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Contents

EDITORIAL
Clinical molecular medicine has finally arrived
M.M. Levi

REVIEWS
Dermatomyositis and polymyositis: new treatment targets on the horizon
A.E. Hak, B. de Paepe, J.L. de Bleecker, P-P. Tak, M. de Visser

Dutch guidelines for diagnosis and treatment of chronic lymphocytic leukaemia 2011
A.P. Kater, S. Wittebol, M.E.D. Chamuleau, M. van Gelder, M.H.J. van Oers

New insights into the pathogenesis of chronic myeloid leukaemia: towards a path to cure
N. Thielen, G.J. Ossenkoppele, G-J. Schuurhuis, J.J.W.M. Janssen

Clinical effects of leucoreduction of blood transfusions
Y.M. Bilgin, L.M.G. van de Watering, A. Brand

PHOTO QUIZZES
Feverless red neck: why worry?
C. Popa, H.M. Dekker, M. van Deuren

Skin lesions in a HIV-positive female
M. Moret, W.E.M. Schouten, R. Hoekzema, G.E.L. van den Berk

A rare cause of abdominal pain
K.E. Verweij, F.E. de Jongh

Fever and persisting cough

ORIGIAL ARTICLE
Equations estimating renal function in patients with diabetes

CASE REPORT
Listeria peritonitis in patients on peritoneal dialysis: two cases and a review of the literature
M. Bierhoff, E. Krutwagen, E.F.H. van Bommel, C.A. Verburgh

SPECIAL ARTICLES
Needlestick injuries and infectious patients in a major academic medical centre from 2003 to 2010
G. Frijstein, J. Hortensius, H.L. Zaaijer

Clinical pathological conference
A non-Hodgkin’s lymphoma patient with persistent anaemia after chemotherapy
M.D. Hazenberg, B.J. Biemond, T. Pals, C.M. Zumpolle, C.J. Schinkel, G.E. Linthorst, J.B.L. Hoekstra, J.M.I. Vos
About a hundred years ago spectacular changes in medicine were occurring. The discovery of hormones and the understanding of clinical endocrinology, the development of antibiotics, early application of radiation for diagnosis and treatment of various diseases, identification of blood groups and initiation of transfusion medicine, and many other new advances rapidly changed the face of medicine. The famous physician William Osler wrote in 1902: ‘Never has the outlook of the profession been brighter. Everywhere the physician is better trained and better equipped than he was 25 years ago. Disease is understood more thoroughly, studied more carefully, and treated more skilfully. The average sum of human suffering has been reduced in a way to make the angels rejoice. Diseases familiar to our fathers and grandfathers have disappeared, the death rate from others is falling to vanishing point, and public health measures have lessened the sorrows and brightened the lives of millions.’

It is fair to say that medicine is in a similar situation of very rapid progress in the present era. Advances in technology present us with a fascinating imaging potential that is improving every few months. Similarly, minimally invasive interventions are swiftly developing and getting better each year. Simultaneously, the dazzling elucidation of molecular genetics is supposed to provide medicine with a similar boost. Indeed, the exponential increase in knowledge about genes, DNA variants, polymorphisms and mutations, as well as transcription regulation, gene-environment interaction and epigenetics, has certainly been translated into mounting knowledge about biology and disease. However, clinical medicine has derived very few advantages from the genetic revolution in biomedicine so far, even for relatively simple genetic disorders such as sickle cell disease, which affects hundreds of thousands of people worldwide. The genetic mutation of this monogenetic disorder was elucidated more than 50 years ago, yet this very precise molecular knowledge has had no effect at all on clinical management. In fact, despite all genetic preciseness patients with painful sickle cell crises are managed with intravenous fluids and painkillers. Similarly, patients with primary haemochromatosis due to a precisely defined gain of function mutations in genes involved in iron absorption are managed by blood letting, a therapy that has been with us since the Middle Ages. Another clear example is the genetics of thrombophilia. Factor V Leiden was discovered in 1994 as the genetic defect responsible for activated protein C resistance, leading to a prothrombotic state and an increased risk of thrombosis. However, almost 20 years later we do not have a clue how to provide adequate differential primary or secondary prophylaxis for affected individuals or how to precisely handle common thrombotic complications in patients with factor V Leiden or similar genetic thrombophilic defects. Apparently, the gap between the discovery of the genetic base of a disease and the consequences for clinical management is large and it takes a lot of additional research and time before this gap can be bridged. And the examples given all represent monogenetic and relatively simple diseases, let alone the clinical consequences in terms of management of multigenetic disease, such as atherosclerosis and cancer.

