Chest X-ray shows fine reticulonodular opacities. What is your diagnosis?
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To serve the needs of the physician to practice up-to-date medicine and to keep track of important issues in health care. To promote and to enhance clinical knowledge by publishing editorials, original articles, reviews, papers regarding specialty training, medical education and correspondence.

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An overview of strategic differences for internal medicine care in Dutch Emergency Departments

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Internists in the Netherlands are shedding more light on the organisation of Emergency Departments (EDs) in order to improve its structure and thus, quality of care. Cases presenting to the ED have become more complex, and the number of elderly patients presenting to EDs is increasing. These factors contribute significantly to ED overcrowding, which has a considerable impact on quality of care. There is certainly a need to restructure the acute care system in EDs, however, to date, it is still a matter of discussion on how to achieve this, since there is significant disagreement between hospitals and healthcare insurers.

In the current issue of NJM, Kremers et al. are the first to describe a nationwide detailed overview of the organisation and structure of different EDs. The first step is simply wanting to re-organise where needed, especially with respect to care of internal medicine patients. These patients pose a significant challenge for Emergency Physicians (EP) given their complex medical background with multi-morbidity and polypharmacy; it requires more time to perform a diagnostic work-up than other similar presentations. Since our population is expanding and growing older, this will lead to more crowding and longer wait times in the ED, especially if the required knowledge and expertise is lacking or absent.

The study by Kremers et al. highlights the important finding that ED staffing (consisting of residents, EPs, medical specialists) is considerably different between EDs in the Netherlands, and this has an impact on workflow. They also show that the presence of acute care specialists in EDs improves the quality of care and patient flow. This topic remains a matter of debate, since it may be difficult to pursue a uniform organisational structure for all EDs, where specialists are present at all times. Nevertheless, having the right expertise and knowledge present will definitely improve our healthcare services. Therefore, we should evaluate and improve the role of internists in the current structure of EDs more carefully. One solution, for example, could be the presence of an internist in the ED to review cases, especially in EDs where there are no EPs present.

Investigating the current structure of EDs in the Netherlands, and obtaining more details as to when and where medical specialists (mainly internists) are needed and how we can implement this, increases and improves collaboration with the EPs, residents, and medical specialists. This would be the first step to a more uniform organisational structure nationwide, and is crucial to improving our current acute healthcare system. In the future, we should start by implementing the presence of an (acute) internist in EDs, especially during ‘office’ hours, to be able to compare the quality of care and workflow with our current knowledge.

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Clinical significance of soluble interleukin-2 receptor measurement in immune-mediated diseases

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ABSTRACT

A soluble form of the interleukin-2 receptor (sIL-2R) is secreted upon T-cell activation. Increased blood levels of sIL-2R occur in a variety of immunological diseases. Although the biological function of sIL-2R is incompletely understood, both in health and disease, sIL-2R serum measurements are commonly conducted in clinical practice as it may help to facilitate diagnosis of specific immune-mediated diseases, such as haemophagocytic lymphohistiocytosis and sarcoidosis. In these, and in other immune-diseases, sIL-2R levels may be used as a biomarker to monitor/predict disease activity and treatment response. In this review, we will give a brief overview of the biology of the IL-2/IL-2R system and will subsequently discuss the clinical utility of sIL-2R measurement, especially in the context of haemophagocytic lymphohistiocytosis, sarcoidosis, rheumatoid arthritis, systemic lupus erythematosus, juvenile idiopathic arthritis, adult-onset Still’s disease, ANCA-associated vasculitis, and IgG4-related disease.

KEYWORDS

Haemophagocytic lymphohistiocytosis, immune-mediated diseases, sarcoidosis, soluble IL-2 receptor, T-cell activation

INTRODUCTION

Interleukin 2 (IL-2) represents one of most significant cytokines in the immune system as it is involved in the regulation of protective immunity, as well as maintaining immune tolerance mediated by CD4+ regulatory T lymphocytes (Treg). IL-2 acts on cells that express either the trimeric high-affinity IL-2 receptor (IL-2R) or dimeric low-affinity IL-2R (figure 1). The dimeric low-affinity IL-2R consists of the IL-2Rβ chain (also known as CD122) and the cytokine receptor common γ-chain (γc, also known as CD132). The low-affinity dimeric IL-2R is hardly expressed by naive CD4+ T lymphocytes, expressed at low levels by naive CD8+ T lymphocytes and memory CD4+ T lymphocytes, and at high levels on memory CD8+ T lymphocytes and NK cells. Cells that express high levels of low-affinity IL-2R are susceptible to activation by IL-2 in vitro, yet this requires stimulation with (non)-physiological IL-2 concentrations. The third chain of the trimeric high-affinity IL-2R is IL-2Ra (also known as CD25 or TAC antigen). IL-2Ra does not actively participate in receptor signalling but rather, enhances the receptors affinity for IL-2. Tregs are characterised by strong constitutive expression of IL-2Ra which enables these cells to constantly express the high-affinity trimeric IL-2R (IL-2Rαβγ) and thereby use the low physiological level of IL-2 as is present in vivo. The high-affinity IL-2R is transiently expressed at high levels by activated CD4+ and CD8+ T lymphocytes. First, following signalling induced by T-cell receptor (TCR) activation and co-stimulatory molecules, IL-2Ra is induced to moderate expression levels which is subsequently further enhanced in a positive feedback loop through IL-2/IL-2R signaling. Rubin and colleagues were the first to demonstrate that after in vitro activation, T lymphocytes not only enhanced cellular IL-2R expression but also released soluble IL-2Rα in and similar to cellular IL-2R expression, the release of soluble IL-2R required de novo protein synthesis rather than cellular proliferation. Other studies demonstrated a significant correlation between surface membrane IL-2R expression on activated CD4+ and...
CD8+ T lymphocytes and the amount of secreted sIL-2R following in vitro activation. In addition, CD4+CD25+ Tregs were found to secrete sIL-2R upon in vitro activation with certain stimuli. Soluble IL-2R most likely originates from enzymatic cleavage and release from the cell surface membrane. Several enzymes, including neutrophil-derived elastase, matrix metalloproteinases-9, and the house dust mite protease Der p 1, have been proposed for cleavage of membrane IL-2Ra. However, although the exact nature of the involved proteolytic factor(s) remains largely unknown, it is most likely endogenous to the sIL-2R-producing cell as suggested by occurrence of sIL-2R production under serum-free cell culture conditions. Since its initial in vitro description in 1985, elevated sIL-2R blood levels have been found in different pathological conditions, including autoimmune diseases, infectious diseases, transplant rejection, and malignancies. Associations between genetic variants in IL2RA (the gene encoding IL-2Rα) and autoimmune diseases have been described. However, correlating this directly to serum sIL-2R levels may be difficult given that sIL-2R is produced through membrane cleavage which, in turn,
depends on several different processes which may be influenced by disease activity.\textsuperscript{17-22} Currently, sIL-2R is generally regarded as a marker of T-lymphocyte activation; however, the cellular source of sIL-2R may not be restricted to T lymphocytes as other types of activated immune cells, including monocytes, dendritic cells, and B lymphocytes may release sIL-2R as well.\textsuperscript{6,16,23-27}

Despite the recognised association between immune activation and increased sIL-2R release under pathological conditions, the biological actions of sIL-2R are still far from understood. Several mechanisms of action, ranging from immune-inhibitory to immuno-stimulatory effects, have been proposed (figure 2). Soluble IL-2R binds IL-2 efficiently, and based on in vitro experiments, it has been proposed that sIL-2R may limit activation and proliferation of T lymphocytes by sequestration of available IL-2.\textsuperscript{1,27-30} However, conflicting data have been reported.\textsuperscript{8}

Alternatively, sIL-2R complexed with IL-2 prolongs IL-2 half-life which may enhance the immune-stimulatory properties of IL-2, even by activation of low-affinity dimeric IL-2R.\textsuperscript{31,32} It has been proposed that IL-2 can be presented to CD4+ T lymphocytes through sIL-2R, which then induces differentiation into Tregs (rather than differentiation into Th1-helper (Th1) or Th17 lymphocytes) that subsequently can suppress immune activity.\textsuperscript{33} On the other hand, there are reports to support observations that sIL-2R may promote autoimmune processes in association with enhanced Th17 generation, which involves sequestration of the IL-2 that normally inhibits early Th17 differentiation.\textsuperscript{21,34} Although the exact mechanism(s) of action of sIL-2R, as well as their in vivo occurrence and final biological effects, remains to be determined, the data available so far do support a role for sIL-2R in regulating IL-2-dependent cell function.

**SOLUBLE IL-2 RECEPTOR IN DISEASE AND CLINICAL PRACTICE**

Elevated blood sIL-2R levels have been reported in a variety of human diseases, including autoimmune and inflammatory diseases, solid cancers, haematological malignancies, and infections.\textsuperscript{13-16} This clearly indicates the disease aspecificity of elevated sIL-2R. Nevertheless, serum sIL-2R measurement has increased substantially in routine clinical practice over the last decade. Below we discuss laboratory tests for sIL-2R and several conditions where serum sIL-2R measurement can be of clinical use.

**Laboratory tests for soluble IL-2 receptor**

To date, no gold standard technique and standard reference sera are available for sIL-2R measurement. Current available laboratory tests for sIL-2R comprise enzyme linked immunosorbent assays (ELISA) from different suppliers, but an automated immune assay based on chemoluminiscence (CLIA) is also available. A comparison between an ELISA (Diaclone, Besancon Cedex, France) and the automated Immulite chemiluminescent method (Siemens Healthcare, Germany), both commonly used by clinical laboratories in the Netherlands, is shown in figure 3. Although both detection systems report different absolute values in different units (pg/ml vs U/ml) there is perfect correlation between both methods.
Haemophagocytic lymphohistiocytosis (HLH) is a complex inflammatory and often very serious disease. Two different HLH forms exist. Primary HLH, also referred to as genetic HLH, has a typical disease onset during infancy or early childhood in individuals with gene mutations that hamper the cytotoxic function of NK cells and T lymphocytes. Secondary HLH tends to occur in older patients in association with another condition, most commonly malignancy, infection, autoimmune disease, and without an identifiable genetic abnormality. Despite the genetic difference between primary HLH and secondary HLH, the clinical manifestations (e.g., fever, hepatosplenomegaly, generalised lymphadenopathy, pancytopenia), and most likely the pathophysiological mechanisms involved, are highly comparable. Although the exact mechanisms are not always clear, it is currently thought that all forms of HLH result from impaired cytotoxic T-lymphocyte and NK-cell functioning. This cellular dysfunction prevents efficient antigen removal with subsequent uncontrolled immune activation with a cytokine storm (including IL-6, IL-18, IFN-γ, TNF-α), uncontrolled macrophage activation, and haemophagocytosis.

Serum sIL-2R level is strongly elevated in both primary and secondary HLH and is considered to originate from excessively activated T cells. The updated criteria HLH-2004 from the Histiocyte Society included an elevated serum sIL-2R receptor (≥ 2400 U/ml, with a sensitivity of 93% and specificity of 100%) as an additional laboratory diagnostic criterium for paediatric HLH. However, relevant disease controls that most likely would have lowered specificity were lacking in the HLH-2004 study cohort. A slightly lower sensitivity (89%) for HLH was reported in another paediatric cohort when applying the HLH-2004 serum sIL-2R cut-off value. Currently, the HLH-2004 guidelines contain the standard diagnostic criteria for paediatric HLH. Although developed for paediatric HLH, the HLH-2004 criteria are widely applied to patients with secondary HLH, including adults, as well. There is data to support that the HLH-2004 criteria may be inadequate to accurately diagnose HLH in adults. Nevertheless, serum sIL-2R has also been found to display a good to excellent diagnostic performance in diagnosing HLH in adults, with optimal sensitivity (100%) and specificity (72-5%) at a cut-off value of ≥ 2515 U/ml. Although this cut-off value is slightly higher than the 2400 U/ml described in the HLH-2004 criteria, the data do demonstrate that a serum sIL-2R level ≤ 2400 U/ml can be helpful in ruling out HLH in adults with high sensitivity (100%). Furthermore, a serum sIL-2R level > 10000 U/ml was found helpful for ruling in HLH in adults with high specificity (93%), but with limited sensitivity (45%).

Currently, serum sIL-2R is considered a valuable tool in the diagnostic work-up of HLH, yet data on sensitivity and specificity are so far only available from a limited set of studies on paediatric and adult HLH. Establishing a cut-off value for most optimal diagnostic specificity requires further evaluation, especially when one considers the fact that elevated serum sIL-2R levels occur in many diseases, including different types of cancers, infectious conditions, and haematological disorders. This illustrates that age-related references values are preferable when considering usefulness of serum sIL-2R levels for clinical assessment.

From a practical point of view, it is important to note that sIL-2R measurements in serum and plasma yield comparable results and that sIL-2R levels remain stable at room temperature over a period of at least three days after sample collection (internal validation, Laboratory Medical Immunology, Department of Immunology, Erasmus MC, University Medical Center Rotterdam, the Netherlands). Also, up to three freeze-thaw cycles do not seem to affect sIL-2R concentration (personal experience).

In healthy individuals, serum sIL-2R levels vary with age, with children and elderly (≥ 65 years) having higher levels than (young) adults. This illustrates that age-related references values are preferable when considering usefulness of serum sIL-2R levels for clinical assessment.

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and autoimmune diseases that can overlap, mimic, or trigger HLH. \textsuperscript{45,47-49,54} There are some reports that suggest that serum sIL-2R might be of use to distinguish the aetiology underlying HLH. For instance, higher levels of serum sIL-2R have been described in malignancy-associated HLH compared to HLH associated with infection or (auto)immune disease.\textsuperscript{53,55-57} However, comparable sIL-2R serum levels between malignancy-associated HLH and EBV-associated HLH have been reported.\textsuperscript{37} Moreover, a higher sIL-2R to ferritin ratio was described in lymphoma-associated HLH as compared to infection-associated HLH and autoimmune disease-associated HLH, but conflicting data exist.\textsuperscript{35,37-39}

Likewise, it has been reported that HLH, in the context of (severe)combined immunodeficiency, presents with lower serum sIL-2R levels compared to primary HLH or infection-triggered secondary HLH in infants, which may clearly hamper diagnosing HLH in case of (severe) combined immunodeficiency. Yet, an elevated ratio of serum ferritin/sIL-2R was shown to distinguish HLH in patients with T-cell deficiencies from the other HLH types.\textsuperscript{38} Also, studies reported that primary HLH may present with higher serum sIL-2R levels compared to HLH of other aetiologies, and that the serum sIL-2R/ferritin ratio can distinguish primary HLH from other types of HLH, although data on this is not consistent.\textsuperscript{50,53-60}

Thus, although interesting, data to firmly support a role for serum sIL-2R in distinguishing between HLH types and aetiologies are limited and further studies on this are required.

In addition to its application in diagnosing HLH, serum sIL-2R measurement may also provide a tool to monitor disease activity as serum sIL-2R declines with clinical improvement.\textsuperscript{46,53,57-60} Alternatively, an increasing serum sIL-2R concentration has been associated with clinical deterioration.\textsuperscript{45,53-54} Moreover, higher initial sIL-2R serum levels (for instance ≥10 000 U/ml or ≥20 000 pg/ml) have been reported to be associated with decreased survival compared to HLH patients with lower serum sIL-2R, although data on this is inconclusive.\textsuperscript{53,57,59-60} Altogether these data indicate that serum sIL-2R represents a biomarker useful for at least HLH diagnosis as well as monitoring HLH disease activity and potentially prognosis.

**Sarcoidosis**

Sarcoidosis is a multisystem granulomatous disease of unknown aetiology presenting with a wide spectrum of clinical manifestations. The natural course of the disease is highly variable and the outcome difficult to predict. Symptom burden is high, and quality of life and social participation are negatively affected. In patients with pulmonary sarcoidosis, treatment is recommended in cases with significant symptoms and/or impaired or deteriorating lung function. The development and formation of noncaseating granulomas characterises the fundamental abnormality in sarcoidosis, with the lungs, lymph nodes, and skin being the most affected organs.\textsuperscript{55,58}

Although granulomas may often resolve spontaneously, pulmonary fibrosis occurs in 10%-15% of patients with sarcoidosis. Granuloma formation is thought to be initiated by CD4+ T lymphocytes that interact with antigen-presenting cells, that become activated and differentiate into Th1 lymphocytes. CD4+ Th1 lymphocytes secrete predominantly IL-2 and IFN-γ and stimulate macrophage TNF-α production, ultimately leading to the characteristic fierce influx of CD4+ T lymphocytes into the involved organs.

A significant correlation was observed between serum soluble IL-2R values and the influx of T lymphocytes into the lungs by Grutters et al., who reported the absolute CD4+ T-lymphocyte numbers in bronchoalveolar lavage in 47 newly diagnosed sarcoidosis patients.\textsuperscript{69} Moreover, higher sIL-2R values were observed in sarcoidosis patients with more advanced and progressive disease, which may predict need for therapy, and high sIL-2R at therapy initiation could serve as a predictor of relapse after infliximab therapy.\textsuperscript{70-76} Furthermore, sequential measurements of serum sIL-2R could be useful to assess the evolution of disease activity in sarcoidosis. Vorselaars et al. found that decline in serum sIL-2R after six months of treatment with methotrexate correlated with improvement of pulmonary parameters.\textsuperscript{77}

In patients with extrapulmonary sarcoidosis, serum sIL-2R was superior to the serum marker, angiotensin-converting enzyme (ACE) when used as screening tool for the detection of intra-ocular sarcoidosis in patients with uveitis.\textsuperscript{78,79} Moreover, Peterie et al. reported that sIL-2R measurements were superior to the serum marker, angiotensin-converting enzyme (ACE) when used as screening tool for the detection of intra-ocular sarcoidosis in patients with uveitis.\textsuperscript{78,79}

In contrast, in isolated cardiac sarcoidosis compared to non-isolated cardiac sarcoidosis, plasma sIL-2R levels were not elevated.\textsuperscript{81} The diagnostic value of sIL-2R for sarcoidosis was confirmed in a recent retrospective cohort study. In total, 189 patients suspected for sarcoidosis were analysed. The sensitivity and specificity of serum sIL-2R for detection of sarcoidosis was 88% and 85%, respectively, superior to ACE (62% and 88%).\textsuperscript{81} In 2015, the diagnostic criteria for sarcoidosis were updated in Japan by the Japanese Society of Sarcoidosis and Other Granulomatous Disorders (JSSOG), with elevated serum sIL-2R replacing negative tuberculin reaction.\textsuperscript{73} Although considered useful, serum sIL-2R measurement was not included in the recently revised International Workshop on Ocular Sarcoidosis (IWOS) criteria for diagnosing ocular sarcoidosis. The main reason being that it was considered that serum sIL-2R measurement is not (yet) used widely enough in uveitis clinics.\textsuperscript{81} Nevertheless, the data available
so far do show that serum sIL-2R measurement represents a valuable biomarker in the diagnosis of sarcoidosis as well as for assessment of disease activity and for monitoring treatment efficacy.

**Autoimmune disease and other immune-mediated diseases**

Increased levels of serum sIL-2R have been described in a variety of autoimmune/immune-mediated diseases as well as other disease conditions associated with immune dysregulation (table 1). Of these, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), juvenile idiopathic arthritis (JIA), adult-onset Still’s disease (AOSD), ANCA-associated vasculitis, and IgG4-related disease (IgG4-RD) will be discussed in more detail below.

**Rheumatoid arthritis (RA).** RA is a chronic inflammatory joint disease that eventually results in destruction of cartilage and bone, severe disability and premature mortality. RA is considered an autoimmune disease associated with the production of autoantibodies, such as anti-cyclic citrullinated peptide (ACPA) and rheumatoid factor (RF).

Classification as definite RA, using the ACR/EULAR classification criteria, is based upon the presence of synovitis in at least one joint and a total score of at least six points (of a possible 10) achieved in four domains. These domains are number and site of involved joints, serological abnormality (ACPA or RF), elevated acute phase response, and at least six weeks of symptom duration. Earlier reports in RA patients revealed conflicting results concerning the correlation between sIL-2R levels and disease activity scores or correlation with other laboratory markers of inflammation. More recently, Kuuliala et al. reported that low sIL-2R levels may be predictive of a rapid response to treatment with infliximab in patients with RA. However, in their cohort, the marker did not identify the patients in remission after 22 weeks. In addition, Steenbergen et al. reported that lower sIL-2R levels were associated with more disease-modifying antirheumatic drug-free sustained remission in RA.

**Systemic lupus erythematosus (SLE).** SLE is a chronic, severely debilitating systemic autoimmune disease characterised by the production of autoantibodies and multi-organ inflammation. SLE is a multifactorial disease that results from complex interactions between susceptibility genes, epigenetic, environmental, hormonal, and immuno-regulatory factors and can present with a wide spectrum of clinical manifestations with unpredictable...
Recently, Zhang et al. reported that serum sIL-2R may be a useful biomarker for the assessment of disease activity and therapeutic response in AOSD. The utility of serum sIL-2R for the diagnostic work-up of AOSD was confirmed by a larger group of patients. The pathophysiology of AOSD is complex and multimodal, involving the activation of immune cells and the release of pro-inflammatory cytokines such as interleukin-6, IL-1, and IL-18, as well as associated with secondary HLH and cytotoxic functions. Elevated blood levels of sIL-2R were also observed in patients with lupus nephritis and Behçet disease, suggesting a potential role of sIL-2R in the pathogenesis of these conditions.

Juvenile idiopathic arthritis (JIA). JIA is a heterogeneous group of diseases characterised by arthritis of unknown origin with onset before age of 16 years. The current International League of Associations for Rheumatology (ILAR) classification criteria for JIA were developed by consensus and not formally validated. The classification criteria recognise six mutually exclusive categories defined in clinical and laboratory measures: systemic arthritis, oligoarthritis (persistent or extended), polyarthritis rheumatoid factor (RF)-positive, polyarthritis RF-negative, enthesitis-related arthritis, psoriatic arthritis, and a seventh category, undifferentiated arthritis. Recently, an initiative was started to provide new evidence-based classification of JIA using a formal process to validate preliminary criteria. Elevated sIL-2R levels were found in patients with clinically active JIA compared to controls, and sIL-2R levels correlated significantly with pannus thickness and joint count. Furthermore, in addition to other disease characteristics, treatment-refractory disease course may be associated with a higher sIL-2R level. Finally, macrophage activation syndrome (MAS) has been increasingly recognised in association with rheumatic diseases, most commonly in systemic JIA. The clinical features of MAS in JIA are similar to high, non-remitting fever, generalised lymphadenopathy, hepatosplenomegaly, central nervous system dysfunction, and haemorrhagic manifestations and can result in multi-organ failure. Recent reports suggest that (subclinical) MAS in systemic JIA may even occur in 30-40% of patients. The utility of serum sIL-2R for JIA will probably vary per clinical phenotype, but serum sIL-2R may be a promising marker for disease activity in systemic JIA, especially when associated with MAS.

Adult-onset Still’s disease (AOSD). AOSD is a rare systemic inflammatory disorder characterised by high fever that typically spikes once or twice daily, a transient salmon pink skin rash (mostly on the proximal limbs and trunk) that occurs along with the fever spikes, arthritis and arthralgia involving predominantly the wrists, knees and ankles, and frequently a sore throat; as well as less frequent symptoms including myalgias, lymphadenopathies, splenomegaly, hepatomegaly, pleurisy, pericarditis, weight loss, and abdominal pain. The pathophysiology of AOSD is mainly unknown, but involvement of a pro-inflammatory cascade that can be triggered by infectious agents, solid cancers, or lymphomas in genetically predisposed individuals has been proposed. AOSD shares several phenotypic characteristics with systemic JIA, including daily recurring fever, salmon-coloured skin rash and polyarthritis, and is considered to represent the adult counterpart of systemic JIA or even a continuum of a single disease entity.

No definitive diagnostic tool for AOSD exists and its diagnosis is based on extensively excluding diseases with comparable clinical presentation (including, for example, viral and bacterial infections, SLE, RA, myositis, systemic vasculitis, autoinflammatory diseases, sarcoidosis, malignancies), for which several diagnostic criteria sets have been developed. Laboratory findings reflect the (non-specific) systemic inflammatory nature of the disease and increased erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels, neutrophilic leucocytosis (> 80% polymorphonuclear leucocytes), anaemia and thrombocytosis are common findings. Also, serum ferritin is highly elevated in AOSD, while glycosylated serum ferritin is typically low.

Neutrophil and macrophage activation are at the centre of the immunopathogenesis of AOSD and macrophage-derived pro-inflammatory cytokines such as the interleukin-1 family members IL-1β and IL-1α, as well as IL-6, are centrally involved. AOSD is also strongly associated with secondary HLH and cytotoxic functions.
In addition, circulating Th1 and Th17 lymphocytes correlated significantly with clinical activity, and serum IL-18 and ferritin levels in active untreated AOSD, and all these laboratory parameters declined with clinical remission upon treatment. Only limited data on serum sIL-2R in AOSD is available, yet increased levels are detected in active AOSD, thus further supporting T-lymphocyte involvement in this disease. Although AOSD may not display differences in serum sIL-2R levels compared to other diseases, serum sIL-2R level may represent a potential biomarker for monitoring AOSD disease activity and treatment response. Serum sIL-2R levels have been reported to correlate with AOSD disease activity. Moreover, Fuji et al. reported that serum sIL-2R levels were higher in the subgroup of AOSD patients with chronic articular disease, suggesting that serum sIL-2R levels may distinguish between different AOSD disease patterns. Also, several studies demonstrated that upon treatment, disease remission was associated with a strong decline in serum sIL-2R levels, along with decreases in other laboratory parameters such as ESR, CRP, ferritin, and IL-18 levels. In contrast, a rise in serum sIL-2R level can occur in case of disease recurrence. Altogether, the data available so far suggest that serum sIL-2R can be considered as an additional biomarker to monitor AOSD disease activity and therapeutic response.

ANCA-associated vasculitis (AAV). AAV is a necrotising vasculitis that predominantly affects small vessels and is associated with antineutrophil cytoplasmic antibodies (ANCA) specific for myeloperoxidase (MPO-ANCA) or proteinase 3 (PR3-ANCA). The major clinicopathological variants of AAV are microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA), and eosinophilic granulomatosis with polyangiitis (EGPA). Besides necrotising vasculitis, GPA and EGPA show necrotising granulomatous inflammation often involving the respiratory tract. Moreover, EGPA is associated with asthma and eosinophilia. Clinical manifestations that suggest the diagnosis of AAV, when there is no other obvious cause, and indicate ANCA testing include: (rapid progressive) glomerulonephritis, pulmonary haemorrhage, cutaneous vasculitis with systemic features, multiple lung nodules, chronic destructive disease of the upper airways, long-standing sinusitis or otitis, subglottic tracheal stenosis, peripheral neuropathy, retro-orbital mass, and scleritis. Elevated levels of sIL-2R have been detected in the sera of patients with GPA and MPA. Moreover, sIL-2R levels correlated with disease activity at diagnosis and differed between limited and generalised and between active and inactive disease. In addition, positive ANCA serology during follow up was associated with sIL-2R levels. At 18 and at 24 months after diagnosis, higher levels of sIL-2R were found in ANCA-positive patients compared to patients negative for ANCA. Analogous to sIL-2R as marker for T-cell activation, soluble CD163 (sCD163) is considered a systemic marker of macrophage activation. CD163 is abundantly expressed by tissue macrophages and is shed from the macrophage surface under inflammatory conditions by ADAM17, the enzyme that also releases TNF into the circulation. It has been reported that urinary sCD163 levels may reflect active glomerular inflammation. Taken together, these data show that sIL-2R may be a valuable biomarker for assessment of disease activity and for monitoring treatment, and seems to reflect the central role of the T-lymphocyte-driven immune response in ANCA-associated vasculitis.

IgG4-related disease (IgG4-RD). IgG4-RD is a fibroinflammatory disease that can involve various organs, including the lungs, thyroid, lymph nodes, orbital tissue, kidneys, salivary and lacrimal glands, aorta, pancreas, and skin. IgG4-RD is characterised by accumulation of IgG4-producing plasma cells at affected sites, along with the formation of storiform fibrotic lesions. Serum IgG4 levels are elevated in the majority of patients with IgG4-RD, yet IgG4 elevation is not fully sensitive or specific for diagnosing IgG4-RD. Moreover, serum IgG4 levels may not always accurately reflect disease activity. Therefore, additional blood biomarkers for improved diagnosis and evaluation of IgG4-RD disease activity are still needed.

To date, only a limited number of studies have explored serum sIL-2R in relation to IgG4-RD. These studies report elevated serum sIL-2R levels in IgG4-RD, a positive correlation between serum sIL-2R levels with the number of affected organs as well as disease activity. Moreover, these studies reported a decline of serum sIL-2R levels after treatment. In addition, serum sIL-2R levels have been reported to display high accuracy in predicting an individual glucocorticoid requirement. These data indicate that serum sIL-2R level may be a valuable biomarker for evaluating disease activity and treatment response in IgG4-RD. However, additional
(prospective) studies are needed, especially with regard to treatment stratification, monitoring treatment response, as well as sensitivity and specificity of sIL-2R in the context of IgG4-RD diagnosis.

