosis of intoxication. In this respect, GHB/GBL intoxication should strongly be considered, especially as it has been one of the increasing causes of lethal poisoning in past years.

DISCLOSURES

The authors declare no conflicts of interest. No funding or financial support was received. Written informed consent was obtained.

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ABSTRACT
Renal function deterioration is a rather frequent side effect of ticagrelor; this is especially so in patients over the age of 75, with pre-existent mild renal failure and/or taking an angiotensin receptor inhibitor. We describe a patient in whom deterioration of renal function due to ticagrelor led to a rise in serum concentration of rosuvastatin which resulted in rhabdomyolysis. The presented case emphasises the importance to check renal function routinely before and one month after starting ticagrelor and to screen carefully for possible interactions with other drugs.

KEYWORDS
Acute renal failure, drug interactions, rhabdomyolysis

BACKGROUND
The Guidelines of the European Society of Cardiology (ESC) advise the use of antiplatelet agents inhibiting signal transduction to the platelet P2Y₁₂ adenosine diphosphate receptor (ADP) in patients with acute coronary syndrome (ACS).¹² Ticagrelor is a new, oral P2Y₁₂ adenosine diphosphate receptor antagonist. Ticagrelor is metabolised by CYP3A4; 58% of ticagrelor is excreted in the faeces, and 27% in the urine, predominantly as inactive metabolites.³ Unlike clopidogrel and prasugrel, ticagrelor binds reversibly to the receptor. Both the parent drug and first metabolite, with elimination half-lives of 7 and 9 hours respectively, have antiplatelet activity.¹ These specific properties allow a relatively rapid onset of action. The PLATElet inhibition and patient Outcomes (PLATO) trial showed that ticagrelor significantly reduced the rate of death from cardiovascular events in patients with ACS as compared with clopidogrel, regardless of renal function.¹ Patients with chronic kidney disease have a poorer responsiveness to clopidogrel treatment.⁵ Instructor analysis of the PLATO trial showed that the benefits of ticagrelor over clopidogrel are larger in patients with impaired renal function.⁵ Based on the PLATO trial¹ it is now strongly recommended by the ESC to prescribe ticagrelor (loading dose 180 mg orally followed by a maintenance dose of 90 mg twice daily) for all patients at moderate-to-high risk regardless initial treatment strategies.¹

Combined prescription of ticagrelor and statins is common. Statins are notorious for causing rhabdomyolysis. Risk increases with higher doses or interactions with other medicaments.⁹ Both simvastatin and ticagrelor are metabolised by CYP3A4. Ticagrelor is a weak inhibitor of CYP3A4. Combined prescription will result in higher serum concentrations of simvastatin, potentially evoking rhabdomyolysis. The prescribing information of ticagrelor recommends to avoid simvastatin doses over 40 mg.⁴ In contrast to simvastatin, rosuvastatin, which was used

What was known on this topic?
Renal function deterioration is a rather frequent side effect of ticagrelor. Especially patients over the age of 75, with pre-existent renal failure and/or taking an angiotensin receptor inhibitor, are at risk.

What does this case report add?
This case illustrates that deterioration of renal function can lead to serious complications. It is important to check renal function one month after initiation of ticagrelor therapy, although this is not standard in current clinical practice due to unawareness of potential effects on renal function.
in this case, is not metabolised by CYP3A4. However, dosage of rosuvastatin should be adjusted in patients with renal failure, since its plasma levels may increase threefold with an eGFR < 30 ml/min. Therefore, doses over 40 mg daily are contraindicated in patients with moderate renal impairment (< 60 ml/min). The use of rosuvastatin in patients with severe renal impairment (< 30 ml/min) is contraindicated for all doses.  

In the World Health Organisation (WHO) Adverse Drug Reaction Database, rhabdomyolysis was registered 17 times as adverse reaction of ticagrelor; in all cases the patient used a statin.

CASE PRESENTATION

A 78-year-old man was referred to the emergency department with acute renal failure. The medical history revealed hypercholesterolaemia and two myocardial infarctions, the second one month before admission. The patient had been nauseous and vomiting for six days. He complained about myalgia and muscle cramps. Medication included perindopril 2 mg twice daily, amlodipine 5 mg once daily, metoprolol 25 mg twice daily, omeprazole 20 mg once daily, rosuvastatin 40 mg once daily, ezetimibe 10 mg once daily and ticagrelor 90 mg twice daily. His blood pressure was 146/87 mmHg and pulse rate 89 beats/min. Physical examination revealed no abnormalities, except pain upon palpation of the calves.