Nevertheless, and despite the tardiness of the translation of molecular genetic knowledge to clinically applicable improvements, the first changes are visible. In the field of Internal Medicine, this may be most clear in clinical haematology. Indeed, the discovery of the Philadelphia chromosome as the underlying genetic disorder of chronic myeloid leukaemia (CML) stems from more than 50 years ago, but in the last few years this knowledge has indeed translated into a cure for affected patients. In this issue of the Netherlands Journal of Medicine, Thielen and colleagues extensively review the further improvement in the management of patients with CML based on new insights into the molecular genetics of this disease. But also in the management of chronic lymphatic leukaemia molecular insights form the basis for further improvement in clinical management, including better treatment strategies, as reviewed in the guideline...
paper by Kater et al. in this issue of the Journal. And in other forms of malignant haematological disease genetic insights are now starting to translate into improved management strategies or even better treatment options for these disorders. It seems that precise molecular characterisation of malignancies does not only provide more insight into the pathogenesis of disease but can also be of use to stratify patients as high- or low-risk for recurrence and adverse outcome. But ultimately, the goal is that this knowledge translates into a better treatment outcome and it seems that this is happening now in clinical haematology. It is clear that other disciplines within oncology will follow soon, as previous publications in this journal indicate. But also many other fields in medicine, such as rheumatology, have entered the phase in which incisive knowledge on molecular mechanisms will translate into better management options for patients.

It may be fair to say that the ‘genetic revolution’ will indeed change the face of clinical medicine but that these changes take a lot of time and research effort. Fundamental research is crucial for further development of our insight into normal biology and disease but translational research to bring these results to practical solutions for patients is at least as critical and may require major investment. Nevertheless, it seems that we are at the threshold of reaping the rewards of molecular genetics and that Osler’s statement from 110 years ago is by the same token applicable to the present era.

REFERENCES

Dermatomyositis and polymyositis: new treatment targets on the horizon

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ABSTRACT

Polymyositis (PM) and dermatomyositis (DM) are rare idiopathic inflammatory myopathies (IIM) with a presumed autoimmune pathogenesis. Typical features are subacute onset, proximal, symmetric muscle weakness, elevated serum creatine kinase, and mononuclear cell infiltrates in the muscle biopsy. Strong support for an autoimmune pathogenesis comes from histopathological findings in biopsies of affected muscles. Furthermore, the association with autoantibodies supports the notion that immune-mediated inflammation is involved. PM and DM may occur in isolation or in connection with a connective tissue disease (CTD) or cancer. The current treatment for IIM consists of first-line high-dose steroids and various conventional second-line treatments. Improvements in treatment for IIM are hampered by difficulties in the design of trials and the low incidence and prevalence of the disease. Cytokines and chemokines are factors involved in the inflammatory process in IIM, and are candidates for future therapeutic targets. Preliminary data with anti-tumour necrosis factor therapy are not very promising, but results of blockers of the lymphotoxin signalling pathway are to be awaited. Anti-B cell therapy may be a valuable therapeutic option for treatment of refractory IIM. The effects of anti-interferon-alpha in IIM are to be awaited, as are results of other anti-cytokine therapies and anti-chemokine therapy. Outcome measures to be used in clinical trials in IIM include at present the core sets of outcome proposed by the International Myositis Assessment Clinical Study Group (IMACS).