Cancer and treatment

Increased serum sIL-2R levels have been found in a variety of malignancies, mostly haematopoietic malignancies but also solid cancers. These elevated sIL-2R levels most likely derive from the malignant cells, although the host cellular immune response likely contributes to the generation of sIL-2R as well. Immune checkpoint inhibitor treatment is a rapidly expanding field within oncology. High serum sIL-2R in metastatic melanoma patients before initiation of ipilimumab (anti-CTLA-4) treatment has been associated with treatment resistance, most likely by IL-2 sequestration. Despite the anti-tumour efficacy of immune checkpoint inhibitor treatment, secondary development or worsening of autoimmune/inflammatory disorders is commonly observed in cancer patients upon such treatment. Measurement of serum sIL-2R prior and during therapy, potentially along with other cytokines such as IFN-γ, IL-17, and IL-10, may, in the future, prove a valuable tool for monitoring excessive/uncontrolled immune activation and to predict the development of immune-related adverse events in cancer patients treated with immune checkpoint inhibitors.

CONCLUDING REMARKS

The clinical utility of sIL-2R, as reviewed above, lies particularly in evaluating disease activity in a variety of immune-mediated diseases and may add in the diagnostic work-up of especially HLH and sarcoidosis. As sIL-2R level reflects activation status of the T-lymphocyte compartment, its disease specific value is limited. However, in immune-mediated diseases where T-lymphocyte responses play a central role in the pathophysiology, sIL-2R might be a valuable biomarker for predicting or monitoring the efficacy of immune suppressive therapies.

DISCLOSURE

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New diagnostic and treatment strategies in renal artery stenosis: a promising pursuit or disappointment foretold?

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ABSTRACT

Clinical management of renal artery stenosis has seen a major shift, after randomised clinical trials have shown no group benefit of endovascular intervention relative to optimal medical control. However, the inclusion criteria of these trials have been criticised for focusing on a subset of patients with atherosclerotic renal artery stenosis where intervention was unlikely to be beneficial. Moreover, new imaging and computational techniques have become available, which have the potential to improve identification of patients that will respond to interventional treatment. This review addresses the challenges associated with clinical decision making in patients with renal artery stenosis. Opportunities for novel diagnostic techniques to improve patient selection are discussed, along with ongoing Dutch studies and network initiatives that investigate these strategies.

KEYWORDS

Computational fluid dynamics, fibromuscular dysplasia, functional measurements, renal artery stenosis, renovascular disease, secondary hypertension

INTRODUCTION

Renal artery stenosis is, in most cases, a result of atherosclerosis. In ~ 10\% it is caused by fibromuscular dysplasia (FMD), a connective tissue disorder of unknown origin with a predilection for the renal arteries. Renal artery stenosis is a well-established cause of secondary hypertension, but the success of percutaneous interventions on blood pressure, although generally better in patients with FMD, is variable. Second, atherosclerotic renal artery stenosis (ARAS) is associated with an increased risk of renal failure and mortality. In the Netherlands, renovascular disease was the primary cause of renal replacement therapy in 11\% of patients in 2017. Treatment of renal artery stenosis, however, has declined sharply in the past few years following the publication of randomised trials that showed that angioplasty with stent placement of the renal arteries over and above medical treatment was not better compared to conventional (medical) treatment alone. This strong decline in percutaneous interventions is somewhat surprising as the trials explored indications where the benefit of intervention was uncertain and excluded patients with uncontrolled or treatment resistant hypertension, recurrent flash pulmonary oedema, or
refractory heart failure. Although past trials showing advantages of stent placement in patients with resistant hypertension and acute pulmonary oedema had limitations in design and sample size, recent (non-randomised) experiences continue to suggest that stent placement is beneficial if existing selection criteria are applied. While in ARAS blood pressure can significantly improve after stent placement, percutaneous renal intervention in patients with FMD has been reported to completely cure hypertension in 36% of cases. In older patients, a conventional approach for FMD can also be considered, as the chance of curing hypertension after an intervention diminishes significantly with age. In other words, for both ARAS and FMD, better diagnostic strategies are necessary to identify patients who are most likely to benefit from revascularisation. In the present overview, we provide a summary of ongoing initiatives to improve the diagnosis and treatment of patients with renal artery stenosis.

**New diagnostic possibilities for renal artery stenosis**

Animal experiments have shown that acute renal artery stenosis must be above ~60% diameter loss to alter the pressure gradient and above 75% to cause a reduction in renal blood flow, although these cut-off values are known to depend on stenosis eccentricity. Clinical evidence regarding the anatomical degree above which percutaneous interventions would be useful is limited. This may either result from difficulties related to the accuracy of anatomical grading, the paucity of data in patients with severe anatomical lesions (often excluded from randomised trials), and residual kidney function. Efforts to improve patient selection by ultrasound, renal scintigraphy, and renal vein sampling have, in general, been unsuccessful, and combining anatomical grading with the measurement of translesional pressure gradients have, so far, yielded mixed results. New developments that combine pressure and flow measurements, on the other hand, have yielded promising results in patients with coronary artery disease and may be useful in patients with renal artery stenosis. In parallel to these invasive measurements, advances in imaging techniques and computer modelling have generated the possibility to determine the functional consequences of renal artery stenosis using computational fluid dynamics (CFD).

**Fractional flow reserve and renal flow reserve**

Studies performed in the coronary circulation have shown that anatomical abnormalities have a limited association with functional significance. Assessment of functional characteristics by pressure gradient measurements using sensor-equipped wires under hyperaemic conditions is recommended in current guidelines as it is superior over angiography-guided revascularisation, and has significantly improved clinical outcomes in patients with coronary artery disease. In addition to pressure measurements, direct measurement of coronary flow velocity under baseline and hyperaemia can assess microcirculatory status, which has prognostic value in patients with and without significant coronary artery stenosis.

**Figure 1.** Example of haemodynamic measurements in a patient with atherosclerotic renal artery stenosis. The left panel depicts baseline measurements; the right panel shows an increase of the pressure gradient and flow velocity after induction of hyperaemia with intra-renal dopamine.
Using similar techniques, it is possible to reproducibly measure pressure and flow velocity under baseline and hyperaemic conditions in the renal artery. In the renal circulation, maximal hyperaemia can be achieved by an intrarenal bolus of dopamine 30 μg/kg. As depicted in figure 1, the renal fractional flow reserve is derived from the pressure measurements, which quantify the haemodynamic significance of the stenosis. From the flow measurements, the renal flow reserve is determined, which expresses to which extent the microcirculation can increase flow relative to resting conditions. As the stenosis gradient is dependent on the achieved hyperaemia and the maximal flow rate is related to the pressure loss over the stenosis, these measurements are interdependent. This suggests that, similar to the coronary circulation, the combined pressure-flow measurements can be used to distinguish primary macrovascular from predominant microvascular disease, which may aid in patient selection for renal revascularisation therapy (figure 2).

Earlier studies have shown promising results using only hyperaemic pressure measurements, but it has been difficult to validate specific cut-offs. In the recently started Functional Renal Haemodynamics in Patients with Renal Artery Stenosis study (HERA-3, Netherlands Trial Registry NL8408), the predictive ability of combined pressure and flow measurements for treatment success will be investigated using ambulatory blood pressure and iothalamate/hippuran renal function measurements as outcome. HERA-3 will also investigate the validity of non-invasive flow simulation techniques to estimate the translesional pressure gradient.

Computational fluid dynamics

Computational fluid dynamics (CFD) uses numerical analysis and data structures to analyse and solve problems that involve fluid flows. With CFD techniques, the translesional pressure gradient for stenotic lesions and other hemodynamic metrics can be calculated based mainly on an anatomic model of a patient’s arteries. In this three-dimensional anatomical model, CFD solves the physical equations that govern the motion of blood. The computed solution includes details on the acceleration of blood through the stenosis, as well as the disturbed motion of blood downstream of the stenosis and the pressure loss associated with these disturbances. Over the past decade, CFD has seen an increasing adoption for biomedical research. More recently, CFD software has been commercially developed and validated for an improved clinical classification of coronary artery disease. This has been made possible by advances in medical imaging, segmentation techniques, and computational power, all of which are likely to experience further improvements in the coming decade.

A CFD simulation of renal artery stenosis can non-invasively derive the translesional pressure gradient under various levels of renal blood flow. For this CFD simulation, two primary inputs from patient data are needed to accurately inform the model. The first is a three-dimensional geometry of the stenosis, including the juxtarenal aorta. This geometry can be segmented from high-resolution contrast-enhanced CT or MRI scans, preferably with a slice thickness below 1 mm. Second, the renal blood flow rate through the stenosis must be directly or indirectly specified for the simulation. A strength of the CFD model is that both a resting flow rate, as well as a high flow rate mimicking hyperaemia can be simulated. The difference in translesional pressure gradient of a haemodynamically significant stenosis relative to a non-significant stenosis will be amplified at higher flow rates, as the gradient scales quadratically with flow. Therefore, the translesional pressure gradient during maximal flow appears to be a better predictor of treatment success than measurements in the resting state.
For the HERA-3 study, a CFD model will be simulated for all individual patients, and the modelled resting and hyperaemic pressure drop will be compared to the invasive measurements. An example of a CFD simulation of a patient with a moderate RAS is shown in figure 3, which was performed with the open-source SimVascular software (release 23-05-2018, http://simvascular.github.io/). The CFD simulation for this patient predicted a hyperaemic pressure drop of 34 mmHg, compared to an invasively measured gradient of 38 mmHg (with an aortic pressure of 160 mmHg, this corresponds to a renal fractional flow reserve of 0.45 and 0.43 for the CFD simulation and measurement, respectively). After per-patient measurement and simulation, the accuracy of the CFD simulation will be assessed and its predictive value for blood pressure response to treatment will be investigated. If sufficiently predictive, the non-invasive CFD simulations can become a valuable tool in the diagnostic workup in patients with ARAS or FMD, although the anatomical characteristics of FMD may prove more challenging for the CFD technique.

Imaging in FMD
The diagnosis of FMD is based on imaging studies with typical non-atherosclerotic vascular lesions (by definition in the absence of syndromal or inflammatory diseases). FMD is classified into two subtypes by its angiographic presentation: multifocal FMD with a typical string-of-beads appearance and unifocal FMD with one or more focal or tubular stenoses. Distinguishing unifocal FMD from ARAS is often difficult, and therefore, the diagnosis is limited to younger patients (< 40 years) without risk factors for atherosclerosis and without arterial calcifications on imaging studies. In general, patients with unifocal FMD are younger and have more severe hypertension. Moreover, the effect of unifocal FMD on functional parameters of the kidney resembles that of ARAS, with reduced kidney perfusion and an increase in renin secretion. In multifocal FMD, however, kidney perfusion and microvascular function are more or less intact, and renin secretion is comparable to patients with essential hypertension. Therefore, these two FMD subtypes are generally considered to be different disease entities.

The gold standard for the diagnosis of FMD is digital subtraction angiography (DSA). Both renal arteries should be selectively catheterised, as FMD lesions are predominantly located in the middle or distal renal artery which can be overlooked with aortic angiography alone. To obtain a more detailed view, optical coherence tomography or intravascular ultrasound can be used. Over the past years, the resolution of computed tomography angiography (CTA) has improved considerably, which has increased the use of CTA as an alternative to DSA. As CTA is non-invasive, cheaper, and allows imaging of several vascular beds in one scan; it is currently recommended as the first diagnostic step in case of clinical suspicion for FMD. CTA is preferred over magnetic resonance angiography (MRA) as its spatial resolution is higher. However, as the spatial resolution of CTA is inferior to DSA, smaller lesions could still be missed on CTA. This is illustrated by two prospective studies in whom all patients underwent DSA, regardless of the results of previous MRA or CTA. Although these studies used older MR and CT scanners (slice thickness

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**Figure 3.** Example of a computational fluid dynamics simulation in a patient with atherosclerotic renal artery stenosis. The simulation solves the governing equations for fluid motion in the patient-specific geometry, providing the development of pressure and flow velocities during the heart cycle. The left graph shows the pressure field during peak systole, from which the translesional pressure gradient can be derived. The right graph shows instantaneous streamlines of the flow through the stenosis.
of 2.5-3 mm) than the ones currently used, sensitivity for FMD was only 28% for CTA and 22% for MRA (as compared to DSA) in one study. Presumably, sensitivity has been improved with the newer generations of CT and MR scanners (slice thickness ~0.6 mm), but its value has not been evaluated yet. Moreover, we are aware of several cases with (false) negative CTA or MRA imaging, in whom FMD lesions were found with DSA. Therefore, in case of high clinical suspicion of FMD, DSA should be considered even if CTA or MRA imaging are negative.

**Treatment of FMD**

In patients with hypertension due to renal FMD, treatment with balloon angioplasty appears to be safe and effective in lowering blood pressure. Balloon angioplasty can cure hypertension (BP < 140/90 without antihypertensive medication) in ~36% of patients with FMD, and in those patients not fully cured, improvement in renal function, blood pressure, and reduced use of antihypertensive drugs has been reported. However, these data were derived from observational studies, as randomised controlled trials on revascularisation in FMD are lacking. Nevertheless, balloon angioplasty is more effective in lowering blood pressure in patients with FMD than in ARAS. Presumably, this difference in response to revascularisation is caused by atherosclerotic damage to the intrarenal microvasculature in kidneys with ARAS, while microvascular function is more or less intact in kidneys with FMD. Surgical revascularisation in FMD is usually reserved for patients with complex lesions or after failure of balloon angioplasty, as balloon angioplasty is less invasive with a lower risk for major complications (15% with surgery versus 6% with balloon angioplasty). Stent placement is discouraged in FMD as several cases of stent fractures have been reported. Presumably, this is caused by the fact that multifocal FMD lesions are typically located in the middle or distal two-thirds of the renal artery (in contrast to ARAS or unifocal FMD), where the amplitude of movement due to respiration or exercise is higher. However, not placing a stent increases the risk of restenosis, which occurs in 10-38% of patients (depending upon duration of follow up). Hence, for patients whose blood pressure rises over time, a second balloon angioplasty should be considered. Because observational studies suggest that the efficacy of balloon angioplasty decreases with age, duration of hypertension, and the presence of kidney damage, balloon angioplasty should particularly be considered in young patients or in patients with recent onset hypertension. In elderly patients without severe hypertension or hypertension that responds well to antihypertensive drugs, conservative management is often preferred and reasonably effective. As not all patients with renal artery FMD develop hypertension, it is conceivable that the FMD lesions are an innocent bystander.
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DISCLOSURE

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Combined antihypertensive treatment is better than mono-therapy in hypertensive patients

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ABSTRACT

Background: Hypertension (HT) is a chronic condition associated with serious complications. In the present cross-sectional study, we aimed to analyse factors that contribute to blood pressure control in subjects with HT.

Methods: Subjects with HT admitted to outpatient internal medicine clinics of the institution were enrolled in the study. According to the Joint National Committee (JNC) VIII criteria, subjects with a mean blood pressure above target levels were defined as poorly-controlled hypertensive patients and others were grouped as well-controlled hypertensive patients. Clinical and laboratory parameters were compared between study groups.

Results: Smokers were more prevalent in the poorly-controlled HT group compared to the well-controlled HT group (p = 0.001). The number of patients who adhered to dietary and exercise recommendations were greater in well-controlled HT group than poorly-controlled HT group (p < 0.001 for both). The rate of combined therapy was greater in well-controlled HT group compared to poorly-controlled HT group (p = 0.04).

Conclusions: We suggest that, in addition to dietary and exercise recommendations and smoking cessation, treatment with combination therapy could be better in reaching blood pressure targets in patients with HT.

KEYWORDS

Blood pressure, diet, exercise, hypertension, smoking, treatment

INTRODUCTION

Hypertension (HT) is one of the most common chronic diseases in the world with a great burden on communities. It has significant complications which could result in advanced morbidity or mortality. Hypertension is among risk factors of coronary artery disease, stroke, heart failure, chronic kidney disease, peripheral vascular disease, and vascular dementia.1,2 Thus, achieving regulation of blood pressure is crucial in hypertensive subjects.

There are numerous factors associated with better hypertension control in patients with HT. In addition to the reduction of waist circumference and body mass index, education of patients in acknowledging their blood pressure, compliance to the treatment, adherence to the schedule of exercise, diet modification, and reducing the number of pills for hypertension treatment are among factors that modify the regulation of blood pressure.3-5 In present prospective cross-sectional study, we aimed to observe the anthropometric measures, dietary and exercise compliance, treatment options and compliance, and awareness of the disease of the subjects with hypertension.

METHODS

The study was conducted in the Internal Medicine Department of Abant Izzet Baysal University Hospital between January 2019 and December 2019. The study protocol was approved by the local ethics committee (approval number: 2018/288). Subjects with hypertension (HT) who were admitted to outpatient internal medicine clinics of the institution were enrolled in the study. According to the Joint National Committee (JNC) VIII criteria,6 subjects with a mean blood pressure above target levels were defined as poorly-controlled hypertensive patients and others were grouped as well-controlled hypertensive patients. Blood pressure measurements were obtained in two consecutive clinic visits and the mean of these measurements were used in defining poorly- and well-controlled hypertension groups. Exclusion criteria were as follows: recently diagnosed hypertension (less than one year), modification of anti-hypertensive treatment...
within three months, lack of two consecutive visits for blood pressure measurements.

Age, gender, body weight, height, waist circumference, duration of HT, accompanying morbidities, diet and exercise status, smoking habit, awareness of blood pressure, anti-hypertensive medications (mono-therapy or combination), number of pills for hypertension treatment, additional medications for other conditions, systolic and diastolic blood pressures, laboratory parameters including levels of serum creatinine, blood urea, and triglycerides, as well as LDL-cholesterol and HDL-cholesterol levels were noted. Body mass index (BMI) was calculated by division of weight by the square of height. All study parameters were compared between poorly- and well-controlled hypertensive subjects.

Statistical analyses were held with SPSS software (SPSS 15.0 for Windows, IBM Co, Chicago, IL, USA). The Kolmogorov-Smirnov test was used for whether study

Table 1. General characteristics and laboratory data of the study population

<table>
<thead>
<tr>
<th></th>
<th>Well-controlled HT group</th>
<th>Poorly-controlled HT group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male (n,%): 20 (31%)</td>
<td>8 (22%)</td>
<td>0.32</td>
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<tr>
<td></td>
<td>Female (n,%): 45 (69%)</td>
<td>29 (78%)</td>
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<td>Comorbidity</td>
<td>Present (n,%): 42 (65%)</td>
<td>25 (68%)</td>
<td>0.76</td>
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<td></td>
<td>Absent (n,%): 23 (35%)</td>
<td>12 (32%)</td>
<td></td>
</tr>
<tr>
<td>Smoking habit</td>
<td>Yes (n,%): 4 (6.2%)</td>
<td>11 (29.7%)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>No (n,%): 61 (93.8%)</td>
<td>26 (70.3%)</td>
<td></td>
</tr>
<tr>
<td>Diet compliance</td>
<td>Yes (n,%): 56 (86%)</td>
<td>18 (49%)</td>
<td>&lt; 0.001</td>
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<tr>
<td></td>
<td>No (n,%): 9 (14%)</td>
<td>19 (51%)</td>
<td></td>
</tr>
<tr>
<td>Exercise compliance</td>
<td>Yes (n,%): 44 (68%)</td>
<td>11 (30%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>No (n,%): 21 (32%)</td>
<td>26 (70%)</td>
<td></td>
</tr>
<tr>
<td>Medical treatment</td>
<td>Combined therapy (n,%): 51 (78.5%)</td>
<td>22 (59.5%)</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Monotherapy (n,%): 14 (21.5%)</td>
<td>15 (40.5%)</td>
<td></td>
</tr>
<tr>
<td>Combination therapy</td>
<td>One pill (n,%): 29 (57%)</td>
<td>13 (59%)</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Multiple pills (n,%): 22 (43%)</td>
<td>9 (41%)</td>
<td></td>
</tr>
<tr>
<td><strong>Mean ± SD</strong></td>
<td><strong>62.6 ± 9.5</strong></td>
<td><strong>60.7 ± 12.7</strong></td>
<td>0.41</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td><strong>32.3 ± 6</strong></td>
<td><strong>32.4 ± 5.6</strong></td>
<td>0.90</td>
</tr>
<tr>
<td><strong>LDL cholesterol (mg/dl)</strong></td>
<td><strong>119 ± 31</strong></td>
<td><strong>126 ± 38</strong></td>
<td>0.31</td>
</tr>
<tr>
<td><strong>Median (min-max)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HT duration (years)</td>
<td>9 (1-40)</td>
<td>9 (1-30)</td>
<td>0.65</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82 (53-129)</td>
<td>81 (41-116)</td>
<td>0.92</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>105 (86-135)</td>
<td>109 (80-133)</td>
<td>0.11</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>130 (100-148)</td>
<td>150 (140-180)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>80 (60-89)</td>
<td>90 (70-120)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Creatinine (md/dl)</td>
<td>0.8 (0.57-1.18)</td>
<td>0.8 (0.64-1.12)</td>
<td>0.24</td>
</tr>
<tr>
<td>Blood urea (mg/dl)</td>
<td>32 (17-62)</td>
<td>30 (17-73)</td>
<td>0.83</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>142 (59-744)</td>
<td>168 (58-1420)</td>
<td>0.74</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>119 (48-193)</td>
<td>125 (44-250)</td>
<td>0.31</td>
</tr>
</tbody>
</table>

BMI = body mass index; HT = hypertension; LDL = low-density lipoproteins; n = number; SD = standard deviation
parameters have normal distribution within study groups. Variables with normal distribution were compared with an independent samples t-test and expressed as mean ± standard deviation. Variables without normal distribution were compared with Mann-Whitney U test and expressed as median (min-max). Comparison of categorical variables was conducted with chi-square test. Statistical significance was defined as a p-value lower than 0.05.

RESULTS

One hundred and forty-five subjects enrolled to the study; 43 patients were excluded according to the exclusion criteria. The study population was consisted of the remaining 102 hypertensive patients: 65 in well-controlled HT and 37 in poorly-controlled HT groups. Mean ages of the well- and poorly-controlled hypertensive subjects were 62.6 ± 9.5 years and 60.7 ± 12.7 years, respectively. The age of the patients in the well- and poorly-controlled HT groups were not statistically different (p = 0.41).

In well-controlled HT group, 45 (69%) patients were women and 20 (31%) patients were men, while 29 (78%) were women and 8 (22%) were men in the poorly-controlled HT group. Gender was not statistically different between study groups (p = 0.32). Forty-two (65%) of 65 patients in the well-controlled HT group and 25 (68%) of 37 patients in poorly-controlled HT group had comorbid conditions (p = 0.76).

Duration of HT (p = 0.65), weight (p = 0.92), BMI (p = 0.90), waist circumference (p = 0.11), serum creatinine (p = 0.44), blood urea (p = 0.83), triglyceride levels (p = 0.74), LDL cholesterol levels (p = 0.31), and HDL cholesterol levels (p = 0.68) of well- and poorly-controlled HT groups were not significantly different. Table 1 shows the general characteristics and laboratory data of the study population. The differences of systolic and diastolic blood pressures between study groups were statistically significant (p < 0.001 for both systolic and diastolic blood pressures).

Only 4 (6.2%) of 65 patients in the well-controlled HT group were smokers while 11 (29.7%) of 37 patients in the poorly-controlled HT group smoked cigarettes. The difference between groups was statistically significant (p = 0.001). Fifty-six (86%) of 65 patients in the well-controlled HT group and 18 (49%) of 37 patients in the poorly-controlled HT group followed the dietary advice for treatment of HT (p < 0.001); 44 (68%) of the patients in the well-controlled HT group and 11 (30%) of those in the poorly-controlled HT group were on regular physical exercise (p < 0.001).

Fourteen (21.5%) of 65 well-controlled patients received only one anti-hypertensive drug as monotherapy and 51 (78.5%) of 65 received combined antihypertensive agents; while 15 (40.5%) of 37 patients in the poorly-controlled HT group received only one antihypertensive drug as monotherapy and 22 (59.5%) received combined antihypertensive treatment. Combined treatment was significantly more common in the well-controlled HT group compared to the poorly-controlled HT group (p = 0.04). The association between treatment and blood pressure regulation is shown in figure 1.

Of 51 subjects who received combined treatment in the well-controlled HT group, 29 (57%) received a combined treatment in one pill and 22 (43%) received treatment in multiple pills; 13 (59%) of 22 patients in the poorly-controlled HT group received combined treatment in one pill and 9 (41%) received treatment in multiple pills. There was no significant difference between well- and poorly-controlled HT groups according to the number of pills of combined treatment (p = 0.12).

DISCUSSION

The main findings of the present study were: (i) non-compliance with dietary and physical exercise program, (ii) smoking cigarettes, and (iii) treatment with monotherapy antihypertensive regimen instead of combined treatment was associated with poor control of hypertension. Interestingly, combined therapy with one pill was not superior to combined therapy with more than one pill in achieving good control of hypertension.
The association between diet and hypertension has been studied for a long time. While a Mediterranean diet, which is rich in vegetables and fruits, relates to a reduced blood pressure, excessive salt in the diet was introduced as a cause of increased blood pressure.7 Recent studies showed that a Mediterranean diet and a healthy Nordic diet were associated with a reduced risk of stroke, a serious complication of HT.8,9 Similarly, the stop hypertension (DASH) diet, which recommends consumption of fruits, vegetables, whole grains, dairy products with low fat, and nuts, along with low intake of sodium, sweetened beverages, and red meat (especially processed red meat), has been shown to be associated with a reduction in blood pressure and a decrease in the incidence of HT.10,11 Moreover, the DASH diet was also suggested to be related to a lower incidence of coronary heart disease.12 In addition, a recent meta-analysis reported that adherence to the DASH diet was associated with lower risk of stroke in patients with HT.13 The results of this present study report better blood pressure control in subjects who were compliant with dietary advices compared to those who were not, and are in accordance with data in the literature. Regular physical exercise reduces blood pressure in hypertensive patients, especially in the period after recent physical activity. Blood pressure decreases to below pre-activity levels after 20 to 30 minutes of physical exercise.14,15 In subjects adhering to regular exercise, blood pressure reduces considerably compared to those who do not adhere to regular exercise; therefore, physical exercise is often recommended to hypertensive patients in the treatment of HT.16 Exercise of moderate intensity has been reported to be related with a lower incidence of HT.17,18 Moreover, in a recent cohort study, significantly lower HT incidence was reported in subjects who participated in skiing compared to the subjects who did not.19 Larger artery diameter, positive autonomic balance, and improved endothelial function observed in athletes could be responsible for beneficial effects of exercise seen in hypertensive patients.20 In the present study, we showed that patients on regular exercise more commonly have well-controlled blood pressure levels, which is consistent with medical literature.

Studies in literature pointed out a relationship between smoking and cardiovascular risk factors. Smoking has a hypertensive effect as it promotes arterial stiffness and induces sympathetic system; therefore, smokers are more prone to elevated blood pressure levels.21 In the present report, there was an increased number of smoking patients in the poorly-controlled hypertension group compared to the patients with well-controlled HT. Combined treatment with anti-hypertensive drugs is advised instead of monotherapy with an antihypertensive agent in recent literature. Mahmud et al. reported that combined treatment was more effective than monotherapy in treatment of HT.22 In another study, it was found that combination treatment of HT was superior to treatment with a single agent.23 Similar to the literature, blood pressure levels were significantly lower in our study in patients on combined treatment compared to patients treated with a single agent. In conclusion, we suggest that, in addition to dietary and exercise recommendations and smoking cessation, treatment with combination therapy could improve the goals of reaching blood pressure targets in patients with HT.

**DISCLOSURES**

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

**REFERENCES**


Does the Dutch Safety Management Program predict adverse outcomes for older patients in the emergency department?

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ABSTRACT

Purpose: Frailty screening in the emergency department may identify frail patients at risk for adverse outcomes. This study investigated if the Dutch Safety Management Program (VMS) screener predicts outcomes in older patients in the emergency department.

Methods: In this prospective cohort study, patients aged 70 years or older presenting to the emergency department were recruited on workdays between 10:00 AM and 7:00 PM from May 2017 until August 2017. Patients were screened in four domains: activities of daily living, malnutrition, risk of delirium, and risk of falling. After 90 days of follow up, mortality, functional decline, living situation, falls, readmission to the emergency department, and readmission to the hospital were recorded. VMS was studied using the total VMS score as a predictor with ROC curve analysis, and using a cut-off point to divide patients into frail and non-frail groups to calculate positive predictive value (PPV) and negative predictive value (NPV).