Initial laboratory testing showed a serum creatinine of 674 µmol/l (one month previously this was 108 µmol/l), eGFR of 10 ml/min, serum urea of 25.7 µmol/l and creatinine kinase (CK) of 10,872 IU/l. The urine sediment revealed leukocytes (++), red cells (+++) and proteinuria (3.1 g/24 hours). It is a well-known fact that red cells in qualitative urine analysis can be caused by the presence of free haemoglobin or myoglobin.

The working diagnosis was acute renal failure due to rhabdomyolysis and dehydration due to vomiting, aggravated by use of an ACE inhibitor in a dehydrated state. Treatment with infusion of physiological saline solution and bicarbonate was started. Rosuvastatin (the assumed cause of rhabdomyolysis), perindopril and amlodipine were discontinued.

Clinical symptoms remained unchanged over the next four days and the serum CK rose further to 18,545 IU/l and the creatinine concentration to 707 µmol/l. The diagnosis was reconsidered: rosuvastatin and ezetimibe had been used in the current dosage for six years without symptoms. The only recent change in medication was the start of ticagrelor one month earlier, suggesting a role for ticagrelor in the rhabdomyolysis. Ticagrelor was discontinued, as were omeprazole and ezetimibe. From that moment on (day 4) the patient recovered and serum creatinine and CK values decreased gradually (figure 1). At the moment of discharge

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Figure 1. Serum creatinine kinase and serum creatinine. Day 0: hospitalisation. Arrow: discontinuation of ticagrelor therapy

![Graph showing serum creatinine kinase and serum creatinine over time](image-url)
from the hospital the serum concentration of CK was 3286 IU/l and the serum creatinine concentration was 502 µmol/l. In retrospect, one week after the start of ticagrelor, the serum creatinine had risen from 108 µmol/l (eGFR 60 ml/min) up to 124 µmol/l (eGFR 52 ml/min).

DISCUSSION

In the PLATO trial, serum creatinine concentration significantly increased by more than 50% in 25.5% of the patients receiving ticagrelor. In 8.3% of these patients creatinine levels increased more than 50%. Elevations of more than 50% were more pronounced in patients over 75 years (13.6%), in patients with pre-existent renal impairment (17.8%) and in patients receiving treatment with angiotensin type II receptor inhibitors (11.2%).3,12 The European Medicines Agency recommends to check renal function one month after starting ticagrelor; special attention should be paid to patients with additional risk factors.3 The mechanism leading to an increase in creatinine concentrations is unknown. Ticagrelor prolongs the half-life of adenosine and increases its plasma concentration by inhibition of cellular uptake. Adenosine interferes with the tubuloglomerular feedback system and directly influences renal vascular tone. Adenosine and angiotensin II act synergistically to increase renal vascular resistance and decrease renal blood flow.13,16 In contrast with the reports in the WHO database, rhabdomyolysis does not seem to be a side effect of ticagrelor. Here, it is plausible that ticagrelor led to a deterioration of renal function, resulting in accumulation of rosuvastatin, which provoked rhabdomyolysis. Simultaneous use of ezetimibe was possibly an additional risk factor.20 Serum CK levels declined four days after discontinuation of rosuvastatin, corresponding with the elimination time of rosuvastatin.

Unfortunately, in this patient with multiple risk factors, renal function was not checked one month after initiation of ticagrelor therapy. Additionally, the rosuvastatin dose was not adjusted for the pre-existing mild renal impairment.

ACKNOWLEDGEMENTS

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DISCLOSURES

The authors declare no conflicts of interest.
A 64-year-old female presented in October 2013 with thoracic pain and dyspnoea for two days. She had lost seven kilograms of body weight in four months. Her medical history included long-QT syndrome and IgA nephropathy. She had received a pre-emptive living related kidney transplantation in October 2012, which was complicated by ongoing rejection this was followed by alemtuzumab one month later, with partial recovery of kidney function. At presentation, the physical examination was unremarkable. Her body temperature was 37.8°C. Laboratory analysis showed leucocytes 19.2 (4-10) x 10⁹/l, thrombocytes 557 (150-400) x 10⁹/l, creatinine 304 (< 90) µmol/l (stable), lactate dehydrogenase 279 (<250) IU/l and C-reactive protein 65 (< 8) mg/l. Chest X-ray showed a tumour in the right lung (figure 1). Computed tomography (CT) showed a dense inhomogeneous mass with partial atelectasis and air bronchograms, pretracheal lymphadenopathy and bilateral pulmonary nodules (figure 2). Bronchoscopy showed no endobronchial lesions and a bronchoalveolar lavage and brush were performed. No malignant cells were found and cultures were negative for pathogenic microorganisms. A transthoracic biopsy was performed for histology, showing a granulomatous inflammatory reaction. Gram, Ziehl-Neelsen and PAS-D staining and polymerase chain reaction for mycobacteria were negative.