KEYWORDS

Polymyositis, dermatomyositis, idiopathic inflammatory myopathies, immunopathology, therapeutic prospects

INTRODUCTION

Polymyositis (PM) and dermatomyositis (DM) are idiopathic inflammatory myopathies (IIM) with a presumed autoimmune pathogenesis. Typical features are subacute onset, proximal, symmetric muscle weakness and mononuclear cell infiltrates in the muscle biopsy. PM and DM may occur in isolation, or in connection with a connective tissue disease (CTD) or cancer. High-dose prednisone is the treatment of choice on an empirical basis; its effect has not been investigated in a randomised controlled trial (RCT). DM and PM often give rise to severe chronic disability and may be complicated by life-threatening impairment of swallowing and respiratory function. Sporadic inclusion-body myositis (sIBM), also considered to be an idiopathic inflammatory myopathy and a common myopathy in middle age and older adults, is addressed to outline its distinguishing features in the differential diagnosis of IIM.

CLASSIFICATION AND DEFINITION

PM and DM were already described in the 19th century. However, it took nearly a century before diagnostic criteria for DM and PM were proposed based on clinical features (subacute, symmetrical proximal weakness, and typical skin abnormalities for DM) and ancillary investigations. The latter included muscle biopsy abnormalities (necrosis, regeneration, perifascicular atrophy, inflammatory exudates), elevated serum creatine kinase (sCK) and electromyographic changes (short duration, low-voltage motor unit action potentials, and spontaneous activity). These criteria were initially developed for research purposes. The usefulness of this Bohan and Peter classification was challenged when new diagnostic modalities such as myositis-specific antibodies became...
In 1971, 8 was not recognised as a distinct disease entity at the time, and therefore probably misdiagnosed as PM, and the same holds true for muscular dystrophies in which cellular infiltrates may be prominent, such as dysferlinopathy and facioscapulohumeral dystrophy.\(^5,10\) In 1984, Arahata and Engel\(^{11}\) and subsequently Dalakas\(^{12}\) suggested another classification including sIBM. This was based on histopathological and immunohistochemical findings of muscle tissue suggesting that the immune mechanism in DM is humoral and targets the intramuscular microvasculature, whereas PM and sIBM are characterised by an antigen-directed cytotoxic T cell attack on myofibres expressing class I major histocompatibility complex antigens. Recently, at a consensus workshop under the auspices of the European Neuromuscular Centre (ENMC) a new classification was proposed.\(^{13}\) The main differences from previous classifications included the paradigm shift with regard to the prevalence of polymyositis, which appeared to be considerably less frequent in several parts of the world\(^3,14\) and the recognition of two immune-mediated disease entities: necrotising myopathy and non-specific or overlap myositis.\(^3,15,16\) In the ENMC classification non-specific myositis is defined by subacute onset, progressive proximal weakness, elevated sCK, perivascular, perimysial inflammatory cell infiltrate or scattered endomysial CD8+ T cells that do not clearly surround or invade muscle fibres. In a Dutch retrospective study,\(^{14}\) non-specific myositis was found in 39% of the patients with a previous diagnosis of myositis, with subacute onset of symmetric, proximal weakness excluding other neuromuscular disorders and 40% of these patients developed a connective tissue disorder. In a French-Canadian study,\(^{15}\) 67% of the IIM patients were diagnosed as overlap myositis on the basis of clinical features and the presence of autoantibodies. Immune-mediated necrotising myopathy is defined by subacute or insidious onset, progressive symmetrical weakness of the proximal muscles associated with an elevated sCK, and many necrotic muscle fibres as the predominant abnormal histological feature. Inflammatory cells are sparse and perivascularly located or even absent.\(^{16}\) Immune-mediated necrotising myopathy was found in 19% of the Dutch patients with IIM.\(^{14}\) Patients with immune-mediated necrotising myopathies were found to have a strong association with antibodies directed against the signal recognition particle (SRP). Recently, a unique subset of patients with anti-200/100 kd autoantibodies was reported in whom prior statin use was a frequent observation.\(^{17}\) The recognition of non-specific or overlap myositis and immune-mediated necrotising myopathy caused a shift in the prevalence of polymyositis. Some authors found this disease in only 2% and 9% of the patients with IIM, respectively,\(^3,14\) whereas in other populations PM appeared to be rather common.\(^{18}\) There is also some controversy about the clinical profile of polymyositis. Amongst the group of Dutch patients designated as PM on the basis of histopathological findings, it was noticed that a proportion developed progressive muscle weakness despite immunosuppressive treatment and ultimately showed a clinical picture consistent with sIBM. Although the muscle biopsy lacked rimmed vacuoles, a microscopic entity considered to be specific for sIBM,\(^{14}\) these patients were no longer considered to have PM, but designated as having sIBM. Others recognised a new category of IIM, i.e. PM/IBM characterised by clinical features of sIBM but with muscle biopsies that lacked rimmed vacuoles, the canonical feature of sIBM.\(^{18}\)