Results: A total of 249 patients were included. Higher VMS score was associated with 90-day mortality (AUC 0.65, 95% CI 0.54-0.76) and falling (AUC 0.67, 95% CI 0.56-0.78). VMS frailty predicted mortality (PPV 0.15, NPV 0.94, p = 0.05) and falling (PPV 0.22, NPV 0.92, p = 0.02), but none of the other outcomes.

Conclusion: In this selected group of patients, higher VMS score was associated with 90-day mortality and falls. The low positive predictive value shows that the VMS screener is unsuitable for identifying high-risk patients in the ED. The high negative predictive value indicates that the screener can identify patients not at risk for adverse medical outcomes. This could be useful to determine which patients should undergo additional screening.

KEYWORDS

Emergency department, frailty, geriatric screening method, VMS

INTRODUCTION

Life expectancy and the prevalence of frailty in the Netherlands are increasing.1 Up to 25% of emergency department (ED) presentations are patients aged 65 years or older.2 These patients are at risk of adverse outcomes after discharge, such as readmission, functional decline, and mortality.2 After discharge from the ED, 24% of patients are readmitted in the first three months and 44% in the first six months.1 The average 90-day mortality of these patients is about 10%.3 Identifying patients at high risk of adverse outcomes such as death and readmission provides opportunities for preventive geriatric intervention. Frailty is a predictor of adverse medical outcomes in older patients. Frailty is defined as a dynamic syndrome characterised by decreased reserves and resistance to stressors, due to a decline in multiple physiological systems.6 A comprehensive assessment of frailty is difficult...
to measure in the ED. The increasing number of older patients in the ED and prevalence of frailty require the development of frailty screening instruments in the ED. Screening for frailty in the ED is feasible and can improve patient outcomes.\(^5\) To identify frail patients, many screening instruments are available both worldwide\(^7,10\) and in the Netherlands.\(^11\) Studies have been conducted investigating diagnostic accuracy of older adult vulnerability screening instruments, but there is a lack of pragmatic, accurate, and reliable tools.\(^7\) An instrument that may be used for identifying frail older patients at risk for negative outcomes in older patients presenting to the ED is the Veiligheidsmanagementsysteem ‘VMS’ (Dutch Safety Management Program) for frail older patients.\(^12\) This instrument is part of a national program to prevent avoidable injury or death. The complete screener is presented in figure 1. Screening can be performed by a nurse, geriatrician, or physician’s assistant and takes about 4-5 minutes; it identifies patients (aged 70 years or older) at risk for delirium, falls, malnutrition, and functional impairment, who require preventive measures.\(^12,13\) In the Netherlands, all hospitalised patients aged 70 years or older are screened but screening is not routinely performed in the ED. The VMS instrument has been shown to be a good predictor for adverse outcomes in older hospital patients.\(^14,15\) No previous studies have been done to test the predictive value for patient outcomes of the VMS screener in the ED. This study investigated if the VMS screener identifies patients aged 70 years or older at risk for adverse outcomes (i.e., mortality, functional decline, falls, readmission to the hospital or ED, or a change in living situation) in the ED.

**METHODOLOGY**

This prospective cohort study was approved by the medical ethical committee of the Amsterdam Medical Centre, the Netherlands (registration number W17_209) and the institutional review board at Gelre Hospitals, Apeldoorn and Zutphen, the Netherlands. It was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from all eligible patients before inclusion in the study. The inclusion period ran from May 2017 until August 2017. Because the effect size was unknown, a power analysis was not feasible. The authors aimed to recruit a convenience sample of 200 patients. Patients aged 70 years or older were recruited at the ED of Gelre Hospital Apeldoorn presenting for the following specialties: internal medicine, geriatric medicine, general surgery (including trauma), orthopaedic surgery, gastroenterology, pulmonary medicine, and

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**Figure 1. The VMS screener**

<table>
<thead>
<tr>
<th>Risk of delirium</th>
<th>At risk if ≥ 1 questions is answered with “yes”</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Do you have cognitive problems?</td>
<td></td>
</tr>
<tr>
<td>• Did you need help with self-care in the last 24 hours?</td>
<td></td>
</tr>
<tr>
<td>• Have you experienced a previous episode of confusion or delirium before?</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Risk of falling</th>
<th>At risk in case of a positive answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Did you fall at least once in the last six months?</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Risk of malnutrition (SNAQ)</th>
<th>At risk if ≥ 2 points (consult dietician if ≥ 3 points)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Did you have a reduced appetite last month? (yes = 1 point; no = 0 points)</td>
<td></td>
</tr>
<tr>
<td>• Did you lose weight unintentionally? (no = 0 points; ≥ 3 kg in the last month = 2 points; ≥ 6 kg in the last 6 months = 3 points)</td>
<td></td>
</tr>
<tr>
<td>• Did you take nutritional drinks or did you use a feeding pump last month? (yes = 1 point; no = 0 points)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Risk of functional decline (KATZ-ADL)</th>
<th>At risk if ≥ 2 questions are answered with “yes”</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Do you need help with bathing? (yes = 1 point; no = 0 points)</td>
<td></td>
</tr>
<tr>
<td>• Do you need help with dressing? (yes = 1 point; no = 0 points)</td>
<td></td>
</tr>
<tr>
<td>• Do you need help using the toilet? (yes = 1 point; no = 0 points)</td>
<td></td>
</tr>
</tbody>
</table>

KATZ-ADL = Katz index of independence in activities of daily Living; SNAQ = short nutritional assessment questionnaire; VMS = Dutch Safety Management Program
urology. Gelre Hospital Apeldoorn is a level 2 trauma centre in an urban setting. Inclusion hours were between 10:00 AM and 7:00 PM during week days. Exclusion criteria were: logistical impossibility to include patient (i.e., patient missed for inclusion, unstable medical condition), language barrier (patient not proficient in Dutch or English), severe cognitive impairment (diagnosed by physician at ED or as mentioned in patient records) with no proxy present, no permission to approach the patient by their attending nurse or physician. Age, sex, and specialty for which the patient had been referred was documented for patients for which no informed consent was obtained. All measurements were performed within approximately 30 minutes of presentation at the ED. The following baseline data were collected: age, sex, specialty for which the patient had been referred, living situation (at home, in a residential care facility, in a nursing home), diagnosis of dementia (as stated in medical records), number of different medications, use of a walking device, and whether the reason for the ED visit had been a fall.

VMS screening was performed for all included patients by author HS who had received training by a professional geriatrician. The VMS screener consists of four domains (figure 1): risk of functional decline, risk of falling, risk of delirium, and risk of malnourishment.11 Functional decline was measured using the KATZ activities of daily living (KATZ-ADL) score.16 The SNAQ score is a validated screening instrument for detecting malnutrition.17 Risk of delirium was assessed by asking if the patient had cognitive problems, needed help with self-care in the previous 24 hours, or had experienced a previous episode of delirium. In cases with a positive answer to either of these questions, the risk of delirium was considered present. The risk of falling was assessed by asking if the patient had experienced a fall in the previous six months and considered present in cases with a positive answer. Patients with incomplete VMS data were excluded from further analysis. The VMS score was calculated by adding up all positive domains, resulting in a score ranging from zero to four. All domains were given equal weight. Domains were not analysed individually because the VMS is already implemented in all Dutch hospitals as a four-domain tool and this will not change. Additionally, in order to divide patients into ‘frail’ and ‘non-frail’ groups, VMS scores were dichotomised using a cut-off point of two or more positive domains, based on previous studies.18,19 Treating physicians in the ED were blinded for VMS scores. The primary outcome of this study was 90-day mortality, which was determined by consulting the municipal civil registry. After a follow-up period of 90 days after presentation at the ED, all surviving patients were contacted by telephone to determine secondary outcomes. Three attempts on three different dates at different times were made to contact the patient. If the patient could not be reached after the third attempt, the patient was considered lost to follow up. Secondary outcomes were functional decline, defined as a one-or-more point loss of KATZ-ADL, having experienced a fall during follow up, change in living situation, or a hospital or ED readmission during follow up. A change in living situation was defined as moving to a facility with a higher level of care than before presentation at the ED (e.g., from living on their own to a residential care facility). A composite outcome was created, defined as either death or functional decline (loss of points on KATZ-ADL) at follow up, assuming that patients who had died had also inherently experienced functional decline. This decline could not be quantified by the KATZ-ADL, because the patient must be alive at follow up to determine this score. Differences between frail and non-frail patients were analysed with the chi-squared test for categorical data. Normality was tested for continuous variables including VMS score with the Shapiro-Wilks test. All continuous variables were not normally distributed at the p < 0.001 level and thus, A Mann-Whitney u test was performed. Positive predictive value (PPV) and negative predictive value (NPV) was calculated for VMS frailty and each outcome. VMS scores were analysed as a continuous variable using ROC curve analysis for each outcome. All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., 2017, Armonk, NY). The level of statistical significance was set at 0.05 for all analyses. No funding was received for this study. This paper was written in accordance with the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines.20

Results

During the study period, 1203 eligible patients presented to the ED, 379 of whom presented within inclusion hours. A total of 112 patients were excluded. Due to a software error in data registration, 18 subjects had to be excluded because their VMS data was incomplete. After 90 days, 30 patients had died. Of the surviving patients, 32 individuals could not be reached by telephone. Cohort selection is summarised in figure 2.

Baseline characteristics are shown in table 1. The median VMS score was 2 (IQR 1-3) and 168 (68%) of patients were classified as frail. The median age was 80 years (IQR 75-86) and there were 153 (61%) female patients. Median KATZ-ADL score was 1 (IQR 0-2). Patients were mainly referred to the ED for general surgery, internal medicine, and geriatric medicine.

Frail patients were older [median 83, IQR (77-87)] vs. non-frail patients [median 78 (74-83)], p < 0.01. They
were dependent on more activities of daily living, had a higher SNAQ score, and used more medication at baseline. The prevalence of dementia was higher in the frail cohort (n = 18; 11%) vs. non-frail (n = 1; 1%; p = 0.01) and frail patients more often lived in an institutional care facility (frail, n = 31; 19% vs. non-frail, n = 4; 5%; p < 0.01). The results of all ROC curve analyses are summarised in table 2. The ROC curve analysis for VMS score in relation to 90-day mortality had an area under the curve (AUC) of 0.65, with a 95% CI of (0.54-0.76) and a p-value of < 0.01. A higher VMS score was also associated with a fall during follow up, with an AUC of 0.67 and a 95% CI of (0.56-0.78), p = < 0.01. There was no association between a higher VMS score and the composite outcome (functional decline and death), functional decline (KATZ-ADL), readmission to the ED, readmission to the hospital, or a change in living situation during follow up.

The associations between VMS frailty and different outcomes is presented in table 3. Patients classified as frail were more likely to die during follow up in this study: frail, n = 25 (15%) vs. non-frail, n = 5 (6%), p = 0.05 with a PPV 0.15 and an NPV 0.92. There was no association between VMS frailty and KATZ-ADL functional decline (p = 0.83), the composite outcome of functional decline and death (p = 0.13), readmission to the ED (p = 0.18) or the hospital (p = 0.81), or a change in living situation (p = 0.94).

### Table 1. Baseline characteristics, frail (two or more VMS domains positive) vs. non-frail patients

<table>
<thead>
<tr>
<th>Overall</th>
<th>Frail</th>
<th>Non-frail</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 249</td>
<td>n = 168 (67%)</td>
<td>n = 81 (33%)</td>
<td></td>
</tr>
<tr>
<td>Age, median (IQR)</td>
<td>80 (75-86)</td>
<td>83 (77-87)</td>
<td>78 (74-83)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>153 (61%)</td>
<td>107 (64%)</td>
<td>46 (57%)</td>
</tr>
<tr>
<td>KATZ-ADL score, median (IQR)</td>
<td>1 (0-2)</td>
<td>1 (0-3)</td>
<td>0 (0-0)</td>
</tr>
<tr>
<td>SNAQ score, median (IQR)</td>
<td>1 (0-3)</td>
<td>2 (0-3)</td>
<td>0 (0-1)</td>
</tr>
<tr>
<td>ED visit because of fall, n (%)</td>
<td>96 (39%)</td>
<td>70 (42%)</td>
<td>26 (31%)</td>
</tr>
<tr>
<td>Number of different medications, median (IQR)</td>
<td>5.5 (3-8)</td>
<td>6 (4-8)</td>
<td>4 (2-7)</td>
</tr>
<tr>
<td>Diagnosed with dementia, n (%)</td>
<td>19 (8%)</td>
<td>18 (11%)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Living in an institutional care facility, n (%)</td>
<td>35 (14%)</td>
<td>31 (19%)</td>
<td>4 (5%)</td>
</tr>
<tr>
<td>Specialty for which patient had been referred, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General surgery</td>
<td>120 (48%)</td>
<td>79 (47%)</td>
<td>41 (51%)</td>
</tr>
<tr>
<td>Internal medicine</td>
<td>67 (27%)</td>
<td>45 (27%)</td>
<td>22 (27%)</td>
</tr>
<tr>
<td>Geriatric medicine</td>
<td>30 (12%)</td>
<td>23 (14%)</td>
<td>7 (9%)</td>
</tr>
<tr>
<td>Pulmonary medicine</td>
<td>16 (6%)</td>
<td>11 (7%)</td>
<td>5 (6%)</td>
</tr>
<tr>
<td>Gastroenterology</td>
<td>10 (4%)</td>
<td>6 (4%)</td>
<td>4 (5%)</td>
</tr>
<tr>
<td>Orthopaedic surgery</td>
<td>5 (2%)</td>
<td>3 (2%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Urology</td>
<td>1 (0%)</td>
<td>1 (1%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

### Table 2. Summary of ROC curve analysis for VMS scores and outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>AUC</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality</td>
<td>0.65</td>
<td>0.54-0.76</td>
<td>0.01</td>
</tr>
<tr>
<td>Fall during follow up</td>
<td>0.67</td>
<td>0.56-0.78</td>
<td>0.01</td>
</tr>
<tr>
<td>Composite outcome</td>
<td>0.54</td>
<td>0.46-0.62</td>
<td>0.29</td>
</tr>
<tr>
<td>Functional decline</td>
<td>0.49</td>
<td>0.40-0.58</td>
<td>0.85</td>
</tr>
<tr>
<td>Readmission to ED</td>
<td>0.58</td>
<td>0.48-0.67</td>
<td>0.12</td>
</tr>
<tr>
<td>Readmission to the hospital</td>
<td>0.52</td>
<td>0.43-0.62</td>
<td>0.63</td>
</tr>
<tr>
<td>Change in living situation</td>
<td>0.56</td>
<td>0.44-0.67</td>
<td>0.31</td>
</tr>
</tbody>
</table>

AUC = area under the curve; CI = confidence interval; ED = emergency department; ROC = receiver operator characteristic; VMS = Dutch Safety Management Program.
DISCUSSION

In this study, frail patients as identified by the VMS admitted to the ED were more likely to die during 90-day follow up or experience a fall, compared to non-frail patients. ROC curve analysis showed that a higher VMS score was also predictive of mortality and of a fall during follow up. The overall predictive value of the VMS screener for adverse outcomes was low.

No previous studies have investigated VMS score in the ED in relation to adverse medical outcomes in the ED. Previous studies investigating VMS score in relation to adverse outcomes target patients who are already hospitalised or target specific patient populations such as cancer patients or orthogeriatric patients. These cohorts are not comparable to the cohort presented in this study. The 12% mortality in this study corresponds with previous studies, in which 90-day mortality was between 9% and 12% in older patients presenting to the ED.

The ED may provide physicians with an opportunity to screen patients in an early stage and implement geriatric interventions, if necessary. There is currently no gold standard to identify frail patients in the ED. The VMS screener can provide physicians with useful information regarding deficits in the four different domains, but predictive performance as a screener for adverse medical outcomes seems limited. Total VMS score ROC curve had an AUC < 0.7, which represents poor test performance in predicting outcomes. The low positive predictive value of the VMS frailty score means that in practice, some patients would be classified as frail, while not at higher risk for adverse outcomes. The high negative predictive value indicates that the screener can be used to identify patients not at risk for adverse medical outcomes. The ideal screening tool identifies is non-invasive and has a high sensitivity. The VMS meets these criteria and could be a useful first step to determine which patients should undergo additional screening (e.g., comprehensive geriatric assessment). The VMS screener identified 68% of all patients as frail, and it is unlikely that resources are available to provide all these patients with a thorough geriatric follow up. A two-step approach could be considered, using the VMS screening with a high negative predictive value as a first step (i.e., rule-out model), and a second step where patients are screened by a consulting geriatrician by using clinical impression or by using a frailty tool with a high positive predictive value. The patient burden in the ED in the Netherlands is increasing.

Figure 2. Flowchart of cohort selection

ED = emergency department; n = number; VMS = Dutch Safety Management Program

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and screening methods should be both effective and time-efficient. Alternative screening methods, such as the acutely presenting older patient (acuut presenterende oudere patient, APOP) with a PPV of 0.20-0.30 for 90-day mortality and the identification of seniors at risk (ISAR) could also be considered. However, these screeners only give a total score and do not provide information in specific domains. There are three distinct advantages of VMS screening: 1) It can be performed by any nurse, physician’s assistant, or physician at presentation at the ED or anywhere in the hospital; 2) it is non-invasive and takes only about 2-3 minutes to complete; and 3) the screener identifies four different domains for which non-invasive geriatric measures can be taken immediately, such as fall prevention and delirium prevention.

This study has a few limitations. First, due to limited logistical resources, inclusion hours were between 10:00 AM and 7:00 PM on workdays. This resulted in a smaller sample size and the possibility of selection bias. There was no significant difference in mortality between patients included in the final cohort (12%) and excluded patients, including those presenting outside inclusion hours (10%), \( p = 0.48 \). Patients who had been referred to the departments of geriatric or internal medicine were more often excluded. It is possible that these patients were more ill and refused to participate in the study. Second, it is possible that patients with a higher VMS score received different treatment then patients with a lower VMS score. Although treating physicians were blinded for VMS score, factors such as comorbidity and older age may have guided decision making, which may introduce bias. Third, external validity of this study may be limited as it was a single centre study and patients presenting to the departments of cardiology and neurology were excluded.

To our knowledge, this is the first study to investigate the use of the VMS screener in relation to adverse medical outcomes in the ED. No previous studies have investigated VMS score as a continuous outcome in relation to outcomes in the ED. An important strength of this study was the use of many different important patient outcomes during follow up, such as functional decline and change of living situation. Another strength was that functional decline was determined in two different ways to reduce survival bias. KATZ-ADL is frequently used in follow-up studies to measure a degree of functional decline or functional outcomes. The authors advise caution regarding this approach for two reasons. First, KATZ-ADL follow-up can only be obtained for patients who are alive after the follow-up period. This holds true for any functional outcome measure and is especially challenging when investigating functional decline in older patients. Second, patients who are not ADL dependent can more easily lose points in KATZ-ADL than patients who are fully dependent on others at baseline. This means that one-point loss of KATZ-ADL does not represent an equal loss of function among patients. Patient-reported outcome measures such as the patient-reported outcome measurement information system for physical function (PROMIS-PF) may be more accurate and patient-centred outcomes and should be used in future investigations.

In conclusion, the predictive performance of the VMS screener in relation to several important patient outcomes in the ED was studied. Using a cut-off point of two or more positive domains predicts 90-day mortality (PPV 0.15, NPV 0.94) and falls (PPV 0.22, NPV 0.92), but

<table>
<thead>
<tr>
<th>Table 3. Outcomes of VMS frail vs. non-frail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outcome</td>
</tr>
<tr>
<td>90 days mortality, n (%)</td>
</tr>
<tr>
<td>Functional decline*, n (%) (composite outcome)</td>
</tr>
<tr>
<td>Functional decline**, n (%) (KATZ-ADL)</td>
</tr>
<tr>
<td>Readmission to ED***, n (%)</td>
</tr>
<tr>
<td>Readmission to the hospital****, n (%)</td>
</tr>
<tr>
<td>Change in living situation**, n (%)</td>
</tr>
<tr>
<td>Fall during follow up**, n (%)</td>
</tr>
</tbody>
</table>

* Total, n = 217; Frail, n = 151; Non-frail, n = 66
** Total, n = 187; Frail, n = 126; Non-frail, n = 61
*** Total, n = 186; Frail, n = 125; Non-frail, n = 61
ED = emergency department; KATZ-ADL = Katz activities of daily living; n = number; VMS = Dutch Safety Management Program
none of the other outcomes. The low PPV shows that many patients classified as frail do not experience adverse outcomes, making the screener less suitable to identify high risk patients. The screener can still be used to get a quick impression of the functional and nutritional and cognitive status of a patient, which can help guide decision making. The high negative predictive value indicates that the screener can identify patients not at risk for adverse medical outcomes, which could be a useful first step to determine which patients should undergo additional screening by comprehensive geriatric assessment.

ACKNOWLEDGEMENTS

Compliance with ethical standards

This prospective cohort study was approved by the medical ethical committee of the Amsterdam Medical Centre, the Netherlands and the institutional review board at Gelre Hospitals, Apeldoorn and Zutphen, the Netherlands. It was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments.

DISCLOSURES

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

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Organisation of internal medicine in acute care in the Netherlands: a detailed overview

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ABSTRACT

Background: Organisation of the emergency department (ED) is gaining attention due to an increased demand on emergency services, leading to crowding and influencing the quality of care. It is known that the organisation of acute care influences the performance of the ED. In the Netherlands, the organisation of EDs differs between hospitals. However, detailed information about the various organisational structures is lacking. This study aims to determine the organisational structures and the different roles and responsibilities of internists and emergency physicians (EPs) in the EDs.

Methods: We performed a nationwide observational study between January 2018 and February 2019. All hospitals with an ED in the Netherlands were identified, contacted, and surveyed. Requested information was retrieved from internists and complemented with local administrative hospital data.

Results: 76 out of 89 EDs responded to the questionnaire (84%); 93% of EDs were operational 24/7. A registered acute internist was present at 47 locations (62%) and an EP at 60 EDs (79%). At 10 locations (13.2%), internists reported not being physically present at the ED. Supervision and working agreements between EPs and internists differed between the hospitals. Collaboration between EPs and internists was graded satisfactory (7.4/10).

Conclusion: This is the first study providing a detailed overview of the ED organisation in the Netherlands regarding internal medicine patients. This organisation differs in terms of staffing, presence of EPs and internists, and working agreements. The influence of the various organisational structures of EDs on quality of acute care should be the subject of future research.

KEYWORDS

Acute care, emergency department, healthcare organisation, internal medicine

INTRODUCTION

The demand on the health care systems and the emergency department in particular, is increasing.1,2 This leads to crowding and queuing, negatively influencing the quality of care.3 It manifests as long lengths of stay in EDs, adverse clinical outcomes, and poor patient experience.3-5 Over the last years, it has been stated that this could impede the accessibility of the acute care, locally and internationally.4,6,7

For several years, there has been ongoing discussion about the reorganisation of the acute care system in the Netherlands, creating disagreement between hospital organisations and health insurers.8 Many reports have been published on this topic, aiming to gain insight into the use and accessibility of Dutch emergency care.9-13 Factors such as patient flow, healthcare costs, and predicted future demographic changes are subjects of these reports and are used in decision making processes by policymakers. Key issues for shaping the acute landscape with intended preservation of quality are, for
example, centralisation of complex care and the presence of a doctor in the ED with at least one year of working experience. However, organisation of emergency care for acute medical patients and of EDs especially, differs between hospitals, mainly in number of physicians, the presence of (supervising) internists in the ED, working agreements between internists and emergency physicians (EP), the presence of an Acute Medical Unit (AMU) and collaboration with the general practitioner out-of-hours services, known as General Practitioner Cooperatives (GPC). Insight into similarities or differences of these characteristics and eventually their influence on patient outcomes would help to make well-founded choices in reshaping the acute care chain for acute medical patients.

It is known that the organisation of the acute care chain may influence the quality of delivered care and performance of the ED. Internal factors such as staffing, number of patients, and number of treatment bays, and external factors such as demographics and underlying financial resources, have already been identified as having an influence on ED performance. Moreover, these factors will also complicate evaluation and comparison of ED performance. In addition, many acute medical patients, especially the elderly, have multimorbidity or polypharmacy and present themselves to the ED with undifferentiated problems which makes it challenging to differentiate between the influence of internal factors of the ED and patient-related factors on outcomes. Given the complexity of this patient group increasingly presenting in the acute care chain, the Netherlands Association of Internal Medicine has stated in their strategic vision that internists should play a coordinating role in the acute care of patients with multimorbidity and polypharmacy. Yet, an important subject in order to improve the quality of acute care, is identifying potential actors on performance and outcomes of the local hospital organisation, such as the physical presence of internists in the ED. Therefore, it is important to create a sufficient overview of ED care, focusing on (acute) internal medicine, in the Netherlands.

When investigating the influence of organisation on patient outcomes, it is essential to first evaluate the organisation, including staffing and working arrangements between EPs and internists. We believe that this detailed overview is necessary for creating a foundation for scientific research nationally and also internationally. In addition, this will also make comparisons between care for acute medical patients in the Netherlands and internationally more insightful. Finally, we will reflect on the public discussion regarding acute care and formulate critical notes for future organisational models based on this overview, aiming to improve the quality of care for acute medical patients.

METHODS

Design
We performed a nationwide observational study, identifying the organisational structure of EDs in the Netherlands. All hospitals with an ED in the Netherlands were identified in January 2018. At the start of the study, we identified 91 EDs within 76 hospital organisations and 89 EDs at the end of the study (February 1st, 2019) due to the closure of two hospitals. An acute internist, if present, or a consultant internal medicine physician with an affinity for acute care, was contacted by e-mail to participate in the study and an online questionnaire was distributed (using Qualtrics XM, U.S.A.). In addition, administrative hospital data of patient numbers between January 1st, 2013 and December 31st, 2017 in a predefined format (supplementary data) were collected. The total number of patients visiting the ED, the number of patients older than 65 years, the number of patients visiting the ED for internal medicine, and admissions for internal medicine were requested. The results of the questionnaire including patient numbers, were directly transferred to the study database in SPSS Statistics 25.0 for Windows.

The study period of the online questionnaire was between January 2018 and February 2019. Reminders were sent every 2-3 months by e-mail, to all identified physicians at the beginning of the study. Three researchers (MK, HH, PN) contacted the invited physicians who did not respond to the questionnaire by telephone. This was done in December 2018 and January 2019, in an ultimate effort to collect as much data as possible. No effort was made to retrieve missing data. A full overview of definitions used in the questionnaire is provided in appendix 1. Words that are associated with a definition in the appendix are marked with an asterisk (*). Our goal was to obtain participation of at least 66% of all EDs, divided over the country. Participation was voluntary and the study protocol was approved by the Medical Ethics Committee of Máxima MC (study number N17.122).

Setting
In the Netherlands, 2.4 million ED visits were registered in 2016 and 840,000 (35%) patients were admitted (total country population of 17 million). To gain access to hospital care in the Netherlands, including EDs, patients are required to have a referral from a GP or directly transferred by an ambulance. Self-referral is possible, however a deductible reduction has been introduced to discourage self-referrals. During out-of-hours, GPs in the region cooperate to provide urgent primary care on a rotation basis, taking care of each other’s patients in GPCs. This ensures a gatekeeping function of the GP, around the clock. GPCs can collaborate with the local ED, varying between no collaboration to an integrated GPC in the ED.
In general, residents of different medical specialties staff the ED in collaboration with residents in emergency medicine, supervised by medical specialists and EPs, depending on the local organisation and working agreements. All residents are qualified doctors who are either in training to become specialists or non-trainees who are working in the hospitals to gain experience with the aim of entering a specialist training programme. Only since 2009, has emergency medicine been recognised as a specialty, however in 2000, the first hospitals started to train EPs aiming to introduce EPs into the ED. However, until now, EPs are not fully integrated into every ED. Acute internal medicine has been recognised as subspecialty within internal medicine since 2010. Internists are present in each hospital, whereas acute internists are not.

Statistical analysis
Descriptive statistics were executed using IBM SPSS Statistics 25.0 for Windows. Missing data were categorised as ‘missing’.

RESULTS
In total, we gathered data from 76 out of 89 EDs (84%) within 67 hospital organisations of different types (table 1). The EDs were evenly spread over the country as is shown in Figure 1.