**WHAT IS YOUR DIAGNOSIS?**

See page 141 for the answer to this photo quiz.
Intermittent abdominal pain and melaena in a 64-year-old man

X. Zhou¹, Q. Zhou³, C. Huang³

Departments of ¹Surgery, ²Radiology, The Dingli Clinical Institute of Wenzhou Medical University (Wenzhou Central Hospital), Wenzhou, Zhejiang, P.R. China, ³Department of Surgery, Yongjia County People’s Hospital, Yongjia, Zhejiang, P.R. China, *corresponding author: tel.: 86-577-88070316, fax: 86-577-88070100, email: bobzxccc@163.com

CASE REPORT

A 64-year-old man presented with a four-day history of intermittent abdominal pain and melaena. The patient had no past history of cancer and no family history of colorectal cancer. Physical examination revealed mild periumbilical and right lower quadrant abdominal tenderness without rebound tenderness. Laboratory examination showed no abnormality. Contrast-enhanced computed tomography (CT) (figure 1A, 1B and 1C) showed an intussusception in the right lower quadrant: a segment of bowel accompanying blood vessel density and adipose tissue density within the lumen of caecum (arrows). Colonoscopy (figure 1D and 1E) revealed a sausage-like mass with superficial hyperaemia and mucosal erosion in the ileocaecal valve.

WHAT IS YOUR DIAGNOSIS?

See page 142 for the answer to this photo quiz.

Figure 1A, B, C. An intussusception in the right lower quadrant: a segment of bowel accompanying blood vessel density and adipose tissue density within the lumen of caecum (arrows)

Figure 1D, E. Colonoscopy revealed a sausage-like mass with superficial hyperaemia and mucosal erosion in the ileocaecal valve
ANSWER TO PHOTO QUIZ (PAGE 139)

AN IMMUNOCOMPROMISED WOMAN WITH A LUNG TUMOUR

DIAGNOSIS

The patient developed fever for which she received ceftriaxone. A second biopsy was performed for histology and microbiology. Again, histology showed a granulomatous inflammatory reaction. Gram staining showed mixed flora. Cultures initially were negative but after eight days the buffered charcoal yeast extract (BCYE) agar became positive for *Nocardia* species, susceptible for trimethoprim-sulphamethoxazole (TMP-SMX), amoxicillin-clavulanic acid and tetracycline. Determination by 16S rRNA sequencing showed *Nocardia abscessus*.

*Nocardia* is a bacterium which belongs to the family of aerobic actinomycetes, characterised as Gram-positive branching filamentous rods that produce fungus-like colonies in culture. *Nocardiosis* occurs mainly in immunocompromised patients, e.g. renal transplant recipients. This has previously been discussed in this journal. *Nocardia* species are ubiquitous in soil and aquatic environments. The main route of acquisition is by inhalation or the skin. Nocardiosis can manifest solely in the lungs or cutaneously, but *Nocardia* species can also disseminate to distant organs. They can be recovered on growth media for bacteria, mycobacteria or fungi but may be obscured by more rapidly growing commensal bacteria; therefore, a selective medium should be inoculated for at least two weeks. *Nocardia* species are susceptible to different antibiotics: sulphonamides and trimethoprim work synergistically, therefore TMP-SMX is regarded as the preferred treatment. In pulmonary or systemic nocardiosis, treatment should be at least three months, whereas some authors advocate life-long prophylaxis afterwards, also depending on the necessity of life-long immunosuppression.

In our patient, who turned out to be a frequent gardener, the diagnosis of pulmonary nocardiosis was made based on the positive biopsy culture and the absence of other findings suggesting systemic nocardiosis including a negative brain CT scan. She was treated with doxycycline and amoxicillin-clavulanic acid as TMP-SMX was contraindicated because of her long-QT syndrome. After three months of therapy, an excellent clinical and radiological response had been achieved. Thereafter, treatment was switched to doxycycline, because of presumed adverse effects of amoxicillin-clavulanic acid (nausea). This secondary prophylaxis was given for an additional three months. In addition, her immunosuppressive medication was diminished in dose. To date, i.e. seven months after doxycycline discontinuation, no signs of recurrence were experienced.