**DIAGNOSIS OF MYOSITIS SUBTYPES**

**Clinical picture**

PM is characterised by progressive, symmetric, proximal muscle weakness. Neck flexor weakness and dysphagia are observed in a fair proportion of the patients. A study assessing the distribution and severity of muscle weakness in DM and PM showed a greater severity of proximal weakness in PM in comparison with DM. The five weakest muscle groups were the hip flexors, hip extensors, hip abductors, neck flexors and shoulder abductors.\(^{19}\) Interstitial lung fibrosis is a common complication especially in patients who have anti-synthetase antibodies. DM is identified by a characteristic rash accompanying or preceding muscle weakness. The skin manifestations include a heliotropic rash (blue-purple discoloration) on the upper eyelids in many cases associated with oedema (sensitivity 67% and specificity 99.6%) (figure 1), Gottron papules (symmetric, livid papules on the dorsal side of interphalangeal and/or metacarpophalangeal joints, on the dorsal side of elbows, knees or medial malleoli, sensitivity 62% and specificity 98.7\%),\(^{20}\) Gottron sign (symmetric erythematous or livid atrophic maculae with or without oedema on elbows, knees and medial malleoli), and an erythematous rash on the face, neck and anterior chest (V sign) or back and shoulders (shawl sign).

**Biochemistry**

In adult DM, sCK is slightly to moderately (2-10 x the upper limit of normal) elevated in 20 to 90% of the patients.\(^{6,21}\) Patients with muscle weakness more often have an elevated sCK than those with amyopathic DM. Generally speaking, there is no relationship between the sCK activity at onset of the disease and outcome. However, in the individual patient who has been in remission, a rise in sCK activity may herald a relapse. There is uncertainty about the sCK activity in PM since the published articles...
are probably contaminated with sIBM cases. In sIBM, sCK is slightly (<5x the upper limit of normal) elevated in 90% of the cases and in only 7% is it ≥10x elevated. Of note, an inflammatory myopathy with perimysial pathology amenable to steroid treatment was recently reported, which was characterised by high aldolase activity while sCK was normal. This observation remains to be confirmed by other groups. Erythrocyte sedimentation rate is usually normal or only mildly elevated in IIM and is not a reliable indicator of disease severity.

Electromyography

Only uncontrolled research on the usefulness of electromyography (EMG) for the diagnosis of IIM has been published and studies on groups of patients with PM have not been performed. Short-duration, low-voltage polyphasic motor unit action potentials were observed in nearly 100% of the patients with DM, PM and sIBM, whereas fibrillations and short-wave activity were found in three-quarters of the patients. Spontaneous muscle fibre activity occurred in 80 to 100% of the cases. Therefore, it seems justified to consider EMG as an adjunct to the clinical, biochemical and histopathological investigations, although the findings are non-specific and do not distinguish between the various subtypes of IIM. EMG can be helpful to distinguish between a relapse of DM or PM and steroid myopathy, since spontaneous muscle fibre activity is not found in steroid myopathy.