<table>
<thead>
<tr>
<th>Hospital type</th>
<th>Total</th>
<th>University medical centre</th>
<th>Teaching hospital</th>
<th>Non-teaching general hospital</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opening hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24/7</td>
<td>72 (94.7%)</td>
<td>8 (10.5%)</td>
<td>31 (40.8%)</td>
<td>37 (48.7%)</td>
</tr>
<tr>
<td>Closed during night</td>
<td>1 (1.3%)</td>
<td>0</td>
<td>0</td>
<td>1 (2.7%)</td>
</tr>
<tr>
<td>Closed during weekends</td>
<td>1 (1.3%)</td>
<td>0</td>
<td>0</td>
<td>1 (2.7%)</td>
</tr>
<tr>
<td>Other (undefined)</td>
<td>2 (2.6%)</td>
<td>0</td>
<td>1 (3.2%)</td>
<td>1 (2.7%)</td>
</tr>
<tr>
<td>Acute Medical Unit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>39 (51.3%)</td>
<td>4 (50.0%)</td>
<td>21 (67.7%)</td>
<td>16 (43.2%)</td>
</tr>
<tr>
<td>Absent</td>
<td>29 (38.2%)</td>
<td>4 (50.0%)</td>
<td>9 (29.0%)</td>
<td>20 (54.1%)</td>
</tr>
<tr>
<td>Missing</td>
<td>2 (2.6%)</td>
<td>0</td>
<td>1 (3.2%)</td>
<td>1 (2.7%)</td>
</tr>
<tr>
<td>Cardiac Emergency Department</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>47 (61.8%)</td>
<td>6 (75.0%)</td>
<td>20 (64.5%)</td>
<td>21 (56.8%)</td>
</tr>
<tr>
<td>Absent</td>
<td>17 (22.4%)</td>
<td>2 (25.0%)</td>
<td>6 (19.4%)</td>
<td>9 (24.3%)</td>
</tr>
<tr>
<td>Missing</td>
<td>12 (15.8%)</td>
<td>0</td>
<td>5 (16.1%)</td>
<td>7 (18.9%)</td>
</tr>
<tr>
<td>Collaboration with the GP out-of-hours services</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No collaboration</td>
<td>4 (5.3%)</td>
<td>2 (25.0%)</td>
<td>0</td>
<td>2 (5.4%)</td>
</tr>
<tr>
<td>GP out-of-hours service</td>
<td>6 (7.9%)</td>
<td>1 (12.5%)</td>
<td>2 (6.5%)</td>
<td>3 (8.1%)</td>
</tr>
<tr>
<td>Co-located (parallel)</td>
<td>14 (18.4%)</td>
<td>3 (37.5%)</td>
<td>1 (3.2%)</td>
<td>10 (27.0%)</td>
</tr>
<tr>
<td>Shared entrance (serial)</td>
<td>28 (36.8%)</td>
<td>0</td>
<td>14 (45.2%)</td>
<td>14 (37.8%)</td>
</tr>
<tr>
<td>Integrated</td>
<td>19 (25.0%)</td>
<td>2 (25.0%)</td>
<td>10 (32.3%)</td>
<td>7 (18.9%)</td>
</tr>
<tr>
<td>Missing</td>
<td>5 (6.6%)</td>
<td>0</td>
<td>4 (12.9%)</td>
<td>1 (2.7%)</td>
</tr>
</tbody>
</table>

GP = general practitioner
in figure 1. Thirteen EDs (16%) did not respond to the online questionnaire and we were unable to reach an internist at these locations by telephone. Of these, four EDs were located in a teaching hospital* and nine EDs in a general hospital*.

**ED characteristics**
From a total of 76 EDs, 72 were operational full time (24/7). The remaining four EDs were closed during the night or reported another (undefined) schedule, as is shown in table 1. At 47 EDs (62%), a separate cardiac ED was present; 17 EDs did not have a separate cardiac ED and 12 EDs did not mention their organisation for acute cardiac patients.

The type of collaboration between EDs and the GPC is shown in table 1. A GPC serial* to the ED or integrated* within the ED were the most frequently reported types of collaboration. The presence of EPs or internists in the ED was not associated with the type of collaboration with the GPC (Fisher’s Exact Test: 11.08; p = 0.47, respectively). An AMU* was present at 39 locations (51%), not present at 29 locations (38%), and 8 locations (11%) did not report if an AMU was present. At locations equipped with an AMU, an acute internist was present* in 72% of these locations, while at locations without an AMU, an acute internist was only present at 55% of these locations.

**Patient numbers**
Between 2013 and 2017, 41 EDs reported numbers of patients visiting the ED. Of these, eight EDs did not provide numbers beyond 2016. Patient numbers were collected from eight EDs located in university medical centres*, 18 in teaching hospitals*, and 15 in general hospitals*. Since 2013, there has been a decrease in the total number of patients visiting the ED. In 2013, on
average 22,359 patients (range: 7,857-42,488) visited the ED compared to 20,818 patients (range: 7,775-42,488) in 2017. This is a decrease of 6.9%. The number of patients ≥ 65 years has increased over the years by 7.3%. In 2013, 6,699 patients (range 3,685-10,245) older than 65 years visited the ED and 7,230 (range: 3,404-13,389) in 2017.

EDs located in university medical centres and teaching hospitals are similar in the total number of patients visiting the ED (± 23,500 on average in 2017), while EDs in general hospitals had fewer ED visits (± 16,500 on average in 2017).

The number of patients presenting for internal medicine increased slightly from 2013 to 2016, but showed a decrease in 2017. In 2013, 3,824 patients (range: 1,227-10,403) presented to the ED for internal medicine, compared to 4,343 patients (range: 1,418-29,426) in 2016 and 3,855 patients (range: 1,505-20,832) in 2017. This decrease is only visible in EDs in general and teaching hospitals, while university medical centres showed an increase of patients presenting to the ED for internal medicine. In addition, there is a slight overall decrease in the number of patients ≥ 65 years presenting for internal medicine, but only between 2016 and 2017. The percentage of patients ≥ 65 years presenting for internal medicine, as part of the total number of patients ≥ 65 years visiting the ED, has also decreased over the last years (figure 2).

**ED Staffing**

EPs were present in 60 EDs (79%), of which there were EPs 24/7 in 32 EDs (46%). If EPs were not present 24/7, they were mostly absent during night hours or had another (undefined) working schedule (table 2). At 51 locations (67%), a registered acute internist is employed. Absence of a registered acute internist was most common in general hospitals*: 68% absence versus 32% in teaching hospitals* and 0% in university medical centres*. While internists are employed and present in all hospitals, their presence* in the ED differs: at 10 locations (13.2%) internists reported not being able to present in the ED for supervision because of other tasks at the same time. In 17 EDs (22.4%), the internist is on call, without a working place near the ED, and therefore may or may not be present when a patient arrives. In 6 EDs (8%), the internist was physically present at least during office hours and evenings in the ED. In absence of an EP, the internist is more often present in the ED during office hours [11 out of 16 EDs (68.8%)], compared to EDs with 24/7 coverage of EPs [18 out of 31 EDs (58.1%)].

### Table 2. Staffing and presence of internal medicine residents, internists, and EPs in the ED

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>University Medical Centre</th>
<th>Teaching Hospital</th>
<th>General hospital</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Presence of internal medicine residents in the ED</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only residents in training</td>
<td>2 (2.6%)</td>
<td>2 (25.0%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Only residents not in training</td>
<td>12 (15.8%)</td>
<td>0</td>
<td>1 (3.2%)</td>
<td>11 (29.7%)</td>
</tr>
<tr>
<td>Both residents not in training/in training</td>
<td>51 (67.1%)</td>
<td>6 (75.0%)</td>
<td>30 (96.8%)</td>
<td>15 (40.5%)</td>
</tr>
<tr>
<td>No residents</td>
<td>11 (14.5%)</td>
<td>0</td>
<td>0</td>
<td>11 (29.7%)</td>
</tr>
<tr>
<td><strong>Presence of any internist in the ED</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not present</td>
<td>10 (13.2%)</td>
<td>0</td>
<td>2 (6.5%)</td>
<td>8 (21.6%)</td>
</tr>
<tr>
<td>Present during office-hours</td>
<td>38 (50.0%)</td>
<td>4 (50.0%)</td>
<td>14 (41.2%)</td>
<td>20 (54.1%)</td>
</tr>
<tr>
<td>Present during office-hours and evenings</td>
<td>9 (11.8%)</td>
<td>3 (37.5%)</td>
<td>5 (16.1%)</td>
<td>1 (2.7%)</td>
</tr>
<tr>
<td>On call during office hours</td>
<td>17 (22.4%)</td>
<td>1 (12.5%)</td>
<td>9 (29.0%)</td>
<td>7 (18.9%)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (2.6%)</td>
<td>0</td>
<td>1 (3.2%)</td>
<td>1 (2.7%)</td>
</tr>
<tr>
<td><strong>Presence of the EP in the ED</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>16 (21.1%)</td>
<td>2 (25.0%)</td>
<td>4 (12.9%)</td>
<td>10 (27.0%)</td>
</tr>
<tr>
<td>24/7 present</td>
<td>32 (42.1%)</td>
<td>4 (50.0%)</td>
<td>13 (41.9%)</td>
<td>15 (40.5%)</td>
</tr>
<tr>
<td>Present during office hours and evenings</td>
<td>22 (28.9%)</td>
<td>2 (25.0%)</td>
<td>11 (35.5%)</td>
<td>9 (24.3%)</td>
</tr>
<tr>
<td>Other</td>
<td>6 (7.9%)</td>
<td>0</td>
<td>3 (9.7%)</td>
<td>3 (8.1%)</td>
</tr>
</tbody>
</table>

ED = emergency department; EP = emergency physician
Internal medicine residents treat patients for internal medicine in 65 EDs (86%), as shown in table 2. In 11 EDs (14%), all located in a general hospital, there are no internal medicine residents treating patients in the ED. Patients in these hospitals are treated by emergency care residents, EPs, or internists. However, in the majority of university medical centres and teaching hospitals (92%), patients are seen by residents in training as well as residents not in training. Residents in training are present in 15 EDs in general hospitals (48%), while residents not in training are present in 26 EDs (84%). Supervision is provided by internists and/or EPs.

Roles and responsibilities
Internists reported having various roles in the ED: 68 internists (89.5%) were practitioners*, 57 consultants* (75.0%), 22 coordinators* (28.9%), and 15 managers (19.7%); 3 internists did not report their role. In addition, 6 internists (7.9%) mentioned other roles, such as supervisor and trainer. Internists working in a university medical centre seemed to be more frequently a coordinator and manager in comparison with internists in teaching or general hospitals. Furthermore, the presence of an acute internist was associated with reporting these coordinating and managing roles frequently. In hospitals where EPs were not present, internists more often reported a role as practitioner compared to hospitals where no EPs were present (100% vs. 87.1%) and consultant (93.8% vs. 64.5%).

We assessed working agreements between internists and EPs by taking inventory of who was in the lead during the initial care* of medical, haemodynamically instable patients. These arrangements were different for referred* and not referred* patients. In general, the initial care of referred patients is led by the internist (39%) or internal medicine resident (46%). Non-referred patients are most often treated by the EP (60%), if present. Secondly, we assessed supervision agreements. Patients who were referred and assessed by residents were supervised by internists at 71.1% of the EDs, by an internal medicine fellow* at 7.9% of the EDs, and by EPs at 14.5% of the EDs. Fourteen EDs (18.4%) did not report their supervision agreements. Supervision of residents treating non-referred patients is equally divided between EPs and internists. Furthermore, EPs must contact the internist to admit acute medical patients at all of the 47 responding EDs. Discharging patients directly from the ED without contacting the internist is only acceptable in cases of non-referred patients at 18 of the 36 responding EDs (50.0%).

Lastly, internists graded the collaboration with EPs in the ED with a mean of 7.4 out of 10 (range: 1 to 10). There was no difference in grading in presence or absence of an acute internist. Transparent working agreements, being approachable, and logistic support were mentioned as strengths. Internists experienced variation between EPs in the quality of delivered care, especially in complex multimorbid patients. For example, one internist’s opinion, “There’s a continuous conflict of domains and EPs have a poor knowledge of internal medicine”. In contrast, another internist reported, “We experience a perfect interprofessional collaboration and we make use of each other’s expertise”. In addition, some internists preferred to be contacted in an earlier stage by the EP for consultation.

DISCUSSION
We provide a detailed overview of the organisation of the Dutch acute care in the ED, focusing on acute internal medicine combined with the roles and presence of EPs. Our study shows that there is a decrease in the number of patients arriving at the ED, while there is an increase in patients of 65 years and older. However, in 2016, there was a slight decrease in patients of 65 years and older presenting to the ED for internal medicine. Furthermore, we identified differences in the presence of (acute) internists and EPs in the ED and a variability in working and supervision agreements. Internists reported their roles at the ED most often as practitioner and consultant. Internists experienced the collaboration with EPs as satisfactory.

We showed that patients 65 years and older are an increasing population in the ED in the Netherlands, which is also an international trend. In general, internists are trained to provide complex care to acute patients regarding aging, multimorbidity, and polypharmacy. However, we assessed that patient visits for internal medicine among patients 65 years and older decreased in 2017, which is the contrast with the overall growth of this population in the ED. This decrease remained also present when correcting for missing data. Several reasons may be suggested for this decline, such as older patients encounter problems other than acute illness, or patients are triaged to specific disciplines (i.e., pulmonology, cardiology, or even geriatrics) by EPs. However, this discussion is beyond the scope of this article.

The most notable finding of this study is the number of internists (14%) not physically present in the EDs. In addition, some internists did not identify themselves as a practitioner* (10.5%) nor a consultant* (25%). These statements are a remarkable finding, because both observations are in contrast with the current strategic vision of the Netherlands Association of Internal Medicine, which states that internists should be the central contact for acute medical patients with multimorbidity and polypharmacy. The literature has not shown best
practices on this matter yet, although one Dutch study has shown that the presence of medical specialists, including internists, leads to improved patient flow and satisfaction. In addition, internists are specialised to take care of multimorbid patients with polypharmacy. Given the increased case complexity of acute patients presenting to the ED due to multimorbidity and polypharmacy, internists can play a central role in the care for these patients. In addition, most patients suffer from an acute deterioration of a chronic disease. These patients need a specialist with knowledge of the disease course prior to the ED visit, diagnostic and treatment possibilities considering comorbidities and medication use, and coordination of follow-up. As acute care needs teamwork, EPs can play an important role in the initial care of acute medical patients. It has been shown that activities in the patient care process and patient flow differ between internists and EPs, which may suggest that internists and EPs could be complementary to each other. However, in this study, we found signs of suboptimal interprofessional collaboration between EPs and internists in some hospitals. A qualitative study about interprofessional collaboration between internists and EPs as well as a quantitative study on outcomes, could provide useful insight in this subject and the effects on quality of care.

We showed many organisational and staffing differences between different EDs across the Netherlands, such as the presence of internists at the ED, variability in working agreements in initial care of haemodynamically instable patients, and collaboration choices with the GPCs. These data concur with research in the field of acute medical care from, for example, the United Kingdom (UK), which also showed differences in structure and staffing (in this case AMUs) and even more interesting, that patient flow also vary per hospital. However, in contrast to the recently developed Dutch quality standards for acute care, it may not be achievable and desirable to pursue one uniform organisation for all EDs in the Netherlands. It has been shown that regional and local external factors are known to influence performance of the ED and differences in organisation could be beneficial, if adapted to the local characteristics. In this study, we identified these differences in organisational structure, which should be investigated further in order to evaluate impact on the quality of acute care. The yearly Society for Acute Medicine Benchmarking Audit performed in the UK is an interesting tool which can be used as an example to provide insight into the performance of acute medical care, which also take organisational differences into account. In addition, the identified differences in organisational structure should have a place in the interpretation of scientific research concerning acute care and used as context, assuring benefit of potential changes in treatment or organisation in the local situation. Finally, we would recommend to use this overview to interpret and evaluate international differences in acute care.

Recommendations and future directions

We emphasise further research on the influence of the organisation of acute care on the quality of care, aiming to make well-founded choices in the future organisation of acute medical care, at least in the Netherlands. In order to evaluate these organisational factors, we recommend a national registry for acute medical care including patient outcomes, ED characteristics, and regional organisational characteristics of the acute care chain. In addition, we believe that relevant Patient Reported Outcomes for acute care should be evaluated regularly and incorporated in this registry. Structural measurements of performance in acute medical care could help to make sensible and evidence-based organisational choices.

Secondly, we recommend that internists increase their presence in the ED and availability for ED care, and aim to be the central contact for acute medical patients with multimorbidity and polypharmacy in accordance with the current strategic vision of the Netherlands Association of Internal Medicine. Internists have the knowledge and expertise to treat this specific group, however, we demonstrated that in 2018, their presence was suboptimal in EDs in quite a few hospitals. As case complexity increases, patients deserve specialised care provided by a doctor who is capable of overviewing all problems and able to arrange and provide proper follow-up. Therefore, the presence of internists in the ED and their influence on the quality of care should be investigated further. This observational study could be used as a reference.

Limitations

Unfortunately, we were unable to receive responses from all EDs in the Netherlands. However, we achieved a response rate of 84% by sending reminders and even trying to reach internists by telephone. As the responding EDs were fairly divided over the country and representing university medical centres, teaching hospitals, and general hospitals, we postulate that the selection bias is minimal. Only 41 EDs reported on patient numbers and of these EDs, patient numbers beyond 2016 were not provided by eight EDs. Therefore, interpretation of these data demands some caution.

In addition, due to the use of multiple-choice questions, it was difficult to interpret answers in the local context or identify motives in organisational choices.

Conclusion

To our knowledge, this is the first study that provides a detailed overview of ED organisation in the Netherlands.
regarding internal medicine patients. Our study shows that organisation of ED care for internal medicine patients differs in terms of staffing, presence of EPs and internists, and working agreements between EPs and internists. Some of these differences, such as the presence of internal medicine residents in the ED, seem to depend on the type of hospital.

As it is known that regional and local external factors influence performance of the ED, local and regional differences in the organisation of acute medical care should be taken into account when developing nationwide quality standards for acute care and future research should be used to create a more evidence-based policy. Given the assumed increased case complexity of medical patients, we believe that internists should be the central contact for these patients and therefore should be present frequently at the ED.

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REFERENCES


### Appendix 1. Definitions per chapter in alphabetic order

#### ED characteristics

<table>
<thead>
<tr>
<th>Acute Medical Unit</th>
<th>A dedicated facility within a hospital that acts as the focus for acute medical care for patients who have presented as medical emergencies to the hospital and have to be admitted</th>
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#### Collaboration with GP out-of-hours services (17)

| No collaboration | The GP out-of-hours service and ED are located separately. No working agreements are in place. |
| Located separately | The GP out-of-hours service and ED are located separately, but there is a form of collaboration (for example, working agreements). |
| Parallel | The GP out-of-hours service is located at the hospital and has its own reception desk. There is a separate triage procedure for the ED and GP out-of-hours service. |
| Serial | The GP out-of-hours service is located at the hospital, with the reception desk earlier in line than the EDs. Self-referred patients are encouraged to visit the GP first. There is a separate triage procedure. |
| Integrated | The GP out-of-hours service and ED share a common reception desk. There is a common triage procedure. |

| General hospital | A hospital with the aim to provide basic specialised care and treat non-specific populations or diseases. Some of these hospitals provide a part of the training of medical specialists. |
| Teaching hospital | A hospital providing basic specialised care and complex care in one or more specific areas. A teaching hospital performs research and all hospitals provide (a part of) the training of medical specialists. |
| University medical centre | A hospital affiliated to a university, aiming to provide high-complex care, perform research and train medical specialists. |

#### ED staffing

| Acute internist | An internist trained in acute medicine and registered for the subspecialty acute internal medicine. |
| Fellow internal medicine | A resident in internal medicine, specialising in a specific subspecialty within internal medicine during the last two years of residency, such as acute medicine. |
| Presence in the ED | Having a working place at or nearby the ED, facilitating presence in the ED before or during arrival of the patient. The working schedule facilitates timely presence, without having other clinical or teaching tasks at the same time. |

#### Roles and responsibilities

| Consultant | A medical specialist, such as an internist, providing consultation of a patient, as requested by another medical specialist. |
| Coordinator | Any healthcare professional streamlining the patient flow at the ED |
| Non-referred | Patients arriving at the ED without a referral from the general practitioner, i.e., self-referral or arrival by ambulance. |
| Practitioner | A medical specialist, such as an internist, primarily accountable for the care of a patient at the ED. |
| Referred | Patients arriving at the ED with a referral from the general practitioner. |
| Initial care | Primary care for a patient presenting at the ED with threatened vital functions. |

ED = emergency department; GP = general practitioner
Expression of the matrix metalloproteinases MMP-2 and MMP-9 and their inhibitors TIMP-1 and TIMP-2 in systemic lupus erythematosus patients

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ABSTRACT

Background: The study aimed to look at alterations in expression of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) and their potential use as biomarkers in the pathogenesis of SLE.

Methods: SLE patients (n = 41) and healthy controls (n = 50) were recruited. Quantitative RT-PCR/ELISA assays were performed for expression of MMP and TIMP mRNA in whole blood and PBMC; and corresponding serum protein levels. Intracellular levels of MMP-2 and MMP-9 proteins were analysed by flow cytometry.

Results: Based on SLEDAI scores patients were grouped into active (SLEDAI ≥ 10) and inactive cases (SLEDAI < 10). In active cases, MMP-2 expression significantly increased and TIMP-2 expression was decreased (p < 0.0001) both at serum secretion (p = 0.0003) and mRNA (p < 0.0001) levels as compared to inactive cases. MMP-9 and TIMP-1 showed significantly reduced serum secretion and mRNA expression (p < 0.0001) in active cases as compared to inactive cases. Intracellular concentration of MMP-9 was reported to be higher in neutrophils, while MMP-2 was mainly found in lymphocytes of SLE patients as compared to controls. MMP/TIMP ratio profile was altered as SLE disease progresses.

Interpretation & conclusions: Findings suggest disturbed MMP and TIMP levels have a role in the pathogenesis of SLE.

KEY WORDS

Expression, MMP-2 and -9, secretion, SLE, TIMP-1 and -2

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease characterised by altered autoantibody formations against various autoantigens resulting in direct cellular and immune complex-mediated tissue damage. Multiple studies have analysed the role of matrix metalloproetinases (MMPs) and their endogenous inhibitors (TIMPs) in the pathogenesis of autoimmune disorders such as Wegener’s granulomatosis, multiple sclerosis, scleroderma, and Sjogren’s syndrome. MMP-9, MMP-2, and their tissue inhibitors TIMP-1 and TIMP-2 have been implicated and linked to inflammation in autoimmune disorders, in particular, SLE. Earlier studies have shown conflicting results. The studies done in China and Israel reported higher levels of both MMP-9 and MMP-2, where as studies carried out in Poland and Iran reported a higher MMP-2 level but lower MMP-9 levels in the serum of SLE patients. A recent meta-analysis of levels of serum MMP-9 in SLE based on more than 700 patients with SLE and healthy controls did not find any significant association. However it did find that the sample size (n ≥ 60) group had lower MMP-9 levels as compared to controls. MMP-9 and MMP-2, also known as gelatinase B and gelatinase A, cleave denatured collagens (gelatins) and type IV collagen, the major component of basement membranes. This results in the migration of various inflammatory players including lymphocytes and other leucocytes, which enter and leave the blood circulation. In addition, MMP-9 cleaves some proteins leading to remnant epitopes that can generate autoimmunity. Recently, an abnormal regulation of neutrophil extracellular traps (NETs) has been reported in
SLE. They are released by activated neutrophils by a process called ‘NET formation’.16 We hypothesised that the abnormal expression and secretion in neutrophils and lymphocytes of MMP-9 and MMP-2 may lead to disease exacerbation and may play a role in perpetuation of autoimmunity in SLE. It may act as a potential marker for SLE disease progression.

MATERIALS AND METHODS

A total of 41 diagnosed SLE cases (38 females and 3 males) fulfilling the revised American College of Rheumatology (ACR) classification criteria for SLE (1997)17 were recruited along with 50 age and sex matched healthy controls (47 females and 3 males) without any concurrent infections between November 2013 to December 2015 (three years and one month). The study was approved by the Institutional Ethics Committee (IEC) and written consent was obtained from each patient and controls recruited. Anti-nuclear antibodies (ANA) and anti-dsDNA were detected by indirect immunofluorescence (IIF) method and high-sensitivity C-reactive protein (hs-CRP) levels were measured by nephelometry analysis (BN Prospec, Germany).

Peripheral blood mononuclear cell (PBMC) culture
PBMCs were isolated from fresh peripheral blood by Histopaque-1077 (Sigma-Aldrich, USA). After purification, one part of the 1 x 10⁶ PBMCs/ml was directly used for RNA isolation, and another part was cultured at 37 °C and 5% CO₂ under serum-free conditions in RPMI 1640 (Sigma Chemical, USA) supplemented with Nutridoma-SP (Boehringer Mannheim, Mannhein Germany) in the presence of antibiotic-antimycotic solution (HiMedia, India); all the PBMC cell populations (active, inactive, and controls) were activated with 10 µg/ml of phytohemagglutinin (PHA). After 24 hours (hrs), conditioned media was harvested and culture supernatants stored at -20 °C until use.

ELISA Analysis of serum and conditioned media
Matrix metalloproteinases (MMP-2 and MMP-9) and tissue inhibitors of MMPs (TIMP-1 and TIMP-2) were analysed in SLE cases (n = 41) and controls (n = 50) in their respective serum and PBMC culture supernatant conditioned media samples using the RayBio human sandwich enzyme-linked immunosorbent assay system (RayBiotech, Norcross, Georgia, USA) as per the manufacturer’s protocol and instructions.

mRNA expression analysis of MMPs and TIMPs
Fresh whole blood (WB) (500 µl) and 1 x 10⁶ PBMCs/ml (500 µl) were used for RNA isolation by the RiboPure RNA Purification Kit (Ambion, Life Technologies) for 41 samples of SLE patients and 50 controls and converted to cDNA. The mRNA expression was studied using Taq Man Hydrolysis FAM-MGB labelled probes MMP-2 (Hs01548727_m1), MMP-9 (Hs00234579_m1), TIMP-1 (Hs00171558_m1), TIMP-2 (Hs00234278_m1), and 18s (Hs99999901_s1) was used as a reference gene. Data was acquired using the Step-one software version 2.2.2. Data expression levels were recorded as Cq. The mean Cq values of the duplicate reactions were used in further analysis.

Intracellular Assessment of MMP-9 and MMP-2 by flow cytometry
Forty-one SLE cases and 50 controls were included for whole blood flow cytometry work. The permeabilisation protocol was used to detect the intracellular as well as the surface protein antigen in the whole blood. Cells were stained with the following primary monoclonal antibodies: mAb anti-MMP-9 (EP 1255Y) and mAB anti-MMP-2 antibody (EP 1183Y; Novus Biologicals, Littleton, USA). A 1:100 diluted secondary conjugated goat anti rabbit IgG H & L (AlexaFl 488; Abcam, Cambridge, USA) antibody was then added. The results were calculated dividing signal to noise (noise: the reading obtained from the secondary tube that was run with each sample, which contained only secondary antibody and sample) and thus acted as an adjustment for background staining. Cells were analysed using a BD FACS Aria (BD, San Jose, CA, USA) flow cytometer and the obtained data were analysed and visualised using the BD FACSDiva (BD, USA) software. The output for the intracellular MMP-2 and MMP-9 was given in terms of mean fluorescence intensity (MFI) and percentage positivity(%).

Statistics:
GraphPad Prism 6.0 was used for statistical analyses. Data was presented as mean ± SD. The statistical significance between two groups was tested by using Mann-Whitney U-test. Wherever more than two groups were analysed, ANOVA analysis was performed and the results represented in Median-IQR. Spearman’s rank correlation was used for correlation analyses. A P-value < 0.05 was considered statistically significant.

RESULTS

The mean age of SLE patients at the time of evaluation was 28.34 ± 9.25 years. The mean disease duration was 2.20 ± 2.1 years. Severity of the disease was assessed by calculating the SLE Disease Activity Index (SLEDAI).18 The mean SLEDAI score for patients was found to be 13.0 ± 5.2. Based on the SLEDAI score, patients were categorised into active (SLEDAI ≥ 10) and inactive (SLEDAI < 10) groups. Accordingly, there were 22 patients (21 females
and 1 male) in the active group and 19 patients (17 females and 2 males) in the inactive group. In active cases, 12 (54%) cases had lupus nephritis (LN). Of the 41 disseminated lupus erythematosus patients, 39 patients (95.1%) were on corticosteroids, 33 patients (80.48%) were on anti-inflammatory drug (HCQ), 5 patients (12.1%) were on cyclophosphamide, and 4 patients (9.7%) were on alternative biologics.

Serum levels of MMPs and TIMPs
We analysed the circulating protein concentration of MMPs and TIMPs in serum. As shown in figure 1A, circulating MMP-2 concentrations and TIMP-1 secretion were highest in cases with active disease compared to inactive disease ($p = 0.0003$, $p < 0.0001$, respectively). In contrast, circulating MMP-9 concentrations were found to be lowest in active cases as compared to inactive cases and healthy controls ($p < 0.0001$). TIMP-2 secretion was reported to be highest in inactive cases. This result indicated an alteration of MMP/TIMP secretion profiles in the SLE disease activity state.