In conclusion, in immunocompromised patients with clinical signs suggestive of a malignancy, opportunistic infections such as nocardiosis should always be considered. To prevent missing the diagnosis, a biopsy should be performed for histology, including Gram staining, and for appropriate cultures, incubated on special agars and for an appropriate time.

REFERENCES

D I A G N O S I S

The patient underwent exploratory laparotomy following the initial diagnosis, at which time an appendiceal intussusception to the caecum was identified (figure 2A) (arrows). The intussusception could not be manually reduced, and so a completely inverted appendix was taken from a caecal incision (figure 2B and 2C) and an appendectomy was performed. Histopathological examination of the resected appendix showed acute suppurative appendicitis with periappendicitis, and high-grade intraepithelial neoplasia of the appendiceal tip (figure 2D), suggesting it as the cause of the intussusception. The postoperative course was uneventful. Appendiceal intussusception is a very rare entity, with an incidence of 0.01% in 71,000 human appendix specimens.1 It occurred less often in adults than in children, and the diagnosis is rarely made preoperatively.2 It has also been reported that appendiceal intussusception in adult women is mainly due to endometriosis, and in children is mainly due to inflammation.3 Appendiceal intussusception may mimic an ileocolic intussusception. Contrast-enhanced CT and colonoscopy may help to precisely identify the causative lesion preoperatively.4 A firm diagnosis of an appendiceal intussusception to the caecum induced by high-grade intraepithelial neoplasia could be established fundamentally. It was based on the intraoperative exploration and the histopathological examination. In adults, intussusception is usually associated with a neoplastic lead point and requires surgical exploration with resection.5,6 Our case was considered intraoperatively to be caused by a pure inflammatory lesion and not a neoplastic entity. Thus a simple appendicectomy with a caecal incision was performed. As a result of the postoperative histopathological examination, which revealed acute suppurative appendicitis with periappendicitis, and high-grade intraepithelial neoplasia of the appendiceal tip, a second unnecessary radical resection was avoided. Open surgery should be recommended for this type of appendiceal intussusception, which is described as a completely inverted appendix into the caecum and cannot be manually reduced. Otherwise, when dealing with this type of appendiceal intussusception, a laparoscopic procedure should be completed by the experienced laparoscopic surgeons.

R E F E R E N C E S

To the Editor,
Recently in your journal, Van IJzendoorn et al. presented a post-hoc analysis comparing Dutch with European ICU ventilation practices. Apart from the finding that tidal volumes were lower and levels of positive end-expiratory pressure (PEEP) were higher in Dutch compared with European ICUs, there are some other prominent differences.

The incidence of unplanned extubations in Dutch ICUs is higher than in European ICUs (28.6 versus 13.9%, p < 0.01), and the percentage of reintubations after unplanned extubations is lower (2.4% versus 20%, p < 0.01). This low incidence of reintubations after unplanned extubations suggests there is a group of patients whose planned extubation has been delayed. But the most remarkable difference between Dutch and European ICUs is the PEEP level used in patients without ARDS (8.0 [6.0-9.5] versus 6.0 [5.0-8.0] cm H$_2$O, p < 0.01). Van IJzendoorn et al. suggest that ‘a certain PEEP level is needed to achieve the optimal lung volume at which the alveoli stay open’. This finding in preclinical studies, however, may not at all translate into benefits in patients. Indeed, higher PEEP levels have been found beneficial only in patients with moderate or severe ARDS. While randomised controlled trial evidence for higher PEEP levels in ICU patients without ARDS is absent, a recent randomised controlled trial in patients with healthy lungs receiving short-term ventilation during general anaesthesia for surgery shows that higher PEEP levels were not beneficial, and maybe even harmful. The suggestion that higher PEEP levels in patients with healthy lungs was recently confirmed in an individual patient data meta-analysis. Could it be that use of higher PEEP levels in Dutch ICUs is associated with longer durations of ventilation? Indeed, intensivists tend to extubate ICU patients at the ‘lowest’ PEEP level, which is generally 5 cm H$_2$O. A recent post-hoc analysis of two randomised controlled trials showed that a change from using higher PEEP to lower PEEP levels was associated with a shorter duration of ventilation in post-cardiac surgery patients in a Dutch ICU.

Evidence for harm from mechanical ventilation is rapidly growing. Use of too large tidal volumes causes harm. Unrestricted use of high PEEP levels (i.e. using higher levels of PEEP in patients who do not have moderate or severe ARDS) could also worsen outcome.

REFERENCES
INFORMATION FOR AUTHORS

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