Gene expression of MMPs and TIMPs
We analysed the mRNA expression in WB as well as fresh PBMCs. WB MMP-2 gene expression was highest in the active cases as compared to inactive cases and controls ($p < 0.0001$) (figure 1B). These results are in accordance with the protein secretion pattern seen in serum. Similarly, the whole blood mRNA expression of TIMP-2 was found to be highest in inactive cases as compared to active cases and controls with no significance. The results were in concordance with the protein secretion of TIMP-2 seen in serum. The MMP-9 gene expression was highest in inactive cases; the MMP-9 gene expression of active cases

**Figure 1.** 10-90 Box and Whisker’s plot of (A) serum concentrations of MMPs and TIMPs in patients and healthy controls; (B) mRNA expression of MMP-9, TIMP-1, and TIMP-2 in whole blood; (C) freshly isolated PBMCs (F-PBMC); (D) secreted MMPs and TIMPs levels in PBMC culture supernatant after 24 hrs (expressed in median-IQR and analysed by ANOVA test).

**MMp = matrix metalloproteinases; p = P-value; PBMC = peripheral blood mononuclear cells; TIMp = tissue inhibitors of MMPs; WB = whole blood; ***p < 0.001; **p < 0.01; *p < 0.05; No astrisk for any P-value < 0.05**

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was also higher as compared to controls, which was in contrast to the secretion profile in serum. TIMP-1 gene expression was highest in inactive cases. This led us to analyse the PBMCs gene expression profile.

In PBMCs, the expression of MMP-2 was 12-fold higher in active cases and 6-fold higher in inactive cases compared to controls (figure 1C). Similar observation was reported for TIMP-1 gene expression in active cases [Median -
interquartile range (IQR); [Relative Quantification (RQ) = 14.91 (14.56 – 15.49)], and TIMP-2 expression in inactive cases [RQ = 3.41 (3.0 – 3.78)] as compared to controls [RQ = 1.13 (1.05 – 1.29)].

**Secretional status of MMPs and TIMPs in PBMC-cultured cells**

We measured secreted MMPs and TIMPs levels in culture supernatants (CS) after 24 hours (figure 1D). Data showed that, even in the absence of any external stimulation, PBMCs spontaneously released significantly higher quantities of MMP-2 in active cases [15.97 ng/ml (15.51 – 16.51)] as compared to inactive cases [10.14 ng/ml (9.36 – 10.55)] which in turn were higher than healthy controls [7.37 ng/ml (7.09 – 7.98)]. The secretional levels of MMP-9 were significantly reduced in active cases [7.36 ng/ml (6.97 – 7.94)] as compared to inactive cases [14.08 ng/ml (13.61 – 14.44)]. For endogenous inhibitors the secretion of TIMP-1 was higher in active cases [11.93 ng/ml (11.40 – 12.86)] as compared to inactive cases [5.49 ng/ml (4.7 – 6.22)].

**Intracellular staining of MMP-2 and MMP-9**

The altered expression pattern in WB and PBMCs led us to analyse the presence of MMP-2 and MMP-9 in circulating neutrophils and lymphocytes by WB flow cytometry (figure 2A). Among SLE patients, MFI values of MMP-9 were significantly higher in neutrophils (6.11 ± 0.12) as compared to healthy controls (2.96 ± 0.10, p < 0.0001), but no difference was observed in the lymphocytes (p = 0.32); the MMP-2 values were higher in lymphocytes (5.51 ± 0.72) as compared to healthy controls (1.65 ± 0.12, p < 0.0001). A similar observation was reported in neutrophils (p < 0.0001) (figure 2B). Among SLE patients, the majority of the lymphocytes showed positivity for MMP-2. In neutrophils, the positivity for MMP-2 was less in SLE cases than in controls. Among the control group, majority of neutrophils showed positivity for MMP-9 (79.98 ± 3.82%) as compared to SLE cases (70.67 ± 3.21%). In lymphocytes, the positivity for MMP-9 was decreased in SLE cases (30.36 ± 4.71%) as compared to controls (48.72 ± 4.57%) (figure 2C).

**Gelatinase/Inhibitor ratios**

To calculate the relative inhibition of the two MMPs, we calculated the ratio between MMPs and TIMPs (table 1). The MMP-2/TIMP-1 ratio showed a consistent pattern of decrease in ratio for active cases as compared to inactive cases for serum secretion. Similar results were obtained for the MMP-9 /TIMP-1 ratio. The ratio of MMP-2/TIMP-2 showed a consistent pattern of increase in active cases as compared to inactive cases which in turn, were significantly higher than controls for serum secretion. The MMP-9/TIMP-2 ratio showed a pattern of increase in controls as compared to active and inactive cases for serum secretion.

| Table 1. Ratios of MMPs to TIMPs in serum |
|-----------------|-----------------|-----------------|
| Ratio           | Serum (ng/ml)   |
|                 | A       | I       | C       |
| MMP-2/TIMP-1    | M 0.19  | 0.27   | 0.16   |
|                 | SD 0.007 | 0.01   | 0.01   |
|                 | P-value < 0.0001< | < 0.0001< | < 0.0001< |
| MMP-9/TIMP-1    | M 1.18  | 2      | 2.98   |
|                 | SD 0.03  | 0.02   | 0.2    |
|                 | P-value < 0.0001< | < 0.0001< | < 0.0001< |
| MMP-2/TIMP-2    | M 0.32  | 0.25   | 0.2    |
|                 | SD 0.01  | 0.02   | 0.006  |
|                 | P-value < 0.0001< | < 0.0001< | < 0.0001< |
| MMP-9/TIMP-2    | M 2      | 1.84   | 3.78   |
|                 | SD 0.05  | 0.16   | 0.11   |
|                 | P-value < 0.0001< | < 0.0001< | < 0.0001< |

P-value using the Mann-Whitney test: < = Active (A) vs. Inactive (I); < = Inactive (I) vs. Controls (C); < = Active (A) vs. Controls (C); M = mean; MMP = matrix metalloproteinases; p = P-value; SD = standard deviation; TIMP = tissue inhibitors of MMPs.
Correlation SLEDAI with markers

Figure 3. Spearman’s correlation analysis of serum levels of MMP-2 (A), TIMP-1 (B), MMP-9 (C), TIMP-2 (D), MMP-9/TIMP-1 ratio (E), and MMP-2/TIMP-2 (F) ratio against SLE disease activity index (SLEDAI).

The mean serum MMP-2 and TIMP-1 levels showed a significant positive correlation with SLEDAI (MMP-2: $r = 0.534$, $p = 0.003$ and TIMP-1: $r = 0.723$, $p < 0.001$) (Figures 3A and 3B). A similar observation was reported in the gene expression of PBMCs ($r = 0.780$) and culture supernatant ($r = 0.727$) for MMP-2 and TIMP-1 ($r = 0.687$, $r = 0.794$, respectively) with SLEDAI. Among the culture supernatants, mean MMP-9 and TIMP-2 levels were 10.48 ± 3.41 and 3.18 ± 2.77 ng/ml, respectively. A significant negative correlation was observed between SLEDAI and MMP-9 ($r = -0.740$, $p < 0.001$) and TIMP-2 levels ($r = -0.731$, $p < 0.001$) (Figures 3C and 3D). The SLEDAI showed a significant negative correlation with MMP-9/TIMP-1 ($r = -0.721$, $p < 0.001$) and a positive correlation with MMP-2/TIMP-2 ($r = 0.735$, $p < 0.001$) in the serum (Figures 3E and 3F).

CRP levels showed a negative correlation with MMP-9 ($r = -0.285$, $p = 0.003$) and TIMP-2 levels ($r = -0.309$, $p = 0.001$). It correlated positively with MMP-2 ($r = 0.148$, $p = 0.122$), TIMP-1 ($r = 0.221$, $p = 0.03$), as well as MMP-2/TIMP-2 ($r = 0.254$, $p = 0.007$) and MMP-9/TIMP-1 ratios ($r = 0.244$, $p = 0.01$).
DISCUSSION

Recent evidence suggests that MMPs are involved in many types of kidney disease. A few studies have been carried out in SLE patients to understand the role of MMPs and TIMPs in the pathogenesis of SLE. Studies carried out in the Chinese SLE cohort by Jiang et al. reported significantly higher levels of serum MMP-2 in advanced stages of the disease. A similar observation was reported by Bahrehmand et al. in Iranian SLE cases. In our study, the serum concentrations of MMP-2 were found to be highest in active cases as compared to inactive SLE cases. The results were in concordance with a study conducted in Greece in kidney biopsies of lupus nephritis patients, which showed a marked increase in expression of MMP-2 and significantly correlated with the higher activity index as compared to absent or weak staining (p < 0.001).

A study carried out in Polish population by Robak et al. in 41 SLE cases and 20 healthy controls reported a decrease in serum levels of MMP-9 in active SLE cases as compared to controls (p = 0.03). Similarly, in accordance with our results, a study done by Liu et al. in 31 SLE cases in PBMC cell culture supernatants reported a decrease in MMP-9 levels in relapsed cases (5.46 ± 4.27 ng/ml) as compared to the patients in remission (12.98 ± 8.07 ng/ml). A study done in Iranian populations by Bahrehmand et al. showed a significant decrease in serum levels of SLE cases as compared to controls (p < 0.001) which are in line with our study. Similar results were obtained in our study, showing lowest MMP-9 serum concentrations in active cases than inactive cases. Serum concentrations of TIMP-1 were higher in active cases as compared to inactive cases. TIMP-1 levels were significantly lower in inactive cases as compared to controls, indicating an alteration in the serum secretion of TIMP-1 as compared to its substrate MMP-9.

A study by Matache C et al. also reported a decrease in the PBMC secretion of TIMP-1, which was not sufficient to inhibit the MMP-9 activity. Further, we revealed that TIMP-2 levels were higher in SLE patients as compared to controls; a similar observation was reported by Jiang et al. in Chinese population. The marked alterations were observed in the gene expressions of MMPs and TIMPs in WB and PBMCs among SLE patients. MMP-2 and TIMP-2 showed a similar mRNA expression pattern in WB, Fresh (F)-PBMCs, and PBMC culture supernatants as seen in serum levels. A study carried out by Matache et al. reported a consistent pattern of increase in mRNA expression of MMP-9 in F-PBMCs and protein secretion in culture supernatants in inactive cases as compared to active cases. Similar findings were observed in our study which extended further to WB mRNA expression and a profile similar to secretion of MMP-9 in serum of inactive cases. The studies conducted by Jonsson et al. which compared mRNA expression of MMP-9 and TIMP-1 in PBMCs with circulating protein concentration failed to find any correlations. This disparity was further reported by Jonsson et al. in myocardial infarction patients and Lichtinghagen et al. in chronic hepatitis C patients, in which they assessed mRNA expression of MMP-9 and its inhibitors in cultured cells but found no significant correlation. Similar results were obtained in our study, which are suggestive of the fact that the MMP-9 and TIMP-1 mRNA is being converted into proteins.

Also, MMP-9 is mainly found in the intracellular tertiary granules of neutrophils. Due to the altered expression and secretion pattern of PBMCs and WB in SLE cases, we decided to analyse this difference in the intracellular levels in neutrophils and lymphocytes. Results confirmed that neutrophils were the predominant source of MMP-9, and lymphocytes as the predominant source of MMP-2 in SLE cases. Although TIMP-1 is an endogenous inhibitor of MMP-9, studies have shown that TIMP-2 has been more effective in inhibiting MMP-9. A study carried out by Jonsson et al. reported contrasting findings to our study in post myocardial infarction patients, in which they reported a significant increase in MMP-9 mRNA expression in PBMCs, which showed a positive correlation with TIMP-1 and TIMP-2, and between TIMP-1 and TIMP-2 at mRNA and protein levels. We found a significant positive correlation between MMP-9 and TIMP-2 in fresh PBMC mRNA and culture supernatants (CS) which were similar to the results obtained by Jonsson et al. We also reported a positive correlation between WB mRNA and CS levels for TIMP-1 and TIMP-2. MMP-2 and TIMP-2 negatively correlated at the serum level and positively at the CS level; an explanation for this would be that in our study, MMP-2 has been mainly found to be expressed and secreted in PBMCs of SLE cases. This study signifies a marked alteration in matrix degradation resulting in enhanced matrix deposition in SLE cases, hence we calculated the ratios between MMPs and TIMPs. Low MMP-2/TIMP-1 and MMP-9/TIMP-2 has been reported in previous studies. The study carried out by Lichtinghagen et al. in peripheral blood cells of hepatitis C induced cirrhosis (Cj) patients reported a lower ratio of MMP-2/TIMP-1 and MMP-9/TIMP-2 as compared to controls. Also a study carried out in chronic hepatitis C patients before therapy, according to alanine transaminase (ALT) responses to interferon, reported a significantly lower MMP-2/TIMP-2 ratio in patients with sustained response than in those with transient response and no response. The authors therefore concluded that the ratio of serum MMP-2/TIMP-1 levels may act as a new predictor for interferon response in patients with chronic hepatitis C patients. Our study also showed a consistent reduction in the pattern of MMP-2/TIMP-1 and MMP-9/TIMP-1 in active cases at all levels, which is suggestive of the increase in TIMP-1 expression and secretion. In the...
MMP-2/TIMP-2 ratio, a consistent pattern of decrease in the order of active, inactive, and controls conveys the fact that MMP-2 expression and secretion increases as the disease progresses, which is in agreement with previous studies.14 The studies carried out by Ahmad et al. in the Egyptian population15 and Liu et al. in the Chinese population16 showed a significantly negative correlation of MMP-9 levels with the SLEDAI scores. A similar negative correlation of MMP-9 levels with the SLEDAI scores was observed in our study. Also, a study conducted by Cauwe B et al. in a gelatinase B/MMP-9-deficient mouse model (B6lpr/fr*MMP-9−/) showed that the MMP-9 deficiency aggravated lymphoproliferation-induced a lupus-like systemic autoimmune disease.16 The above findings were confirmed in a study conducted in LPR/MMP-9−/ double knockout mice that showed an increase in the level of immune-complex in the plasma and also local complement activation in spleen and kidney. It further showed that MMP-9 dissolved immune complexes from plasma of the lupus-prone mice and from blood samples of SLE patients.17 Also, a significant positive correlation of serum CRP levels with TIMP-1 levels (r = 0.221, p = 0.02) in our study are in accordance with the study carried out in Wegener’s granulomatosis patients by Bjerkeli V et al., which reported a significant positive correlation of TIMP-1 levels with CRP (r = 0.74, p = 0.004).3 MMPs have been shown to be modulators of inflammation and autoimmunity, especially SLE.19–25 In this study, we found a decrease in MMP-9 and an increase in MMP-2 as SLE disease progresses. We tried to analyse these alterations by studying their indigenous inhibitors TIMP-1 and TIMP-2, respectively. To make a complete analysis, we studied the mRNA expression in WB and F-PBMC; secretion of proteins in serum and PBMC CS.

Our findings support the theory by Robak et al. that lower MMP-9 concentrations in serum may be suggestive of the fact that in SLE, MMP-9 is transported from blood to the lupoid tissues, especially the blood vessels of active SLE patients. We further extend the theory to neutrophil extracellular traps (NETs), which have been shown to be abnormally regulated in SLE.26,27 There, clearance is impaired, leading to abnormal alteration in MMP-9 secretion and expression, which has been proven for the first time in our study in intracellular levels of neutrophils and lymphocytes of WB for MMP-2 and MMP-9. Our study suggests a significant increase of MMP-9 in neutrophils and MMP-2 in lymphocytes in SLE cases. Therefore, an alteration of MMP/TIMP ratios suggests its importance in the pathogenesis of SLE disease progression.

In summary, our study suggests that the gene expression and serum proteins of MMP-9 and MMP-2, and their natural inhibitors TIMP-1 and TIMP-2 can be used as a potential biomarkers for SLE pathogenesis and they correlate with the SLE disease activity. Therefore, an alteration of MMP/TIMP ratios suggests its importance in the prognosis of SLE disease progression.

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DISCLOSURES

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Lipegfilgrastim for prophylaxis of chemotherapy-induced neutropenia in Dutch patients


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ABSTRACT

Background: Chemotherapy (CT)-induced neutropenia and febrile neutropenia (FN) can lead to changes in the treatment plan, potentially worsening the cancer outcome. This study evaluated the effect of the glycopegylated granulocyte-colony stimulating factor lpegfilgrastim, used as primary (PP) or secondary prophylaxis (SP), on treatment modifications in adult patients receiving cytotoxic CT with or without biological/targeted therapy (BT) for solid and haematological tumours.

Methods: This phase 4, prospective, observational study was conducted in eight centres in the Netherlands, in 2015-2017. Other study objectives were to characterise the population of cancer patients receiving lpegfilgrastim, to evaluate the incidence of CT-induced neutropenic events, and to assess safety.

Results: Of 142 patients, 73.94% had breast cancer and 55.63% received CT in the adjuvant setting. Most patients received lpegfilgrastim as PP (74.65%) and were at low (34.51%) or high risk (39.44%) of FN. CT dose delays were recorded for 22.64% and 36.11% of patients receiving lpegfilgrastim for PP and SP, respectively. CT dose reductions were recorded for 2.11% of patients; no CT dose omissions and one BT dose omission occurred. FN and grade III/IV neutropenia were reported for 5.63% and 9.86% of patients, respectively; associated hospitalisations were rare. The most frequently lpegfilgrastim-related adverse events (AE) were myalgia, bone pain, and back pain. Serious AEs (55) were reported for 30 (21.13%) patients. There were two deaths, unrelated to lpegfilgrastim administration.

Conclusion: Administration of lpegfilgrastim in routine clinical practice in the Netherlands results in limited CT/BT dose modifications and low incidence of neutropenic events, with no new safety concerns.

KEYWORDS

Chemotherapy dose modification, chemotherapy-induced neutropenia, febrile neutropenia, lpegfilgrastim, real-world evidence

INTRODUCTION

Occurrence of neutropenia is a well-known risk of chemotherapy (CT), and in particular, severe and febrile neutropenia (FN) can significantly impact the outcome of cancer treatment, resulting in an increase in infection-associated morbidity and mortality.1 Moreover, neutropenic events may lead to changes in the treatment plan, such as dose reductions and/or delays in subsequent cycles or even switching to less effective treatment options, ultimately decreasing overall response and life expectancy.1,2

To reduce the risk of CT-induced neutropenia, preventive treatment is required and granulocyte-colony stimulating
factors (G-CSFs) have been recognised as effective therapies as they stimulate neutrophil production and differentiation. In a meta-analysis of randomised clinical trials, administration of G-CSFs as primary prophylaxis (PP) was shown to also reduce all-cause mortality compared with patients not receiving G-CSFs, with the decrease in risk varying with CT dose and schedule. Current European guidelines recommend G-CSFs support for all patients receiving a CT regimen associated with a high (> 20%) or intermediate (10–20%) risk of FN, for dose-dense or dose-intense CT strategies, or in subsequent CT cycles following a FN episode. Recently-identified factors such as older age (≥ 65 years) or low neutrophil count should also be included in an assessment of risk carried out before each cycle. Real-world data on the routine use of G-CSFs prophylaxis for CT-induced neutropenia and FN are now available from the United States, where an increase in the use of G-CSFs from 2010 to 2016 led to a decrease in the incidence of FN among cancer patients at intermediate and high risk in the first cycle of CT, despite a clear underutilisation of the recommended prophylaxis.

Lipegfilgrastim (Teva Pharmaceutical Industries Ltd, Israel), a glycopegylated G-CSF, has previously been shown to reduce the duration of neutropenia and incidence of FN in adult cancer patients treated with cytotoxic CT and was approved for use in the European Union in July 2013. In a large prospective, non-interventional study conducted in several European countries, the effectiveness and safety of lipegfilgrastim as PP or secondary prophylaxis (SP) in real-world settings was evaluated in adult patients with different tumour types receiving cytotoxic CT. Here we report data on treatment delays and modifications and incidence of neutropenic events in cancer patients in the Netherlands who received lipegfilgrastim in routine clinical settings.

METHODS

Study design and participants

This phase 4, prospective, observational study was conducted in eight centres in the Netherlands between October 2015 and August 2017. Male and female patients who received cytotoxic CT with or without biological/targeted therapy (BT) for solid and haematological malignant cancer in real-world settings were eligible for enrolment in the study. Lipegfilgrastim was administered as prescribed by the treating physician and according to medical indication, independent of the intention to include the patient into the study. Inclusion criteria were age ≥ 18 years and administration of lipegfilgrastim as PP or SP (following a neutropenic event in a previous CT cycle) of CT-induced neutropenia. Patients were excluded from study enrolment if they had chronic myeloid leukaemia and myelodysplastic syndromes, were pregnant or lactating women, or if they received investigational products as part of a clinical trial.

The study was conducted in full accordance with the Helsinki Declaration and the guidelines for Good Clinical Practice, Good Epidemiological Practice, Good Pharmacovigilance Practices, and Good Pharmacovigilance Practices and was approved by institutional ethics committees at each site. All patients signed an informed consent form prior to enrolment in the study.

Patients were followed from the first use of lipegfilgrastim until 6–8 weeks after the last dose. Data pertaining to patient demographics and baseline characteristics, FN risk factors, CT/BT data (dose delay, reduction, omission), incidence of FN or neutropenia, FN-related hospitalisation and use of anti-infectives and antimycotics, and safety were recorded on electronic case report forms. In agreement with the European Society for Medical Oncology (ESMO) guidelines, FN was defined as an oral temperature of > 38.3 °C or two consecutive readings of > 38.0 °C for two hours and an absolute neutrophil count of ≤ 0.5 × 10⁹/l, or expected to fall below 0.5 × 10⁹/l. The relationship of AEs with lipegfilgrastim administration (not related/related) was assessed by the investigators.

Study objectives

The primary objective of the study was to describe the effect of lipegfilgrastim used in prophylaxis on CT dose modifications, including delay, omissions, and reduction of CT dose administration, in patients receiving cytotoxic CT for solid and haematological cancers, according to routine clinical practice in the Netherlands. Secondary objectives included the description of cancer patients treated prophylactically with lipegfilgrastim in terms of tumour type and stage, CT setting, demographic characteristics, comorbidities and FN risk, and assessment of safety.

Safety assessments

Adverse events (AEs) and serious AEs (SAEs) were coded with the Medical Dictionary for Regulatory Affairs version 20.0 and summarised by preferred term. All AEs were classified by severity as grade 1 (mild), 2 (moderate), 3 (severe or medically significant), 4 (life-threatening, urgent intervention needed), and 5 (fatal), according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. The relationship of AEs to lipegfilgrastim administration (not related/related) was assessed by the investigators.

Statistical analyses

A sample size of 150 participants was estimated based on feasibility considerations in the entire European population.
Analyses were carried out on the full analysis set, which included all enrolled patients meeting eligibility criteria. Patients were stratified by subgroups, as follows: type of prophylaxis (PP versus SP), tumour type, tumour stage, CT regimen use (adjuvant vs metastatic), gender, age, Eastern Cooperative Oncology Group (ECOG) performance status, and FN risk (low, intermediate, and high). No imputation of missing values was performed. Analyses were mainly descriptive and interpreted in an explorative way.

RESULTS

Table 1. Participant characteristics at enrolment (full analysis set)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Safety set (N=142)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age ± SD, years</td>
<td>57.27 ± 12.09</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>122 (85.92)</td>
</tr>
<tr>
<td>Primary tumour, n (%)</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>105 (73.94)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>26 (18.31)</td>
</tr>
<tr>
<td>Haematological malignancies</td>
<td>3 (2.11)</td>
</tr>
<tr>
<td>Prostate</td>
<td>3 (2.11)</td>
</tr>
<tr>
<td>Ovary</td>
<td>2 (1.41)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2 (1.41)</td>
</tr>
<tr>
<td>Stomach</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>Risk factor for FN</td>
<td></td>
</tr>
<tr>
<td>Any factor present</td>
<td>140 (98.59)</td>
</tr>
<tr>
<td>Female gender</td>
<td>122 (88.03)</td>
</tr>
<tr>
<td>Age &gt; 65 years</td>
<td>42 (29.58)</td>
</tr>
<tr>
<td>History of prior FN</td>
<td>25 (17.61)</td>
</tr>
<tr>
<td>Advanced disease</td>
<td>12 (8.45)</td>
</tr>
<tr>
<td>Poor nutritional status</td>
<td>4 (2.82)</td>
</tr>
<tr>
<td>Poor performance status</td>
<td>3 (2.11)</td>
</tr>
<tr>
<td>Other</td>
<td>3 (2.11)</td>
</tr>
<tr>
<td>No factor present</td>
<td>2 (1.41)</td>
</tr>
</tbody>
</table>

FN = febrile neutropenia; n (%) = number (percentage) of patients with the specified characteristic; N = number of patients; SD = standard deviation

Patient characteristics

In total, 144 patients were enrolled in the study and 142 were evaluated (two patients did not receive lipegfilgrastim during the study period). All 142 patients received at least one dose of lipegfilgrastim followed by a post-cycle efficacy evaluation.

The patients’ baseline characteristics are presented in table 1. The mean age was 57 years, with 49 (34.31%) patients being aged ≥ 65 years (table 1). Most patients (94.37%) were Caucasian and the large majority (85.92%) were women. Lipegfilgrastim was received as PP for 106 (74.65%) patients and as SP for 36 (25.35%) in 596 cycles (there were a total of 613 cycles for the entire study population). Lipegfilgrastim was administered as SP due to the occurrence of FN (for 15 patients) or neutropenia (in 30 patients), with most patients starting to receive lipegfilgrastim as SP in the second (10, 7.04%) or third (15, 10.56%) CT cycle.

Forty-nine (34.51%) patients were classified as low risk, 37 (26.06%) as intermediate risk, and 56 (39.44%) as high risk of FN. The most frequent individual risk factors for FN were female gender (122 patients, 88.03%) and age > 65 years (42 patients, 29.56%) (table 1). Two participants (1.4%) had liver disease and 8 (5.6%) had cardiovascular disease.

CT was planned for all patients, with most participants receiving it in the adjuvant (79 patients, 55.63%) and neo-adjuvant (51 patients, 35.92%) setting. Eight patients (5.63%) received CT in a metastatic setting, 2 (1.41%) patients as maintenance, and 2 (1.41%) as other setting. For 28 patients (19.72%), BT was also planned. Most patients received docetaxel/doxorubicin/cyclophosphamide (37 patients, 26.06%), doxorubicin/cyclophosphamide (37 patients, 26.06%), or doxorubicin/cyclophosphamide/vincristine with or without prednisone (17 patients, 11.97%) as CT treatment.

Chemotherapy dose modifications

Overall, 607 administered cycles were analysed, and the delay of CT ranged between 0 and 3.5 days, with a mean delay of 0.8 ± 3.34 days. The mean delay period was higher in patients using lipegfilgrastim as SP than as PP (1.17 versus 0.71 days) and in patients aged ≥ 65 years compared with those aged < 65 years (table 2). Overall, delays were recorded for 37 (26.06%) patients, with higher proportion of patients reporting delays in SP versus PP, blood tumour versus breast tumour, and intermediate/low risk versus high risk of FN subgroups (table 2).

Dose reductions were recorded for 3 (2.11%) female patients with breast cancer using lipegfilgrastim as PP (one reduction each in cycle 1, 2, and 4). All three had an Eastern Cooperative Oncology Group (ECOG) status < 2 and a low risk of FN.

No CT dose omissions occurred.

No dose reduction of BT treatment was reported in any patient. Only one BT treatment omission was recorded, for a male patient aged ≥ 65 years, receiving lipegfilgrastim
as PP; the patient had lymphoma, used CT in the adjuvant setting, and presented intermediate risk of FN.

**Incidence of neutropenia and hospitalisation/treatment**

Overall, 8 (5.63%) patients reported FN. The majority (six) were female with an ECOG score < 2 and three received lipegfilgrastim as PP. FN was reported in four patients with lymphoma, two with breast cancer, one with pancreatic cancer, and one with stomach cancer. Most participants with reported FN used adjuvant CT (five patients) and were at low (three patients) or intermediate (four patients) risk of FN. Grade III/IV neutropenia at a given time were recorded in 14 (9.86%) patients, with 9 (6.34%) and 5 (3.52%) patients reporting grade III and grade IV neutropenia, respectively. Four (2.82%) patients were hospitalised due to FN, 2 (1.41%) due to CT-induced neutropenia; 39 (27.46%)

### Table 2. Summary of chemotherapy treatment delays, by subgroup (full analysis set, n = 142)

<table>
<thead>
<tr>
<th></th>
<th>n (%)</th>
<th>Number of observations</th>
<th>Mean CT delay ± SD (min-max) (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of prophylaxis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>24 (22.64)</td>
<td>487</td>
<td>0.71 ± 3.32 (0–35)</td>
</tr>
<tr>
<td>Secondary</td>
<td>13 (36.11)</td>
<td>120</td>
<td>1.17 ± 3.04 (0–21)</td>
</tr>
<tr>
<td><strong>Tumour type</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haematological malignancies</td>
<td>2 (66.67)</td>
<td>19</td>
<td>2.32 ± 7.06 (0–30)</td>
</tr>
<tr>
<td>Breast</td>
<td>25 (23.81)</td>
<td>440</td>
<td>0.47 ± 2.33 (0–28)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>9 (34.62)</td>
<td>119</td>
<td>1.35 ± 4.64 (0–35)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1 (50.00)</td>
<td>8</td>
<td>6.13 ± 8.72 (0–21)</td>
</tr>
<tr>
<td><strong>CT setting</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjuvant</td>
<td>24 (30.38)</td>
<td>340</td>
<td>1.04 ± 3.94 (0–35)</td>
</tr>
<tr>
<td>Neo-adjuvant</td>
<td>9 (17.65)</td>
<td>216</td>
<td>0.21 ± 1.13 (0–8)</td>
</tr>
<tr>
<td>Metastatic</td>
<td>1 (25.00)</td>
<td>30</td>
<td>0.93 ± 3.24 (0–16)</td>
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<tr>
<td>Maintenance</td>
<td>2 (50.00)</td>
<td>12</td>
<td>4.08 ± 7.59 (0–21)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (50.00)</td>
<td>9</td>
<td>1.00 ± 2.12 (0–6)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7 (35.00)</td>
<td>91</td>
<td>1.89 ± 5.88 (0–35)</td>
</tr>
<tr>
<td>Female</td>
<td>30 (24.59)</td>
<td>516</td>
<td>0.60 ± 2.61 (0–28)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 65 years</td>
<td>23 (24.73)</td>
<td>405</td>
<td>0.66 ± 3.06 (0–30)</td>
</tr>
<tr>
<td>≥ 65 years</td>
<td>14 (28.57)</td>
<td>202</td>
<td>1.08 ± 3.82 (0–35)</td>
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<tr>
<td><strong>ECOG status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2</td>
<td>35 (25.36)</td>
<td>593</td>
<td>0.71 ± 3.02 (0–30)</td>
</tr>
<tr>
<td>≥ 2</td>
<td>2 (50.00)</td>
<td>14</td>
<td>4.50 ± 9.35 (0–35)</td>
</tr>
<tr>
<td><strong>FN risk</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Low</td>
<td>17 (34.69)</td>
<td>190</td>
<td>0.97 ± 3.63 (0–30)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>11 (29.73)</td>
<td>146</td>
<td>1.42 ± 4.47 (0–35)</td>
</tr>
<tr>
<td>High</td>
<td>9 (16.07)</td>
<td>271</td>
<td>0.34 ± 2.14 (0–23)</td>
</tr>
</tbody>
</table>

CT = chemotherapy; ECOG = Eastern Cooperative Oncology Group; FN = febrile neutropenia; n (%) = number (percentage) of patients with CT treatment delays in each subgroup; N = number of patients included in the analysis; SD = standard deviation

Note: Subgroups with no CT treatment delays were not presented.
patients were hospitalised for other reasons, not related to neutropenic events. Anti-infective treatment was used in 54 (38.03%) patients, with a mean duration of 13.97 ± 16.84 days. For 5 (3.52%) and 1 (0.70%) of these patients, the use was related to FN and CT-induced neutropenia, respectively (Supplementary material, table S1). Five (3.52%) patients were administered intravenous anti-infectives for FN. Antimycotic treatment was used a total of 49 times in 23 (16.31%) patients (Supplementary material, table S2) and the mean duration was 27.41 ± 28.72 days; the treatment was not related to FN and CT-induced neutropenia for any of the patients.

Safety
At least one treatment-emergent AE (TEAE) occurred in all patients, the most frequently reported being myalgia (22.53%), bone pain (17.61%), pyrexia (14.79%), and back pain (11.27%). Most TEAEs (88.66%) were mild or moderate in nature and 41.06% were considered related to study medication. Myalgia (19.01%), bone pain (16.20%), back pain (7.75%), and arthralgia (5.63%) were the most frequent TEAEs related to lipegfilgrastim administration. Three patients (2%) discontinued lipegfilgrastim prematurely due to adverse events. Overall, 55 SAEs were recorded in 30 (21.13%) patients, with the most frequent being pyrexia and pneumonia (each in seven cases), and FN and lung infection (each in four cases); most of them were mild to moderate. In total, 14 SAEs in 10 (7.04%) patients were considered as possibly related to lipegfilgrastim administration. Fatal events were reported for 2 (1.41%) patients, with the AEs leading to death being advanced disease and cancer progression for one patient and ureteral stent infection (urosepsis) for the other. Neither deaths were considered related to lipegfilgrastim.

Discussion
This is the first study assessing CT dose modifications following administration of lipegfilgrastim as prophylaxis for CT-induced neutropenic events in real-world settings in Dutch patients. Our findings confirm effectiveness and raises no new concerns about safety when compared to clinical trial results. This study showed that dose delay was the most common modification of CT treatment following lipegfilgrastim administration, occurring in 26.06% of patients, while CT dose reductions were rare, reported for only 2.11% of patients. No CT dose omissions occurred. Dose delays were reported more frequently in patients receiving lipegfilgrastim as SP than those receiving it as PP (36.11% versus 22.64%). In the real-world prospective study assessing the same objectives in a larger, European population which included Dutch patients, slightly lower rates were observed for overall dose delays, reductions, and omissions, but similar to our study, dose modifications were reported with higher frequency, when lipegfilgrastim was used for SP rather than for PP (for 28.1% and 20.1% of patients). In contrast to our observations, dose modifications in Belgian patients occurred in 33.3% and 52.4% of patients receiving lipegfilgrastim as PP and SP, respectively, with dose reductions being more common. Of note, most Belgian patients had breast cancer and more than half (54.7%) received dose-dense regimens, unlike in our study. In another non-interventional study conducted in 2489 German patients undergoing CT in routine clinical practice (NADIR), dose delays were observed at lower rates than in the current study for 11.2% of patients. However, in the NADIR study, 16.3% of patients received lipegfilgrastim as SP, compared to 25.35% of the Dutch population, which may constitute one of the possible explanations for the difference in dose delay rates. In contrast, dose reductions were reported more frequently in the NADIR study in 19.6% of patients. However, these comparisons are hindered by differences in the characteristics of patients in each study, which varied, also in terms of tumour type, stage, metastasis, age, performance status, or FN risk. Of note, a higher percentage of Dutch patients were considered at low risk of FN (34.51%), compared with <10% in the NADIR study. In clinical trial settings, the rates of dose delays ranged from 16.2% to 30.7% of patients in the first cycle after initial administration of lipegfilgrastim, while no dose omissions/reductions were reported, suggesting that dose modifications can vary greatly with demographics, tumour type, and prescribed CT. Overall, FN was observed in 5.63% of patients in our study and grade III and IV neutropenia occurred in 6.34% and 3.52% of patients. Compared to our study, a lower incidence (2.7%) was observed in the NADIR study for FN and higher rates of severe neutropenia (26.8%) were reported. Despite differences in the proportion of patients at low risk of FN and lipegfilgrastim prophylactic administration for the two populations, both studies show that lipegfilgrastim is effective in reducing CT-induced neutropenic events and further confirm effectiveness data observed in phase III clinical trials. Randomised head-to-head trials have been conducted between lipegfilgrastim and pegfilgrastim. A meta-analysis concludes lipegfilgrastim showed a lower, nonsignificant risk of febrile neutropenia compared with pegfilgrastim (risk ratio (RR) = 0.34, 95% CI: 0.05, 2.14). Lipegfilgrastim has a statistically significantly shorter absolute neutrophil count recovery time without significant differences in bone pain. Moreover, in the current study, the rates of
FN/neutropenia-related hospitalisations, intensive care unit stays, and use of anti-infectives/antimycotics were low, suggesting that the associated cost can be decreased considerably when lipegfilgrastim is used as prophylactic treatment of CT-induced neutropenic events. According to several modelling studies, lipegfilgrastim was estimated to be a likely cost-effective alternative to other G-CSFs in patients with breast cancer.

Lipegfilgrastim was well tolerated, with a safety data comparable to that observed in the NADIR study. A comparable proportion of Dutch and German patients experienced SAEs (21.13% versus 18.0%, respectively), and none of the fatal events occurring during the two studies were related to lipegfilgrastim administration. Myalgia, bone pain, and back pain were the most frequent lipegfilgrastim-related TEAEs in the current study, consistent with observations from real-life studies and the safety profile assessed in pivotal clinical trials. Of note, in the NADIR study, patients evaluated the administration as easy to handle and the most frequently documented reason for discontinuing lipegfilgrastim prematurely was that prophylaxis with lipegfilgrastim was no longer considered necessary.

Our study has several potential limitations. Specific reasons for changes in CT treatment were not collected and therefore, we cannot conclude on a potential association between lipegfilgrastim administration and CT treatment modifications. Analyses were mainly descriptive and the sample size was relatively low, although patients from eight centres in the Netherlands were enrolled in the study which is likely to ensure a representative sample. Selection bias and confounding by indication are potential biases, but the majority of the included patients were at risk of FN (table 1) and lipegfilgrastim support was according to current guidelines. Data in the entire European population will soon be available and will provide an improved interpretation of the results for the Dutch patients in the larger context of clinical practice in Europe. In conclusion, the use of lipegfilgrastim as either PP or SP during cytotoxic CT treatment led to a relatively low proportion of patients with CT dose modifications or with neutropenic events, in real-world settings in the Netherlands. Administration of lipegfilgrastim was well tolerated, with no new safety concerns arising during the study.

ACKNOWLEDGEMENTS

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DISCLOSURES

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*Supplementary materials are available from the authors upon request.

REFERENCES


Symptomatic rebound methaemoglobinaemia after treatment with dapsone

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ABSTRACT

A 28-year-old female patient was admitted to our hospital with severe dyspnoea and hypoxemia due to methaemoglobinaemia caused by dapsone. The patient recovered completely after repeated infusions of methylene blue and cessation of dapsone. However, 12 days after cessation of dapsone, the patient was readmitted due to recurrence of symptoms based on a relapse of methaemoglobinaemia. Toxicological analysis revealed a toxic dapsone level at readmission and no other explanation for methaemoglobinaemia. Several possible mechanisms as explanation for the recurrence of methaemoglobinaemia are listed and additional tests were performed. In addition to supportive care, treatment consisted of methylene blue; furthermore, cimetidine and ascorbic acid were added. An overview of the pathophysiology, diagnostics, treatment, and possible explanations for this relapse of methaemoglobinaemia caused by dapsone are given. This case shows the importance of considering the possibility of a late rebound methaemoglobinaemia after discontinuation of dapsone.

KEYWORDS

Dapsone, methaemoglobinaemia, methylene blue, rebound

CASE DESCRIPTION

A 28-year-old woman visited the Emergency Department of our hospital with acute hypoxic respiratory distress. The patient was experiencing progressive dyspnoea for a couple of days and had experienced multiple fainting spells. On examination, she had an oxygen saturation measured with pulse oximeter of 80-85% (SpO₂), and central and peripheral cyanosis, which did not improve with supplementation of 15 litres of oxygen/minute using a non-rebreather mask. Because of her condition, the patient was immediately intubated and ventilated with pressure support. Unfortunately, SpO₂ also did not improve despite maximum fraction of inspired oxygen (FiO₂) of 100%. A CT scan ruled out pulmonary embolism and intraparenchymatous abnormalities explaining the low saturation. Transthoracic ultrasound showed normal ventricular function and no signs of cardiac shunting. Arterial blood samples were drawn, which had a striking chocolate brown colour, and blood gas analysis showed a normal oxygen saturation of 98%, in comparison with the measured 85% with pulse-oximetry. Surprisingly, blood gas analysis showed that the methaemoglobin (MetHb) level was 21.6% (0.216 mol/mol), almost 15 times higher than the cut-off normal value of 1.5% (0.015 mol/mol).

After initial stabilisation and treatment, additional information about her medical history became clear. She was diagnosed with coeliac disease and was also suspected of dermatitis herpetiformis, for which the antibiotic dapsone was prescribed and dosage was increased from 50 to 100 mg, once daily, four days prior to her visit to the Emergency Department. Based on these results, the most likely diagnosis was thought to be symptomatic methaemoglobinaemia caused by dapsone. Methylene blue was given intravenously, after which her MetHb level quickly declined (figure 1) and shortly after infusion, she was extubated because of substantial clinical improvement, in particular, the normalisation of her blood oxygenation. Dapsone was discontinued.

During the next two days, however, her levels of MetHb slowly increased without any symptoms. Treatment with methylene blue was repeated, returning MetHb values to a normal level. Four days after presentation, the patient was discharged from the hospital.
Surprisingly, seven days after discharge and 12 days after discontinuing dapsone, the patient presented again with symptoms of progressive dyspnoea, which appeared to be based on a recurrent symptomatic methaemoglobinaemia. MetHb level was 15.6% (0.156 mol/mol), a 3-fold increase compared to the levels at time of discharge. The patient was readmitted and again treated with methylene blue, and after two doses, her MetHb level stayed within normal ranges (figure 1). Toxicological analysis of a blood sample taken 12 days after discontinuation of dapsone showed a dapsone concentration of 20.8 mg/l, which is within the toxic range since the reference therapeutic concentrations are between 0.5-2.0 mg/l. Unfortunately, no toxicological analysis was performed on blood samples taken during the first episode. The patient assured us she had not taken any dapsone, or any other medication or drugs of abuse such as ‘poppers’, which was confirmed by toxicological screening. Her pharmacy confirmed that she had turned in the remaining tablets after discharge from the previous admission; furthermore, she used no medication influencing pharmacokinetics of dapsone. The toxicological analysis supports our theory that dapsone is the most likely agent for the relapse of the methaemoglobinaemia with a score of 7, as assessed by the Naranjo algorithm for assessment of adverse drug reactions.1,2

Due to the toxic concentration, we hypothesised that the elimination of dapsone was delayed or that there was a rebound. Based on literature which will be reviewed later, cimetidine and ascorbic acid were added to the treatment to prevent a new relapse and to further diminish MetHb levels. Two days after readmission, the patient could be discharged from the hospital free of symptoms. Frequent outpatient clinic follow-up showed no increase of MetHb levels thereafter, and the concentration of dapsone decreased to 2.7 mg/l in five days. Since then, there has been no more relapse of symptomatic methaemoglobinaemia (figure 1).

Methaemoglobinaemia by dapsone
Dapsone is an antimicrobial agent registered for the treatment of leprosy and dermatitis herpetiformis. It can sometimes be used for other inflammatory conditions or as prophylaxis for pneumocystis jiroveci pneumonia on an off-label basis. Serious side effects are rare but include agranulocytosis, methaemoglobinaemia, and haemolysis, especially in patients with G6PD-deficiency.3 Methaemoglobinemia is a congenital or acquired functional anaemia in which the ferrous iron (iron(II) or Fe$$^{2+}$$) of haemoglobin is oxidised to ferric iron (iron(III) or Fe$$^{3+}$$). Under normal conditions, iron is reduced to Fe$$^{2+}$$ to maintain a MetHb level of < 1%. This reduction takes place by two pathways involving nonenzymatic antioxidants: the cytochrome b5 and NADPH reductase pathways (figure 2). Medication and toxins are the major cause of acquired methaemoglobinaemia. Dapsone, chloroquine, primaquine, and local anaesthetics such as benzocaine and lidocaine are most common; the latter, even if administered topically, can cause significant methaemoglobinaemia. Chemicals known to cause methemoglobinemia are anilines, hydrogen peroxide, chlorates, and nitrates, ranging from polluted water and high-nitrate food to party drugs and medicinal nitrates.4 In dapsone, the toxic metabolite hydroxylamine is responsible for oxidising haemoglobin and thus the formation of MetHb.5
Oxidised iron in haem is not capable of dissociating oxygen because it changes the conformation of the remaining haemoglobin, resulting in a higher oxygen (O$_2$) affinity. The oxygen dissociation curve shows a left shift and O$_2$ delivery to the tissues is decreased, leading to cellular hypoxia and cyanosis. Symptoms vary based on MetHb concentration, ranging from asymptomatic cyanosis and headache to coma and severe hypoxic symptoms, as summarised in table 1.

Clinical clues of methaemoglobinaemia are chocolate brown blood, no improvement of blood oxygen saturation measured by pulse-oximetry despite supplementation of oxygen with 100% FiO$_2$ and a so called ‘saturation gap’: a difference between saturation measured with pulse-oximetry and blood gas analyser. A simple bedside test can distinguish deoxyhaemoglobin from MetHb: Blood containing concentrations of MetHb of 20% and larger can appear chocolate brown; the colour gets darker with an increase in MetHb concentrations. When blood is exposed to atmospheric oxygen, for example, in cases of MetHb, its appearance does not change in contrast to deoxyhaemoglobin, in which its colour changes to bright red. This test is used in low resource settings to establish methaemoglobinaemia; it is also used to estimate the percentage of MetHb.

Pulse oximeters measure the ratio of absorbance from pulsatile light transmission through vascular beds at two wavelengths, typically 660 and 940 nm, based upon the spectral properties of oxyhaemoglobin and deoxyhaemoglobin. The spectral properties of MetHb cause an underestimation of O$_2$ saturation by pulse oximetry in increasing MetHb levels and eventually a stable plateau of approximately 85% saturation. Additionally, after administration of methylene blue, it becomes impossible to measure O$_2$ saturation by pulse oximetry since methylene blue also absorbs light at the same wavelengths, and is not confined to the vascular system as it is also present in other tissues. However, some pulse oximeters use multiple wavelengths and can discriminate methaemoglobin, such as the Rad-57 Pulse CO-Oximeter (Masimo, Switzerland).

**Blood gas analysis**

While pulse oximetry suffers from interference by MetHb and methylene blue, the spectrophotometry-based blood gas analysis by modern analysers is accurate. Unfortunately, there still are misunderstandings about the interference of methylene blue in the measurement of the MetHb concentration. Previously, MetHb was determined from the absorption measured at two wavelengths, analogously to modern pulse oximetry, while cyanide was used to distinguish MetHb from other haemoglobin (Hb) derivatives (Evelyn-Malloy method). Later, carbon monoxide (CO)-oximetry methods were developed using four or more wavelengths, theoretically enabling the simultaneous determination of oxygenated Hb (O$_2$Hb), deoxygenated Hb (HHb), carboxyhaemoglobin (COHb), and MetHb. Unfortunately, various CO-oximetry methods using 4 or up to 17 wavelengths are reported to yield inaccurate MetHb measurements in the presence of methylene blue.

Today however, using modern analysers, the interference of methylene blue on MetHb measurement is negligible.

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**Table 1. Symptoms of methaemoglobinemia**

<table>
<thead>
<tr>
<th>Methaemoglobin%</th>
<th>Severity</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 2</td>
<td>Normal</td>
<td>None</td>
</tr>
<tr>
<td>2-15</td>
<td>Mild</td>
<td>None in healthy individuals</td>
</tr>
<tr>
<td>15-30</td>
<td>Mild to moderate</td>
<td>Cyanosis, headache, fatigue, chocolate brown blood, dyspnoea</td>
</tr>
<tr>
<td>30-50</td>
<td>Moderate</td>
<td>Dizziness, syncope, confusion, weakness</td>
</tr>
<tr>
<td>&gt; 50</td>
<td>Severe</td>
<td>Seizures, coma, dysrhythmias, acidosis, death</td>
</tr>
</tbody>
</table>

---

G6PD = glucose 6 phosphate dehydrogenase; HHb = deoxyhaemoglobin; MetHb = methaemoglobin; NAD = nicotinamide adenine dinucleotide; NADP = nicotinamide adenine dinucleotide phosphate; NADPH = nicotinamide adenine dinucleotide phosphate hydrogen
For example, the ABL90 Flex Plus (Radiometer, Denmark), utilises CO oximetry with a 236-wavelength spectrophotometer in the range of 467-672 nm. The theoretical spectra of O₂Hb, HHb, COHb, MetHb, fetal Hb (HbF), and bilirubin are fitted to the measured spectrum from the patient's sample, enabling the separate determination of the concentration of these six parameters. Due to the large number of wavelengths used to determine the concentrations, the influence of interference is minimised. Therefore, MetHb results obtained using this blood gas analyser are correct and may be used safely in clinical practice.

**TREATMENT**

Treatment is indicated in cases where MetHb concentrations are > 20% or in cases with symptoms of impaired tissue oxygenation. The first reference to methylene blue as a treatment of methaemoglobinaemia is from the early 1930s, but no clinical trials were published. Methylene blue is a cofactor of the nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) reductase pathway and forms leucomethylene blue, which acts as an electron donor and reduces MetHb to Hb (figure 2).

The usual dose is 1–2 mg/kg in a 1% solution, intravenously administered over 5 to 10 minutes. The effect is expected in minutes to one hour. Treatment can be repeated to a maximum of 4 mg/kg/day to prevent toxicity, mainly paradoxical (mild) haemolysis.

Methylene blue is ineffective in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency and can even cause severe hemolysis. G6PD is a rate-limiting enzyme of the pentose phosphate pathway, which reduces nicotinamide adenine dinucleotide phosphate (NADPH) to NADPH; therefore in patients with G6PD deficiency, there are low levels of NADPH. Insufficient levels of NADPH fail to reduce methylene blue to its active metabolite leucomethylene blue and therefore cannot reduce MetHb to Hb. Methylene blue can induce G6PD-related haemolysis due to the formation of free radicals and direct oxidative stress, for which G6PD-deficient erythrocytes are more vulnerable. In cases of G6PD deficiency, ascorbic acid can be used at high doses up to four grams per day, which acts as a nonenzymatic MetHb-reducing agent. In addition, the hydrogen (H₂) antagonist cimetidine can be added in a daily dose of 400 mg once. Cimetidine reduces the activity of several cytochrome P-450 enzymes, which are responsible for the formation of dapsone's toxic metabolite hydroxylamine, and therefore lowers the formation of hydroxylamine. In extreme cases of methaemoglobinaemia, exchange transfusion must be considered, particularly in children.

**REASONS FOR REBOUND**

To explain the relapse in our patient after the first discharge with normal MetHb levels, we performed a literature search and investigated several options. First, delayed renal clearance was excluded since her kidney function was normal (Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) 107 ml/min). Second, G6PD deficiency was unlikely due to the significant drop in MetHb concentration after methylene blue administration. In cases of G6PD deficiency, methylene blue would not have this effect because G6PD is essential for the production of NADPH, which is responsible for the reduction of Fe³⁺ to Fe²⁺. The absence of G6PD deficiency was confirmed by determining the G6PD activity in our patient (172 U/mmol Hb; reference interval 82-130 U/mmol Hb). Third, to rule out delayed metabolism due to pharmacogenetic variants, the enzymatic pathways responsible for the metabolism of dapsone were analysed through assessing genetic variants of the specific enzymes. Dapsone is acetylated by N-acetyltransferase 2 (NAT2) to the inactive metabolite monoacetyldapsone and will therefore be deacetylated. Further metabolism is through CYP3A4 and CYP2C9. The toxic metabolite hydroxylamine, is obtained by CYP2C9 and CYP2C19. Pharmacogenetic analysis revealed that there were no deficiencies in these enzymes; the patient was an intermediate metaboliser for CYP3A4 (*1/*2) and NAT2 (*4/*5), and a normal metaboliser for CYP2C9 (*1/*1). Therefore, we excluded delayed metabolism of dapsone as cause of recurring methaemoglobinaemia.

Furthermore, we found several theoretical explanations in earlier case reports also describing the recurrence of methaemoglobinaemia after the cessation of drug intake. First, dapsone has lipophilic characteristics (Vₘₐₜ = 1 l/kg and Log P = 0.97) and will therefore mostly be found in peripheral tissues. It has been hypothesised that dapsone concentrations in blood could be increased for a prolonged period as a result of the distribution of dapsone from the peripheral tissue to the blood. Additionally, dapsone undergoes enterohepatic circulation, which is responsible for high concentrations in the tissue even after three weeks of discontinuation; however, it is highly unlikely that this mechanism can be responsible for the toxic levels found in our case since most of the elimination of dapsone metabolites is in the urine. Moreover, once dapsone is converted into hydroxylamine and absorbed by erythrocytes, it can be recycled and converted back into dapsone. This recycling pathway may be responsible for the persistence of dapsone in the blood and thus the relapse of methaemoglobinaemia.

A possibility that should always be considered is re-initiation of dapsone, possibly as part of Munchhausen
syndrome. However, the patient denied taking any additional dapsone after the first hospital admission and disposal of the remaining dosages was confirmed by her pharmacy.

**CONCLUSION**

We gave an overview of the pathophysiology; diagnostics, including potential interference of methylene blue with measurement of MetHb in modern blood gas analysers; and treatment of methaemoglobinaemia caused by dapsone. This case shows the importance of considering the possibility of a late rebound methaemoglobinaemia after discontinuation of dapsone and possible pathophysiological explanations for this phenomenon.

**DISCLOSURES**

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

**REFERENCES**


Jonkers et al. Rebound methaemoglobinaemia after dapsone
CASE REPORT

HLH caused by an HSV-2 infection: a case report and review of the literature

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ABSTRACT

Haemophagocytic lymphohistiocytosis (HLH) is a rare hyperinflammatory condition that can be triggered by infections, malignancies, or auto-immune diseases. Here, we present a patient with rapidly progressive HLH triggered by a herpes simplex virus type 2 (HSV-2) primary infection. The patient was successfully treated with intravenous high-dose acyclovir, immunoglobulins, and dexamethasone. This is the first report of HSV-2-associated HLH in an immunocompetent adult patient.

KEYWORDS

Haemophagocytic lymphohistiocytosis, HSV-2

BACKGROUND

Haemophagocytic lymphohistiocytosis (HLH) is a rare, but potentially fatal hyperinflammatory condition that is often not recognised due to a lack of awareness among physicians. It can present with nonspecific symptoms including fever, cytopenias, and multiple organ failure. The diagnostic guidelines by the Histiocyte Society in 1991 are recognised, but their sensitivity and specificity were never validated.

HLH can be triggered by inborn disorders of the cytotoxic leucocytes, haematologic malignancies, auto-immune disorders, medication, or infections. Herpes viruses are associated with HLH: Epstein-Barr virus, cytomegalovirus, and human herpesvirus-8 infections are the most often reported triggers, in both immunocompetent and immunodeficient patients. However, herpes simplex virus type 2 (HSV-2)-related HLH is rare. Here, we present a patient with rapid progressive HLH caused by an HSV-2 primary infection.

What was known on this topic?

Haemophagocytic lymphohistiocytosis (HLH) is a rare, but potentially fatal hyperinflammatory condition which can present with nonspecific symptoms including fever, cytopenias, and multiple organ failure. HLH can be triggered by inborn disorders of the cytotoxic leucocytes, haematologic malignancies, auto-immune disorders, medication, or infections.

What does this add?

HLH caused by a disseminated HSV-2 infection is rarely described in immunocompetent patients. If clinically suspected, HSV serology and PCR should be screened in patients with idiopathic HLH. HSV-2-associated HLH has a relatively good prognosis when adequate anti-viral and immunosuppressive therapy is started at an early stage.

CASE PRESENTATION

A 63-year-old woman presented with acute fever, confusion, and progressive right upper quadrant pain. Her medical history included hypothyroidism and chronic lower back pain. She had consulted her general practitioner two days prior because of fever and dysuria, and amoxicillin/clavulanate was started to treat a presumed urinary tract infection. An additional history revealed unsafe sexual intercourse with a new partner one week before the onset of her current symptoms.

On general examination, a severely ill patient with a fever of 39 °C and genital ulcers were seen. Palpation of the right upper quadrant of the abdomen was painful without signs of peritonitis. She had no lymphadenopathy or organomegaly.
Initial blood investigations showed a mild thrombopenia (thrombocytes of $123 \times 10^9/\text{l}$), leucopenia (white blood cell count of $3.7 \times 10^9/\text{l}$), and a non-icteric hepatitis (alanine aminotransferase (ALT) of 1628 IU/l and aspartate aminotransferase (AST) of 1566 IU/l). Cefuroxime was started to treat a sepsis of unknown origin.

A CT scan of the abdomen showed a normal aspect of the gallbladder but a wide common bile duct and multiple liver cysts, and some prominent lymph nodules around the mesenteric vessels. An endoscopic ultrasound was performed, which showed acalculous cholecystitis but no signs of biliary obstruction.

**Figure 1.** Clinical course. Treatment with acyclovir, IV immunoglobulins, and dexamethasone was started on day 3. Changes of ASAT, ALAT, and ferritin (A). Course of HSV-2 load in plasma (B). Cycle threshold (Ct) values are inversely correlated to the HSV-2 load. A Ct value of > 45 means that the PCR result is negative.

<table>
<thead>
<tr>
<th>Day</th>
<th>ASAT</th>
<th>ALAT</th>
<th>Ferritin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>20</td>
<td>25</td>
</tr>
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<tr>
<td>25</td>
<td>55</td>
<td>60</td>
<td>65</td>
</tr>
</tbody>
</table>

ALAT = alanine transaminase; ASAT = aspartate aminotransferase; HSV-2 = herpes simplex virus type 2; IV = intravenous
During the second day of admission, the liver enzymes and bilirubin level increased rapidly (ALT of 1830 IU/l and AST of 2581 IU/l). The presence of hyperferritinaemia (36,700 ng/ml) and progressive pancytopenia led to the suspicion of HLH that was confirmed by a bone marrow aspirate. Simultaneously, liver failure progressed and the patient was transferred to the nearest academic medical centre. There, a primary infection with HSV-2 was shown: IgM antibodies against herpes simplex virus without IgG antibodies. Additionally, a PCR assay of the EDTA plasma was strongly positive for HSV-2 but negative for herpes simplex virus type 1. A subsequent liver biopsy also showed HSV-2 infection, confirmed by immunohistochemistry, with chronic infiltration and necrosis of 40% of the liver parenchyma with focal haemophagocytosis. Treatment with intravenous high-dose acyclovir (10 mg/kg, 3 times daily), intravenous immunoglobulins and dexamethasone was started on hospital day three. The fever quickly subsided and the liver enzymes started to improve after two days (figure 1, panel A). The HSV-2 load decreased during and after hospital admission, whereby the HSV-2 DNA was still detectable after three months (figure 1, panel B).

The patient showed a rapid recovery and was discharged 32 days after initial presentation. Oral valacyclovir was continued until one month after discharge. The dexamethasone was gradually tapered and was finally discontinued after 74 days of treatment.

**DISCUSSION**

Here, we describe a patient with HLH and acute fulminant liver failure after a primary genital HSV-2 infection. HSV-2 is a common sexually transmitted disease that is usually self-limiting and asymptomatic, but can result in a more serious infection depending on the immune status of the host. Acute hepatitis with liver failure is a rare complication that can be seen in patients with an HSV infection, either both HSV-1 or HSV-2. In our case, the patient developed fulminant liver failure within three days caused by both HSV-2 and reactive haemophagocytosis. The liver enzymes improved, accompanied by a decreasing HSV-2 viral load rapidly after anti-inflammatory and anti-viral treatment.

### Table 1. HLH and HSV-2: five reported cases

<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Sex</th>
<th>Age</th>
<th>Underlying condition</th>
<th>Concomitant immuno- suppressive medications</th>
<th>Primary infection or reactivation?</th>
<th>Treatment</th>
<th>Course of disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>Yamaguchi</td>
<td>Female</td>
<td>Unknown</td>
<td>Pregnant</td>
<td>None</td>
<td>Unknown</td>
<td>Acyclovir, prednisolone, IVIG, and cyclosporine</td>
<td>Patient improved after administration of cyclosporin in a few days</td>
</tr>
<tr>
<td>2006</td>
<td>Ramasamy</td>
<td>Female</td>
<td>33</td>
<td>Bone marrow failure syndrome</td>
<td>Oxy-metholone and prednisolone</td>
<td>Unknown</td>
<td>Acyclovir, immunoglobulins, broad spectrum antibiotics</td>
<td>Patient developed multi-organ failure and died a few days after starting with acyclovir and immunoglobulins</td>
</tr>
<tr>
<td>2016</td>
<td>Ikumi</td>
<td>Male</td>
<td>56</td>
<td>Multiple sclerosis</td>
<td>Gingolimod and methyl-prednisolone pulse therapy</td>
<td>Primo</td>
<td>Unknown, no antiviral therapy because no diagnosis of HSV-2</td>
<td>Patient died 18 days after admission, diagnosis settled with autopsy</td>
</tr>
<tr>
<td>2018</td>
<td>Nasser</td>
<td>Female</td>
<td>36</td>
<td>Pregnant</td>
<td>None</td>
<td>Primo</td>
<td>Acyclovir, dexamethasone</td>
<td>Clinical improvement in a few days</td>
</tr>
<tr>
<td>2019</td>
<td>Kurosawa</td>
<td>Male</td>
<td>46</td>
<td>Hydropsinophilic syndrome</td>
<td>Prednisolone and azathioprine</td>
<td>Unknown</td>
<td>Acyclovir, methyl-prednisolone</td>
<td>Clinical improvement in two weeks, discharge on day 22 after admission</td>
</tr>
</tbody>
</table>

HSV-2 = herpes simplex virus 2; IVIG = intravenous immunoglobulin
We searched the literature for cases of HSV-2-associated HLH. We used the PubMed search engine and selected all cases that fulfilled the current HLH criteria and were written in English. There are five previously reported cases of HLH caused by a disseminated HSV-2 infection, of whom two patients had a certain primary infection (table 1). Three of the five described patients were using immunosuppressive therapies because of their underlying disease. Two cases report a pregnant woman without a relevant medical history. Pregnancy might lead to a reduced immunity to herpes viruses due to the shift of a T-helper 1 to T-helper 2-dominated cell-mediated immunity and is often associated with an exaggerated inflammatory response to viral infections. In three cases, the patients were treated with acyclovir intravenously, and cessation of immunosuppressive therapy and corticosteroids early in the course of the disease, and all three patients recovered with no reported signs of recurrence of HLH. In one case, the diagnosis was established relatively late, and therefore the start of adequate treatment was delayed, leading to the death of the patient. In the fifth case, the diagnosis of HSV-2-associated HLH was made on autopsy. Remarkably, our patient did not use immunosuppressive agents. Her medical history and further analysis did not reveal any signs of immunodeficiency; the patient had normal immunoglobulin levels, normal levels of CD4+ and CD8+ T cells, and no human immunodeficiency virus infection. Furthermore, whole-exome sequencing did not reveal any known mutations that are associated with HLH or immunodeficiency in general. Five months after these episodes, there is still no sign of immune deficiency. Therefore, it is presumed that reactive HLH has complicated a primary HSV-2 infection in a previously immunocompetent patient. Treatment of adult patients with HLH is challenging because no general guidelines exist. It is recommended to treat the underlying cause together with immunosuppressive treatment in a multidisciplinary referral centre with expertise in adult HLH patients. Cautious monitoring and swift adjustment in cases of progressive signs of the HLH is warranted to achieve complete regression of the potentially fatal hyperinflammatory state. In cases of HLH caused by a disseminated HSV-2 infection, which is rarely described in an immunocompetent person, differentiating between HLH and primary severe HSV-induced hepatitis remains challenging. Both cytopenia and hyperferritinemia could be caused by hepatitis due to hepatocellular damage. The differentiation is important because of treatment implications. Evidence of hemophagocytosis in the bone marrow and hypertriglyceridaemia may point towards HLH, although this hypothesis cannot be corroborated with clinical evidence since this has never been studied.

In conclusion, this is the first report of HSV-2-associated HLH in an immunocompetent adult patient. If clinically suspected, HSV serology and PCR should be screened in patients with idiopathic HLH. Although HLH is a severe condition with a high rate of morbidity and mortality, the prognosis of a patient with HSV-2 largely relies on the early recognition and treatment of the HSV infection. Based on previously reported cases and the case reported here, HSV-2-associated HLH could have a relatively good prognosis when adequate anti-viral and immunosuppressive therapy is started at an early stage.

**DISCLOSURES**

All authors declare no conflicts of interest. No funding or financial support was received. Informed consent: written informed consent of the patients was obtained.

**REFERENCES**

**Cryoglobulinaemic vasculitis in a patient with chronic hepatitis C: favourable outcome due to direct-acting antivirals**

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**ABSTRACT**

This case report describes a patient who presented with a debilitating hepatitis C virus-related cryoglobulinaemic vasculitis who was treated with immunosuppression and direct-acting antivirals. After returning symptoms revealed a relapse of the hepatitis C virus infection, treatment with direct-acting antivirals was repeated. Subsequently, he achieved a sustained virological response and his vasculitis subsided.

**KEYWORDS**

Cryoglobulinaemic vasculitis, direct-acting antivirals, hepatitis C virus, immunosuppression

**INTRODUCTION**

Mixed cryoglobulins, circulating immune complexes consisting of polyclonal IgG and monoclonal or polyclonal IgM with rheumatoid factor activity, are detected in the circulation in up to 60% of patients with chronic hepatitis C virus (HCV) infection. Symptomaticcryoglobulinaemic vasculitis of small and medium vessels is observed in approximately 10% of patients with HCV. HCV-associated cryoglobulinaemic vasculitis accounts for more than 90% of cryoglobulinaemic vasculitis worldwide. In the Netherlands, however, the prevalence of HCV is less than 0.1% and HCV-related cryoglobulinaemic vasculitis is extremely rare. In 754 consecutive patients presenting in the southeast of the Netherlands with cryoglobulinaemia, HCV was found in less than 2% (Aendekerk et al., in preparation). Mixed cryoglobulinaemia can be considered as a low-grade, indolent lymphoproliferative disorder at risk for evolution into non-Hodgkin's lymphoma (NHL). The clinical spectrum of cryoglobulinaemic vasculitis is variable and ranges from myalgia, arthralgia and arthritis, skin lesions (purpura), to peripheral neuropathy and renal damage. We present a patient with cryoglobulinaemic vasculitis...
due to an HCV infection and discuss the treatment options and prognosis.

**CASE STUDY**

A 59-year-old male from Azerbaijan with an unremarkable medical history was referred to our Department of Immunology after a 2-month history of fever, fatigue, polyarthritis, myalgia and muscle weakness, abdominal pain, polyneuropathy, and intermittent skin lesions. In the referring hospital, repeated blood cultures and infectious serology remained negative, including HCV. Antinuclear antibodies (ANA) and antineutrophil cytoplasmic antibody (ANCA) were negative. A total body CT scan and bone marrow examination ruled out lymphoma. The electromyography was inconclusive. The patient was referred under the suspicion of a systemic autoinflammatory or autoimmune disease, such as Behcet’s disease. The kidney involvement (dysmorphic erythrocytes, low grade proteinuria, serum creatinine 113 µmol/l) however, suggested small vessel vasculitis or immune complex-mediated disease. Regrettfully the patient refused kidney biopsy. The skin showed purpura at different stages. A CT angiography showed no signs of large or medium vessel vasculitis nor occlusion of abdominal vessels. Lab results showed a mixed cryoglobulinaemia (type II with a monoclonal IgM 0.30 g/l and polyclonal IgG 0.16 g/l), a strongly positive IgM rheumatoid factor (386 IU/ml), and classical complement activation (16%) (table 1). Due to the debilitating nature of his symptoms and the clinical diagnosis of cryoglobulinaemic vasculitis with presumed kidney involvement and vasculitis of the vasa nervorum, prednisolone (1 mg/kg), mycophenolate mofetil, and plasma exchange was started, followed by rituximab, according to local guidelines. PCR showed an HCV infection genotype 3a with a viral load (VL) of 400,000 copies/ml. Liver function tests were normal. A FibroScan showed a score F0-F1 (6.8 kPa) and an echography showed liver steatosis but no signs of cirrhosis or portal hypertension. Upon improvement of his clinical symptoms, the patient was discharged, the mycophenolate mofetil stopped, and a combination of direct-acting antivirals (DAA) was started according to national guidelines, predominantly based on the European Association for the Study of the Liver (EASL) guidelines;\(^8,9\) a pan-genotype HCV NS5A inhibitor (daclatasvir) and a pan-genotype HCV NS5B inhibitor (sofosbuvir) for a duration of 12 weeks were given.

During out-patient follow-up the patient admitted having forgotten to take the medication three days consecutively halfway during the treatment. However, HCV PCR analyses done at weeks 6 and 11 of treatment showed an undetectable VL. Eight weeks after end-of-treatment, the initial presenting clinical symptoms of arthritis and myalgia returned and relapse of HCV infection genotype 3a was confirmed. Presuming possible

<table>
<thead>
<tr>
<th>Table 1. Laboratory results</th>
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<tbody>
<tr>
<td><strong>Lab results (units)</strong></td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
</tr>
<tr>
<td>Cryoglobulins</td>
</tr>
<tr>
<td>Cryoglobulin IgG titre (g/l)</td>
</tr>
<tr>
<td>Cryoglobulin IgM titre (g/l)</td>
</tr>
<tr>
<td>IgM rheumatoid factor (IU/ml)</td>
</tr>
<tr>
<td>Soluble IL-2 receptor (U/ml)</td>
</tr>
<tr>
<td>Classical complement pathway (%)</td>
</tr>
<tr>
<td>Alternative complement pathway (%)</td>
</tr>
<tr>
<td>Complement factor C4 (g/l)</td>
</tr>
<tr>
<td>Complement factor C3d (g/l)</td>
</tr>
<tr>
<td>Urine analyses</td>
</tr>
<tr>
<td>Erythrocytes (ery/µl)</td>
</tr>
<tr>
<td>DYSMORPHIC erythrocytes (%)</td>
</tr>
<tr>
<td>Total protein (g/l)</td>
</tr>
<tr>
<td>n.a. = not applicable</td>
</tr>
</tbody>
</table>
resistance to the HCV NS5A inhibitor, and pending resistance testing, the patient was started on a combination of a pan-genotype HCV NS3/4A-protease inhibitor (pibrentasvir), a pan-genotype HCV NS5A inhibitor (glecaprevir), sofosbuvir and the synthetic nucleoside-analogue ribavirin, with an intended duration of 16 weeks. The patient stopped this treatment after three days due to side effects of extreme fatigue and conjunctivitis attributed to ribavirin. Combination therapy was reintroduced without the ribavirin. This was well tolerated and the patient completed his 16-week treatment without any interruptions.

Meanwhile, resistance testing results showed a NS5A Y93H mutation, not yet present prior to the start of treatment. He most likely developed the mutation during his first treatment, presumably due to non-compliance. The second treatment was successful and follow-up during and 26 weeks after end-of-treatment showed a sustained virological response (SVR), marked reduction of the mixed cryoglobulins titre and normalisation of complement. Urinary abnormalities resolved as well.

**DISCUSSION**

This case illustrates the importance of adding the determination of cryoglobulins, according to strict procedures of blood sampling and processing, to prevent false-negative results in patients with the suspicion of a small vessel vasculitis. In addition, when cryoglobulins are present, underlying hepatitis C should be examined by PCR and in rare cases, even after resolving the cryoprecipitate. Cryoglobulinaemic vasculitis due to hepatitis C in the Netherlands is rare, although the diagnosis may be missed. Important novel treatment modalities for HCV have great impact for the prognosis of these patients.

In patients with a rapid, organ-threatening or life-threatening prognosis, immediate immunosuppressive therapy, in combination with plasmapheresis is indicated regardless of the aetiology of the mixed cryoglobulinaemia followed by treatment of the underlying cause. In moderate to severe cases, such as our patient, glucocorticoids are often combined with immunosuppressants such as cyclophosphamide, azathioprine, or mycophenolate mofetil and in some patients, plasmapheresis is added to more rapidly remove the circulating cryoglobulins and spare the use of glucocorticoids. Glucocorticoids to control inflammatory disease activity, together with rituximab for the depletion of B cells, improve outcome in HCV-associated cryoglobulinaemic vasculitis.

In mild cases, treating the underlying cause is sufficient to control disease activity. Prior to the era of DAA treatment only a few options to achieve an SVR in patients with HCV were available and usually followed by a high relapse rate. In patients with HCV-associated cryoglobulinaemic vasculitis, the goals of antiviral treatment are to achieve SVR, obtain a clinical response, and minimise the use of immunosuppressive therapy.

In the pre-DAA period, patients with an HCV-associated cryoglobulinaemic vasculitis had a poor long-term prognosis with 5-year and 10-year survival rates of 75% and 65%, respectively. In contrast, the 5-year survival rate of HCV infection in the absence of vasculitis is 95%. With the introduction of DAA treatment, prognosis for HCV-infected patients has improved significantly, including for HCV-associated cryoglobulinaemic vasculitis patients. Several studies have shown high rates of clinical remission (70-90%) after DAA therapy after a median follow-up duration of up to two years.

Clinical response was associated with immunologic improvement in patients with HCV-associated cryoglobulinaemic vasculitis, yet circulating cryoglobulins seem to persist in up to 20% of patients despite an SVR and clinical improvement. This suggests that long-lived plasma cells persist and survive in niches long after viral eradication. Virus eradication does not necessarily mean that the immunological memory and signalling has stopped and several patients continue to have B-lymphocyte clonal expansion after SVR. In patients with persistent mixed cryoglobulinaemia with or without cryoglobulinaemic vasculitis symptoms, follow-up evaluation is warranted and a different underlying condition should be considered, especially B-cell lymphoma.

Resistance Associated Substitutions (RASs) occur naturally in HCV infections. A Y93H mutation occurs in < 10% of patients with HCV genotype 3 and corresponds with resistance for a number of HCV NS5A inhibitors including daclatasvir. The current EASL guidelines suggest treatment regimens that do not necessitate any resistance testing prior to first-line therapy. The current Dutch guidelines advise resistance testing to be done in untreated patients with HCV genotype 3 with cirrhosis, and in patients with HCV genotype 3 without cirrhosis but who have been previously treated. The reason for this strategy is the much higher cost of the alternative regimen glecaprevir/pibrentasvir compared to sofosbuvir/velpatasvir, which can be given if there is no corresponding RAS mutation. There is no known resistance against pibrentasvir (another HCV NS5A inhibitor) due to a Y93H mutation and retrospectively, our patient could have been treated with glecaprevir/pibrentasvir for a duration of 12 weeks. In our case, we did not wait for the results of resistance testing before administering the second broader and more robust regimen due to the patient’s returning symptoms; results of resistance testing can take up a couple of weeks.
In conclusion, the introduction of direct-acting antivirals has strongly improved the clinical and immunological outcome for hepatitis C virus-associated cryoglobulinaemic vasculitis patients. Most patients can now be treated for the HCV infection alone without the need for immunosuppressive therapy. In severe cases, immunosuppressive therapy is still warranted.

REFERENCES

CASE REPORT

Persevering syndrome of inappropriate antidiuretic hormone secretion after traumatic brain injury

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ABSTRACT

The syndrome of inappropriate antidiuretic hormone secretion (SIADH) is a known cause of hyponatremia, caused by excessive ADH secretion which, in turn, leads to water retention. SIADH has been associated with multiple etiologies, one of which is traumatic brain injury (TBI). Most cases of SIADH after TBI describe a course in which hyponatraemia develops several days to weeks after the trauma and then resolves within a few weeks. We demonstrate a case of SIADH after TBI, which persisted several years after initial presentation, but eventually did resolve spontaneously after five years.

KEYWORDS

SIADH, syndrome of inappropriate antidiuretic hormone, traumatic brain injury, TBI

BACKGROUND

The syndrome of inappropriate antidiuretic hormone secretion (SIADH) causes excessive antidiuretic hormone (ADH) release, leading to water retention and, as a consequence, hyponatremia. Laboratory results show hyponatremia < 135 mmol/l, together with decreased effective serum osmolality < 275 mOsm/kg. Typically, the urine sodium concentration is above 40 mmol/l, with a concomitant high urine osmolality > 100 mOsm/kg. The patient is clinically euvoletic. Depending on the severity of the hyponatraemia, symptoms can be subtle or severe, comprising nausea, lethargy, an altered mental status, convulsions, and coma. SIADH has been linked to a variety of aetiologies and is most commonly seen in combination with (pulmonary) malignancy, surgery, drugs, and all types of central nervous system (CNS) disruptions. One of these CNS disruptions is traumatic brain injury (TBI). The exact pathophysiology of SIADH after TBI has not yet been clarified. Most hypotheses encompass either damage to the pituitary stalk or the posterior pituitary, leading to an inappropriate secretion of ADH. Risk factors for the development of SIADH after TBI have not yet been elucidated. There is no unambiguous indication to assume that the severity of the trauma is correlated with the development of pituitary dysfunction. The objective of this case report is to draw attention to the sometimes long-lasting nature that SIADH after TBI can display. Literature on the emergence of SIADH shortly after TBI is available, but only one case of ongoing SIADH (six years in our case) has been previously described.

CASE PRESENTATION

In February 2013, a 59-year-old woman suffered a traumatic brain injury (TBI) after falling down the stairs. She suffered a bilateral mastoid fracture, which led to paralysis of the facial nerve and a central scull base fracture. She was admitted to the hospital for one week for supportive care. At admission, her serum sodium
level was 141 mmol/l (reference range: 135-145 mmol/l). Two weeks after the fall, she was admitted to the inpatient clinic again for elective facial nerve decompression. Upon admission, she complained of nausea, vomiting, tiredness, and dizziness. Her blood pressure was 138/92 mmHg with a heart rate of 84 beats per minute, and she had normal urine production, serum creatinine, and blood urea nitrogen (table 1). Laboratory results revealed hyponatraemia with a serum sodium concentration of 114 mmol/l. The results seemed to be consistent with SIADH, showing a low serum osmolality and a high urine sodium and urine osmolality (figure 1, day 0). There were no signs of hyperthyroidism or adrenal insufficiency (table 1), and secondary adrenal insufficiency was determined improbable by a normal metyrapone test. The patient did not use any relevant medication and there were no signs of malignancy, disease of the central nervous system, or pulmonary disease. Furthermore, a CT scan of the cerebrum did not show abnormalities in the hypothalamus/pituitary region or olfactory neuroblastoma. An MRI revealed an absence of the normal hyperintense signal of the posterior hypophysis, which has been correlated with various forms of SIADH in small series (figure 2). Therefore, it was concluded that SIADH caused by TBI was the most likely explanation.

Initially, the patient was treated with a fluid restriction of 1 l/24 h and sodium 3% infusions, leading to the normalisation of sodium levels (135 mmol/l) and fading of symptoms. On day 15, the patient was discharged with a fluid restriction of 1 l/24 h and oral sodium chloride tablets (see figure 1 for details about the dosage and laboratory results following this regimen). Details about the laboratory results in relation to adjustments in therapy and water load tests are summarised in table 2* and table 3*, respectively.

Table 1. Relevant laboratory findings

<table>
<thead>
<tr>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>35 µmol/l</td>
</tr>
<tr>
<td>GFR</td>
<td>&gt; 90 ml/min/1.72m²</td>
</tr>
<tr>
<td>BUN</td>
<td>2.8 mmol/l</td>
</tr>
<tr>
<td>TSH</td>
<td>0.71 mE/l</td>
</tr>
<tr>
<td>Free thyroxine</td>
<td>18.4 pmol/l</td>
</tr>
<tr>
<td>Cortisol at 8 AM</td>
<td>0.34 µmol/l</td>
</tr>
</tbody>
</table>

BUN = blood urea nitrogen; GFR = glomerular filtration rate; TSH = thyroid stimulating hormone

Over the years, the therapy was adjusted to a fluid restriction of 1.6 l, there was an increase in the sodium tablet dosage, and urea powder was added. The patient was able to adhere well to this regimen. In the following

Figure 1. Overview of serum sodium and -osmolality, and urine sodium and -osmolality in relation to therapy and water load tests
years, we planned several water load tests to test whether the SIADH was still profound (figure 1 at 4 months, 14 months, and after 5 years). The first two tests failed, as the serum sodium level decreased as soon as the patient quit taking her sodium and urea supplementation and increased her water intake. Five and a half years after her fall, we observed a spontaneous increase of sodium levels (142 mmol/l) whilst the patient was still on a fluid restriction regimen and taking sodium chloride and urea powder. A third water load test demonstrated that the ability to dilute the urine had been restored, indicating appropriate ADH secretion. In the following months, despite unrestricted fluid intake, the sodium measurements remained normal, and we could declare the SIADH as cured.

**DISCUSSION**

In this report, we demonstrate a case of long-lasting SIADH after traumatic brain injury, which eventually resolved spontaneously after five years. Several cases of SIADH after TBI have been described, but to our knowledge, only one other case has illustrated SIADH withstanding over several years.² Born et al. described 109 patients with a Glasgow Coma Scale < 7 after a severe head injury. Thirty-six patients developed SIADH, all within three weeks after the head injury had occurred. No long-term follow up of the sodium levels was described, but it was assumed that, as is in most cases, SIADH resulting from TBI is of a transient nature.² Chen et al. described four cases of SIADH after TBI, for which SIADH developed within four days after TBI.⁶ These patients all received supportive care consisting of fluid restriction, diuretics and, in some cases, saline supplementation; in all cases, the SIADH resolved within 10 days.

To the best of our knowledge, only one case of persistent SIADH has been published. Dick et al. described the case of a 32-year-old man who developed SIADH after TBI caused by a high-speed motorcycle accident.³ The patient was treated with a fluid restriction of 1.2 l/day. The patient had difficulties adhering to the fluid restriction. He was therefore readmitted several times over the following four years, showing recurrences of hyponatraemia consistent with SIADH. Eventually, demeclocycline was started, which kept serum sodium levels in the normal range, even after fluid restriction was stopped. The authors concluded that the resolution of hyponatraemia was due to treatment with demeclocycline and is unlikely to reflect a spontaneous resolution of the SIADH. However, with our case, we have shown that spontaneous recuperation after more than five years is possible.

Given the observation that SIADH can persist over several years, treatment should be adjusted for the individual patient, especially since adherence to treatment can be difficult. The cornerstone of treatment is fluid restriction. In cases of acute and/or symptomatic hyponatraemia, treatment with hypertonic saline is recommended. Urea powder can increase serum sodium levels by increasing urinary osmolality and water clearance, and can, if necessary, be combined with loop diuretics. The addition of oral salt tablets can increase the serum concentration of sodium. In the setting of persisting hyponatraemia caused by SIADH, the use of salt and urea tablets can somewhat relax the fluid restriction.³ In addition, vasopressin-antagonists (e.g., tolvaptan) can be used to increase the serum sodium levels by inducing free water clearance in the distal parts of the nephron.³ A similar effect can be obtained with the use of demeclocycline, which diminishes the responsiveness of the collecting duct to ADH.³ With our patient, this was not indicated because of her compliance to the fluid restriction and oral salt supplementation and urea powder.

**CONCLUSION**

Having reviewed the current literature, we found that, besides the case reported by Dick et al., no other studies have been published on SIADH lasting several years after the initial TBI. We have demonstrated a case of persistent SIADH after TBI, which resolved spontaneously after over five years of treatment.
DISCLOSURES

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

*Tables 2 and 3 are available from the authors upon request.

REFERENCES

Invasive fungal infections in patients treated with Bruton’s tyrosine kinase inhibitors

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ABSTRACT

Bruton’s tyrosine kinase (BTK) inhibitors are increasingly used in untreated and previously treated chronic lymphocytic leukaemia (CLL) patients. Invasive fungal infections (IFI) were rarely observed in patients treated for CLL in the pre-BTK era. In this article, we describe two patients with CLL who developed an IFI during treatment with the BTK inhibitor ibrutinib. The atypical presentation and the serious course of this complication are described.

KEYWORDS

Immunocompromised patients; invasive fungal infections; tyrosine kinase inhibitors.

INTRODUCTION

Invasive fungal infections (IFI) usually occur in immunocompromised patients such as patients with acute leukaemia and prolonged neutropenia. Although infections represent an important cause of morbidity in chronic lymphocytic leukaemia (CLL), IFIs are rarely observed. Ibrutinib, a small molecule and potent inhibitor of Bruton’s tyrosine kinase (BTK), was registered for the treatment of CLL in 2014. Since the introduction of BTK inhibitors, the number of CLL patients with IFI has increased. In this article, we describe two patients with CLL who developed an IFI during treatment with a BTK inhibitor.

CASE REPORT

Patient A, a 75-year-old man with CLL in remission using the BTK inhibitor ibrutinib, presented with a painful left forearm. Physical examination showed a pale, sick patient with the clinical signs of cellulitis of his left forearm. Blood tests showed a C-reactive protein (CRP) value of 141 mg/l (reference value: < 10) and increased liver enzymes. A CT scan of the chest and abdomen showed a consolidation in the left upper lobe of the lung and two liver lesions (figure 1a). A percutaneous biopsy of the liver lesions revealed inflammation and the presence of fungal hyphae, which raised the suspicion of disseminated fungal infection. Empirical antibiotic treatment and intravenous liposomal amphotericin-B was initiated (5 mg/kg/day) and ibrutinib therapy was discontinued. Despite the initiated treatment, his upper arm had to be amputated. Molecular analysis on the tissue of the amputated arm showed the presence of DNA from Lichtheimia corymbifera and Aspergillus species. Culture on material obtained from the liver abscess showed hyphae. Combination therapy was initiated by adding posaconazole to the treatment. This combination therapy was continued for one month, after which amphotericin B was discontinued. In the meantime, venetoclax was initiated to treat the CLL. Clinically, the patient recovered and was discharged with improved health. At the time of this writing, 17 months later, he continues to receive posaconazole while the lesions in the liver continue to decrease in size (figure 1b).

Patient B, a 72-year-old man with atrial fibrillation and CLL in partial remission using ibrutinib, presented himself at the emergency room with symptoms of a cerebrovascular accident. More than a month before presentation at the emergency room, the patient had been started on prednisone (40 mg/day) due to amiodarone-induced hyperthyroidism. Physical examination showed a latent right-sided hemiparesis. Additional blood tests showed no abnormalities apart from a known normocytic anaemia and thrombocytopenia (Hb 6.6 mmol/l; mean corpuscular
volume 86 fl; platelets 95 x 10^9/l) and mild inflammation parameters (CRP 49 mg/l; leukocytes 13.7 x 10^9/l). A CT scan of the brain was performed which revealed multiple brain infarctions. Clopidogrel was started in addition to the oral anticoagulation that the patient was already taking for his atrial fibrillation. However, his neurological condition worsened and he developed respiratory distress. Next, an MRI of the brain and CT scan of the thorax was performed which revealed multiple brain abscesses and extensive peribronchovascular consolidations. Suspected of having an IFI, the patient was treated with voriconazole and meropenem. PCR testing of bronchoalveolar lavage fluid confirmed a *Serratia marcescens* and *Aspergillus fumigatus* infection. Despite treatment, the patient’s condition soon deteriorated and he died of respiratory failure.

**DISCUSSION**

CLL is the most common form of leukaemia in adults. New drugs have been developed in recent years, including tyrosine kinase inhibitors such as ibrutinib and acalabrutinib. Ibrutinib has also been approved for the treatment of other haematological malignancies such as Waldenström’s macroglobulinemia and mantle cell lymphoma. Acalabrutinib is a more selective BTK inhibitor designed to improve the safety and efficacy of first-generation BTK inhibitors such as ibrutinib. A recently published observational study showed that IFI occurs more often in CLL patients treated with BTK inhibitors than expected. Aspergillus species and cryptococci were most frequently reported. Furthermore, central nervous system involvement was present in a large proportion of these patients (49%) and disseminated infection in multiple organs (60%) was often observed. Interestingly, only a small proportion of patients were neutropenic in the month before the occurrence of the fungal infection (14%).

Why are patients taking a BTK inhibitor at risk of developing IFI? BTK is an intracellular signaling protein that transmits signals from the B-cell receptor (BCR) and is necessary for B-cell survival. Recent preclinical
studies have shown that signalling via BTK is also involved in various other processes such as neutrophil attraction, cytokine production, and phagocytosis by macrophages. Because of these effects on the innate immune system, BTK inhibitors inhibit the effective clearance of fungi that have crossed the patient’s first line of defence. This could explain the risk of IFI when using both non-selective as well as selective BTK inhibitors (i.e., ibrutinib and acalabrutinib, respectively). Indeed, a third case of IFI with Aspergillus species was recently diagnosed by one of the authors of this paper in a patient on acalabrutinib.

The European Organization for Research and Treatment of Cancer (EORTC) and the Mycosis Study Group (MSG) have established guidelines that include risk factors for IFI. Use of BTK inhibitors is currently not listed as a risk factor. Despite established guidelines on the diagnosis and management of IFI, the diagnosis remains challenging. Clinical symptoms and radiological findings are often non-specific, especially when a patient is not neutropenic. Moreover, diagnostic tests are not always immediately available in all hospitals. Furthermore, antifungal agents such as voriconazole interact with many other drugs via CYP450 enzyme-mediated metabolism and enzyme inhibition. An additional problem, particularly in the Netherlands, is the development of resistance to antifungal agents of the triazole class as a result of the use of azoles in agriculture. Resistance of Aspergillus fumigatus to voriconazole was non-existent in the Netherlands before the year 2000 but has been increasing since then. This not only complicates treatment but also increases mortality.

CONCLUSION

CLL patients treated with BTK inhibitors are at risk of IFI. These infections can be fulminant with serious complications and high mortality, as illustrated by the two cases presented here. Early diagnosis is essential but is hampered by the atypical presentation. Future research is needed to evaluate if certain patient characteristics increase the risk for IFI in patients on BTK inhibitors. If this is the case, antifungal prophylaxis could be considered.

REFERENCES

CASE REPORT

Spontaneous remission of unidentified Cushing’s disease revealed by hair cortisol analysis

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ABSTRACT

Pituitary apoplexy is an infrequent but life-threatening complication of pituitary adenomas. When apoplexy occurs in a hormonally active adenoma, this may induce spontaneous remission of the clinical syndrome. In these cases, clinical suspicion of Cushing’s disease or acromegaly may arise at presentation, but due to spontaneous remission of active hormone production, it is not possible to biochemically confirm this diagnosis in retrospect. Resolution of clinical symptoms during follow up retrospectively suggests the diagnosis. However, we describe a patient with Cushing’s disease presenting with pituitary apoplexy, who was biochemically in remission at presentation. The diagnosis could be confirmed in retrospect using hair cortisol analysis, thereby enabling clinicians to adequately anticipate remission of Cushing’s disease.

KEYWORDS

Cushing’s disease, hair cortisol analysis, pituitary apoplexy

INTRODUCTION

Pituitary apoplexy is an infrequent but life-threatening complication of pituitary adenomas. Haemorrhage in the pituitary gland causes immediate mass effects, resulting in severe headaches sometimes accompanied by visual field compromise and hypopituitarism. When apoplexy occurs in a hormonally active adenoma, this might induce spontaneous remission of the clinical syndrome. Rarely, pituitary apoplexy is the presenting symptom of a functioning pituitary adenoma. In these cases, clinical suspicion of Cushing’s disease or acromegaly might arise at presentation. Due to spontaneous remission of active hormone production, it is not possible to biochemically confirm this diagnosis in retrospect. Resolution of clinical symptoms during follow up retrospectively suggests the diagnosis. However, we describe a patient with Cushing’s disease presenting with pituitary apoplexy, who was biochemically in remission at presentation. By using hair cortisol analysis, we were able to confirm the

Van Boven et al. A new tool for diagnosing Cushing’s disease in retrospect. Due to the presence of complete anterior hypopituitarism at presentation, the clinical suspicion of pre-existing Cushing’s disease could not be biochemically confirmed. However, hair cortisol analysis was performed on her 22 cm long hair. The results showed cyclic but significantly and consistently elevated cortisol levels for the last 22 months: average 25 pg/ml (reference value in normal controls 2.7 pg/mg hair, 95% CI 0.7-10.5 pg/mg hair).\(^8\) We therefore confirmed the diagnosis of Cushing’s disease in our patient cured by pituitary apoplexy. After establishing the diagnosis, we were able to taper corticosteroid therapy at a rate that is indicated for Cushing’s disease in remission to prevent steroid withdrawal symptoms. Additionally, anticipating remission of hypertension and type 2 diabetes, the antihypertensive and glucose-lowering medication could be reduced.

**DISCUSSION**

Spontaneous curation of Cushing’s disease by pituitary apoplexy is infrequent and difficult to prove. Hair cortisol analysis can be used to prove the diagnosis in retrospect. Pituitary apoplexy is a rare endocrine emergency resulting from bleeding or infarction in the pituitary gland often resulting in acute loss of pituitary function. It occurs in about 4.8-5.8% of pituitary macro adenomas and mostly it involves non-functioning adenomas.\(^9,10\) Pituitary apoplexy is the first presenting symptom of a pituitary tumour in 7.3% of all cases.\(^10\) Remission of Cushing’s disease by pituitary apoplexy is an infrequent event given that pituitary apoplexy mostly occurs in non-functioning adenoma. A similar course of disease with spontaneous remission of Cushing’s disease after symptomatic pituitary apoplexy has been described in several case reports. In previously described cases, the diagnosis of Cushing’s disease was based on histology after surgery, or on the clinical course of resolution of symptoms of Cushing’s disease after apoplexy.\(^1\) This is the first case in which the diagnosis was proven in retrospect by hair cortisol analysis. Hair cortisol analysis is a relatively new diagnostic tool which is not yet widely used. It is currently being studied for clinical applications.\(^11,12\) To date, it has been studied in the setting of diagnosing cyclic Cushing’s disease, overtreatment with steroid therapy in adrenal insufficiency, as marker of adverse cardiologic risk profile, and in the setting of several psychiatric disorders.\(^11,13\) It is a retrospective test in which cortisol levels in 1 cm of hair represents the average serum cortisol level during the period of one month. The process of hair cortisol analysis is extensively described in the article of G. Noppe et al, 2015.\(^8\) The hair is cut from the posterior vertex and divided into small portions. Then the steroids are

clinical suspicion of Cushing’s disease, thereby enabling us to adequately anticipate remission of Cushing’s disease.

**CASE REPORT**

A 31-year-old woman was referred to our outpatient clinic. She had been admitted to a foreign hospital with thunderclap headache. Additional magnetic resonance imaging (MRI) showed pituitary apoplexy in a pituitary macro adenoma, which had not been diagnosed before (figure 1). At the time of presentation, she had complaints of light-headedness, loss of appetite, and nausea with vomiting. Her medical history reported hypertension, type 2 diabetes mellitus, polycystic ovary syndrome, and hypothyroidism, all diagnosed within a timeframe of three years. She had not been using any steroid medication. Physical examination was suggestive for Cushing’s syndrome, i.e., moon face, acne, facial hirsutism, central obesity, and quadriceps atrophy. Further physical examination was unremarkable. Laboratory testing was consistent with complete anterior hypopituitarism: cortisol 09:00 hr < 28 nmol/l (normal 240-700 nmol/l); thyroid stimulating hormone (TSH) 3.3 mE/l (normal 0.4-4.0 mE/l); free T4 (FT4; thyroxine) 6 pmol/l (normal 10-22 pmol/l); lutenizing hormone (LH) 2E/l; follicle stimulating hormone (FSH) 4E/l; and estradiol < 44 pmol/l. There was no visual impairment due to optic nerve compression, and therefore, no indication for acute pituitary surgery. Hydrocortisone therapy of 80 mg a day was started to treat the secondary adrenal insufficiency.

Gradually, all deficiencies were adequately treated. MRI = magnetic resonance imaging

**Figure 1.** Sagittal MRI T1 weighed. Arrow points towards haemorrhage in the pituitary gland.
extracted and measured using liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS). The sensitivity and specificity are remarkably high compared to other tests for Cushing’s disease. The reported sensitivity and specificity using immunoassay hair cortisol analysis are respectively 93% and 90%.

Although hypocortisolism was identified in the blood samples at initial presentation, this was not reflected in the hair cortisol measurements. In our patient, the average hair cortisol level was, although cyclic, persistently far above the upper limit of normal in the past 22 months as can be seen in figure 2, retrospectively proving that our patient was suffering from a previously unidentified Cushing’s disease. Figure 2 shows a decrease in cortisol level starting from the moment of the pituitary apoplexy (between one and two months prior to the hair sample). These measurements represent average cortisol levels during a period of one month, and in this case, the average of temporary hypocortisolism and relative hypercortisolism due to initial supraphysiologic dosage of hydrocortisone. By proving spontaneous remission of Cushing’s disease using hair cortisol analysis, it is possible to adequately treat patients with initial high dose of steroid for prevention of steroid withdrawal. Additionally, one can anticipate remission of diabetes and hypertension. Therefore, hair cortisol analysis provides a useful tool for the clinician to provide proper care. In our opinion, hair cortisol analysis should be considered in patients presenting with pituitary apoplexy and signs or symptoms of Cushing’s disease.

In conclusion, we present a case of a previously unidentified Cushing’s disease, spontaneously in remission after pituitary apoplexy. This is the first case that is proven in retrospect by hair cortisol analysis.

**PREVIOUS PRESENTATIONS**

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**DISCLOSURES**

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**REFERENCES**


Two cases of a prolonged excited delirium syndrome after chloromethcathinone ingestion

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ABSTRACT

Synthetic cathinones have become popular drugs of abuse. We describe our recent experience with two highly agitated patients following ingestion of the cathinone derivative chloromethcathinone, and cannabis. Both patients suffered from excited delirium syndromes that lasted for over 24 hours. Clinicians should be aware of this phenomenon, especially since routine toxicology screenings do not detect the presence of these agents.

KEYWORDS

Excited delirium syndrome, drugs of abuse, synthetic cathinones

INTRODUCTION

The number of available new psychoactive substances (NPS) has risen dramatically in recent years. Since 2005, the European Monitoring Centre for Drugs and Drug Addition (EMCDDA) monitored more than 733 different NPS. These substances make up a broad range of drugs, including synthetic cannabinoids, stimulants, opioids, and benzodiazepines. Currently, cathinone derivatives are common among hospital presentations involving NPS.12 Cathinone is one of the biologically active alkaloids found in the khat shrub. Because of their structural similarity to amphetamines, cathinone derivatives are often called ‘natural amphetamines’. Similar to amphetamines, cathinone derivatives are characterised by stimulating, euphoric, and empathogenic properties.34

We describe two patients who were admitted to our hospital after the use of the cathinone derivative chloromethcathinone (CMC) which resulted in an excited delirium syndrome with prolonged psychotic symptoms. CMC use was confirmed by qualification of this drug in urine samples of both patients and in the powder substances they had in their possession. To our knowledge, this is the first case report of a prolonged excited delirium after CMC ingestion in a clinical setting.

CASE DESCRIPTIONS

Two 20-year-old Caucasian men arrived at the emergency department (ED) by ambulance. Their medical history was unknown. Both men had shown aggressive behaviour that alternated with a reduced state of consciousness before presentation. They admitted to having smoked cannabis with the possible addition of another substance about 2.5 hours prior to arrival at the hospital.

Patient 1
Patient 1 was very agitated during transport and was given 6 mg of midazolam intravenously in the ambulance. Upon arrival at the ED, he was awake, extremely anxious, and unable to cooperate; Glasgow Coma Scale (GCS) was 4-6-1. He was given an additional dose of intravenous
2.5 mg midazolam to facilitate an electrocardiogram (ECG) and blood sampling. His vital signs showed a blood pressure of 129/100 mmHg; heart rate 110 bpm, temperature 37.1°C, and a respiration rate of 18 breaths/minute with an oxygen saturation of 97% on room air. His pupils were of normal size and unresponsive to light. There were no signs of intravenous drug use. Routine blood tests were normal, including hemocytometry, electrolytes, renal function, liver enzymes, creatinine kinase, and bicarbonate. Blood alcohol levels were unmeasurably low. The ECG was normal. Qualitative analyses of drugs of abuse (Syva® RapidTest d.a.u.®, Siemens Healthcare Diagnostics Ltd., Frimley, Camberley, United Kingdom) in the urine, collected about 12 hours after admission, were positive for THC metabolite levels (cut-off 50 ng/ml) and opiates (morphine cut off 300 ng/ml), but negative for (methyl)amphetamine, methadone, benzodiazepines, and cocaine. Patient 1 was persistently anxious and extremely agitated. He developed severe myoclonus, urine retention, and respiratory depressions and required supplemental oxygen therapy. After transfer to the Medium Care Unit (MCU), he needed to be physically restrained because of combativeness. The consulting psychiatrist diagnosed a psychosis due to withdrawal of unknown drugs of abuse. He remained aggressive and highly agitated and required several doses of intravenous benzodiazepines. After 26 hours, he became calmer and started to respond to verbal instructions.

Patient 2
Patient 2 was lethargic upon arrival at the hospital with a GCS of 3-6-5. His blood pressure was 129/60 mmHg, heart rate 110 bpm, temperature 36.7°C, and he had a respiration rate of 16 breaths/minute with an oxygen saturation of 97% on room air. Physical examination, ECG, and blood test results were similar to patient 1. In his urine, collected about 12 hours after admission, only the use of cannabis was identified (Syva® RapidTest d.a.u.®). About seven hours after admission to the ED, patient 2 woke up agitated, anxious, and aggressive. He developed myoclonus, trismus, and retention of urine. After transfer to the MCU for observation, he remained aggressive and anxious and required several doses of intravenous benzodiazepines. After transfer to the MCU, he needed to be physically restrained because of combativeness. The effects of CMC supposedly last for two hours and are considered to be dependent on both dose and route of administration. Many of its effects, such as psychomotor anxiety, aggression, and psychotic symptoms. However, some typical sympathomimetic properties. 

DISCUSSION
We report on two patients who exhibited prolonged psychotic symptoms requiring intravenous midazolam after the use of cannabis in combination with CMC, a synthetic cathinone. To our knowledge, this is the first observation made by clinicians. CMC is a para-substituted cathinone in which the methyl-group of methylmethcathinone (3- or 4-MMC (mephedrone)) has been substituted for a chlorine halogen. CMC was first detected on the drug market in 2014. The cathinone 4-CMC was one of the five most confiscated synthetic cathinones in 2016 in Europe. So far, information about the effects of CMC is based upon user experiences and observations made by the police. CMC is categorised as a methamphetamine-like cathinone. Its mechanism of action involves preferential reuptake inhibition of catecholamines and liberation of dopamine. The effects reported include a sense of euphoria, increased energy, sociability and sexuality, visual and auditory hallucinations, and strong empathogenetic feelings. It also seems to potentially cause bruxism, nystagmus, near syncope, dizziness, tremor, headache, apathy, psychic and somatic anxiety, jaw tension, and involuntary eye movements. The effects of CMC supposedly last for two to four hours and are considered to be dependent on both dose and route of administration. Recommended care for patients exhibiting toxicity from synthetic cathinones is aimed at controlling the sympathomimetic toxidrome.

Our patients showed many similarities with previous descriptions in the literature. The most prominent signs were psychomotor anxiety, aggression, and psychotic symptoms. However, some typical sympathomimetic
toxicities, such as diaphoresis and hyperthermia, were absent. Furthermore, urinary retention was observed in both patients, which is not expected after the use of stimulants. This might have been a side effect of cannabis. We therefore conclude that both patients had an excited delirium that responded well to intravenous benzodiazepines. Most importantly, the effects of the intoxication lasted well over 24 hours, possibly more than 36 hours. It is possible that the co-ingestion of cannabis had a synergistic effect with regards to the psychotic symptoms.

Among the challenges for clinicians in the diagnosis of cathinone intoxication is the lack of routine toxicology screening for CMC and other synthetic cathinones. Quick assays for these compounds have not yet been developed for widespread routine hospital use. In the Netherlands, one facility provides ‘send-out’ testing, which is not immediately useful for practicing clinicians. The performed test is not able to make a distinction between 4-CMC and 3-CMC, but as the treatment aim for both CMC variants is symptom control, this has no clinical implications.

In conclusion, the use of CMC may lead to a prolonged excited delirium syndrome requiring supportive care. These symptoms could last for 24, and up to 36 hours. Clinicians should keep cathinone derivatives in mind when evaluating substance use in young adults or in anyone presenting with unexplained aggressive behaviour. Treatment of acute intoxication involves supportive care targeting manifesting signs and symptoms. It is recommended to obtain blood and/or urine samples for toxicology screening but one should be aware that many of these compounds cannot be detected with routine toxicology screenings in hospitals.

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DISCLOSURES

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REFERENCES

Septicaemia and liver abscesses after a skin ulcer in the tropics

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CASE REPORT

A 40-year-old, previously healthy man presented to the internal medicine outpatient clinic in Paramaribo, Suriname with a six-week history of intermittent fever. Approximately four days prior to onset of his fever, he had waded through stagnant, fresh water with a leg wound caused by blunt force trauma. Physical examination revealed a non-septic patient with pain in the right upper abdomen and a healing skin ulcer on the left shin (figure 1). Laboratory results showed evident inflammation with leucocytosis 21.9 x 10^9/l [4.5–11 x 10^9/l] and C-reactive protein 285 mg/l [0–5 mg/l]. Liver enzymes were normal and HIV screening was negative. Abdominal CT scan showed multiple liver abscesses, the largest was 41 mm in diameter (figure 2A). Because of the subpleural localisation of this abscess, drainage was deemed too risky. Culture of aspirated pus showed growth of a single species of bacteria with a distinct violet pigment (figure 2B). The blood culture yielded the same microorganism. What is your diagnosis?

WHAT IS YOUR DIAGNOSIS?

See page 304 for the answer to this photo quiz.
DIAGNOSIS

We made a diagnosis of *Chromobacterium violaceum* bacteraemia with liver abscesses. *C. violaceum* is a Gram-negative facultative anaerobic coccobacillus that is motile, catalase-positive, and oxidase positive. The violet pigment violacein is produced by most strains, but unpigmented (sub)strains are also observed. Growth at 37 °C distinguishes *C. violaceum* from *Iodobacter fluviatilis* and *Janthinobacterium lividum*, which also produce violacein but require lower growth temperatures.\(^1\)

*C. violaceum* occurs in soil and stagnant water in tropical and subtropical areas, but very rarely leads to human disease. If it does, it frequently causes rapid sepsis with metastatic abscesses in the liver, lung, or spleen. This explains the high case-fatality rate of approximately 50%. The incubation time is 3-14 days. Pathogen introduction typically occurs via a skin wound. Approximately 70% of patients have skin lesions, either from previous trauma or pustules.\(^2\)

The high virulence of certain strains of *C. violaceum* possibly originates from elevated superoxide dismutase and catalase levels, providing relative protection against phagocytosis by leukocytes.\(^3\) Consequently, patients with chronic granulomatous disease (CGD) have a higher risk of acquiring a symptomatic infection with *C. violaceum*, although over 90% of published cases have occurred in an immunocompetent host.\(^4\)

*C. violaceum* is generally susceptible to antibiotics such as carbapenems, fluoroquinolones and aminoglycosides, among others. Based on one retrospective cohort study describing antimicrobial susceptibility and clinical outcome, septic patients are treated with a combination of a carbapenem and a fluoroquinolone to achieve the highest efficacy.\(^5\)

The site of entry for the *C. violaceum* in our patient was likely the skin ulcer. He was first treated with monotherapy meropenem, but because of persistent bacteraemia, ciprofloxacin was added. After two weeks of combination therapy, he continued with ciprofloxacin monotherapy for three months. As our patient had no history of recurrent infections at his age, we performed no additional test to formally rule out CGD. He recovered completely.

This case describes a rare but potentially fatal cause of liver abscesses in the tropics. It is good clinical practice to account for low-risk/high-probability pathogens as well as high-risk/low-probability pathogens.

We propose that, in cases of liver abscesses in the tropics, especially in the presence of a recent skin trauma, infection with *Chromobacterium violaceum* should be considered, due to its high virulence and significant risk of relapse.

DISCLOSURES

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REFERENCES


PHOTO QUIZ

An unexpected infectious disease in wintertime

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CASE REPORT

A 31-year-old patient, with known alcohol and drug abuse, was admitted on January 2nd, 2020 to our emergency room presenting with fever, stomach ache, vomiting, diarrhoea, myalgia, coughing, and headache for one week. The patient reported that he had cycled into a ditch after drinking too much alcohol, a week prior to admission. Initial laboratory examinations showed the following: C-reactive protein 464 mg/l, leucocytes 14.9 x 10^9/l, thrombocytes 32 x 10^9/l, creatinine 416 µmol/l, alanine aminotransferase 164 u/l, aspartate aminotransferase 281 u/l, gamma-glutamyltransferase 121 u/l, alkaline phosphatase 159 u/l, and bilirubin 123 µmol/l. A few hours after admission, the patient became respiratory insufficient, was admitted to the intensive care unit (ICU), and underwent endotracheal intubation. The chest X-rays and CT scan of the thorax predominantly showed diffuse infiltrative lung disease (figure 1). An ultrasound of the liver demonstrated no liver or bile duct pathology. In the following days, the patient’s vital parameters deteriorated and he developed an acute kidney injury, for which he received continuous renal replacement therapy. He was transferred to the ICU of the nearest academic hospital on day three. The patient’s transaminases were only modestly increased, in contrast to total bilirubin, which peaked at 413 µmol/l (direct 309 µmol/l) at day seven.

WHAT IS YOUR DIAGNOSIS?

See page 306 for the answer to this photo quiz.
DIAGNOSIS

Both the IgM ELISA (Leptospirosis Reference Center, University Medical Center Amsterdam, the Netherlands) and Leptocheck-WB (Zephyr Biomedicals, India) for leptospirosis were positive, which was confirmed by PCR. Cultures from blood and bronchoalveolar fluid remained negative for bacteria and fungi. During intravenous antibiotic treatment with ceftriaxone for seven days, inflammatory markers, thrombocytes, and renal and liver function slowly improved. The patient was discharged in a severely weakened condition.

Leptospirosis is a zoonotic infection that is transmitted to humans by direct or indirect contact with infected animals. Leptospirosis is most common in tropical areas but occur rarely in temperate regions. In the Netherlands, the most common symptoms of leptospirosis are fever, myalgia, headache, diarrhoea, and vomiting, which were also present in our patient. However, our patient also developed septic shock in combination with symptoms typically seen in severe leptospirosis, primarily, kidney failure, thrombocytopenia, isolated bilirubin increase, and alveolar haemorrhage. Potential contributing factors to the severe manifestation of leptospirosis in our patient might be the patient’s delay of one week before seeking help, a potential large amount of ingested fresh water, a possible decreased immunity due to his drug and alcohol abuse, and/or the presence of multiple wounds caused by the accident.

We reported this case as severe systemic leptospirosis after contact with contaminated water in the Netherlands during wintertime. Presentation of this case during the winter period could be due to global warming, which could cause expansion of the (sub)tropic regions where leptospirosis is endemic. In the future, awareness of leptospirosis is warranted in patients presenting with flu-like symptoms after contact with contaminated water or animals in the Netherlands throughout the year.

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REFERENCES

LETTER

Septic patients with cancer: Do prehospital antibiotics improve survival? Do not forget the underlying status influence!

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Dear editor,

In the February 2020 issue of this journal, Nannan Panday et al. reported a significant reduction in 30-day readmissions but no overall survival improvement with prehospital antibiotics in septic patients with cancer. The authors should be complimented for this very interesting study concerning a subgroup of particularly fragile patients due to immunosuppression associated with cancer. This subgroup is probably one of those benefiting from early antibiotherapy.

However, their interpretation of their results requires caution because of some methodological issues. First, the authors do not report the ratio of included patients, independent of the treatment allocation group (usual or intervention group) with a previous 'do not resuscitate' status related to cancer and/or metastatic cancer. Second, the variables included in the multivariate analysis are not reported, and thus we do not know how they were considered potential cofounders of the in-hospital phase for the statistical analysis. These two points are major confounders of overall mortality (28-day, 90-day, and in-hospital mortality) because they affect a physician’s decision. Consequently, during the hospital stage, patients, especially immunosuppressed patients, have a higher risk of limitation of care and/or nosocomial infection occurrence.

Beyond these limitations, we fully agree that early identification of sepsis is the subgroup of sepsis patients where cancer remains a real challenge, particularly in the prehospital setting because of similarities between systemic inflammatory response syndrome related to sepsis and systemic inflammatory response related to cancer. Prehospital emergency medical service intervention, excluding the most caricatural sepsis forms, and early identification of sepsis and severity assessment over the telephone to the emergency medical service dispatch center is a challenge, but remains a prerequisite to determine the appropriate level of care dispatched at the scene (advanced life support vs. basic life support) for an individual patient.

DISCLOSURES

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REFERENCES

REPLY TO LETTER

Septic patients with cancer: Do prehospital antibiotics improve survival? Do not forget the underlying status influence!

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Dear editor,

We’ve read with interest the letter to the editor by Jouffroy et al. We would like to thank the authors for their valuable input and hereby our reaction to their comments.

Firstly, the authors incorrectly state that we did not include data regarding the resuscitation status for the treatment allocation groups (usual or intervention groups). This data was reported, as can be found in supplementary table 3 of our publication.1 In this table, we showed that 19 (21.6%) of the patients with cancer in the usual care group and 36 (27.9%) of the patients in the intervention group had a ‘do not resuscitate’ policy.

Jouffroy et al. mention that the consequences of differences in resuscitation status might explain the fact that there was no difference in survival rate in both the usual care group and intervention group. However, a resuscitation status is always dynamic and dependent on the condition of the patient, and as we have clearly described in supplementary table 3, this was the resuscitation status at admission of the patient. We do not have data on how this changed during the course of the stay of the patients. Therefore, it is impossible to objectively state whether this influenced the mortality rates.

Secondly, the authors state that we did not exactly describe how confounders in our multivariate logistic regression analysis were detected. Although we did indeed not describe this, we used the widely accepted technique, where a variable is considered a confounder when it changes the odds ratio of the outcome by 10% or more.2,3 All variables that had this effect on the outcome were included in our multivariate logistic regression.

We completely agree with the authors that early identification of patients with sepsis in cancer patients remains a challenge. We believe that a data-driven approach to sepsis, using predictive modelling, could play an important role in this regard in the near future.4 The emerging use of wearables to monitor key parameters at home will facilitate these algorithms and hopefully address these difficulties in triage, as described by the authors, to a great extent.5

DISCLOSURES

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