Muscle imaging

Muscle imaging is a promising diagnostic tool in IIM. MRI can demonstrate muscular oedema by showing areas of high signal intensity on STIR (short tau inversion recovery) and fat-suppressed T2-weighted sequences, even in clinically asymptomatic muscles. Recommendations to perform an MRI as a guidance for the muscle biopsy are based on relatively small prospective studies and on studies including only patients with an established diagnosis of IIM.

Histopathology

The predominant histological features in PM are variability in fibre size, scattered necrotic and regenerating fibres, and perivascular and endomysial inflammatory cell infiltrates consisting mainly of CD8+ T cells and macrophages. These inflammatory cells surround and sometimes invade apparently non-necrotic muscle fibres expressing major histocompatibility class I antigens. Since invasion of non-necrotic muscle fibres is actually not very common in myositis patients, from a pragmatic point of view, this feature should not be required for the clinical diagnosis of PM, and perivascular, perimysial or endomysial inflammation without actual invasion of non-necrotic muscle fibres is actually not common in myositis patients, from a pragmatic point of view, this feature should not be required for the clinical diagnosis of PM, and perivascular, perimysial or endomysial inflammation without actual invasion of non-necrotic muscle fibres may suffice for a diagnosis of PM in the proper clinical context. A highly characteristic microscopic feature of DM is perifascicular atrophy (figure 2), which is caused by the degeneration of muscle fibres at the periphery of the fascicles, although this is a late finding and is found in perhaps only 50% of adult cases when biopsied early in the course of their illness. Quantitative morphological analysis suggests that focal capillary depletion is one of the earliest changes in DM. Immunofluorescence studies revealed the deposition of the components of the complement system (complement-membrane complex) in or around microvascular endothelium in a significant proportion of capillaries. These observations support the concept that an immune complex- or antibody-mediated response against a vascular-endothelial component is a primary pathogenetic mechanism in DM. Inflammatory infiltrates are composed primarily of macrophages, B cells and CD4+ cells in the perivascular and perimysial regions. These CD4+
cells also include significant numbers of plasmacytoid dendritic cells as opposed to T helper cells secondary to microvascular damage. Muscle fibres overexpress type 1 interferon (IFN)-inducible genes and proteins, particularly in the perifascicular regions, even before the development of perifascicular atrophy.35,36 Of note, increased expression of type 1 IFN-inducible genes is also evident in peripheral blood and levels appear to correlate with disease activity.37 These observations have led to yet another hypothesis that DM may be caused by overexpression of type 1 IFN by dendritic cells in the perivascular and perimysial regions and this may be toxic to nearby capillaries and the nearby perifascicular muscle fibres. This finding is characteristic of dermatomyositis, mostly associated with the juvenile form but it is also observed in the adult form.

**Epidemiology**

IIM are rare disorders. Most epidemiological data are not accurate since they are based on the diagnostic criteria of Bohan and Peter4 (see above). In the literature, data are found on the incidence of inflammatory myopathies as a whole (DM, PM and sIBM) which is estimated to be between 5.5 and 7.7 per million person-years, not including those overlapping with CTD (see below).41-43 Using Quebec physician billing and hospitalisation databases, the prevalence of PM and DM was estimated to be 21.5/100,000 (95% confidence interval (CI) 19.4 to 23.9) in 2003.44 In children, DM is the most frequent inflammatory myopathy, with an incidence of 1.5 up to 4 x 10^-6 children per year, and occurring at least two times as often among white girls than boys.45-47 There are no good epidemiological data on the occurrence of adult DM. The relative prevalence of DM (in comparison with PM together with sIBM) is higher in areas closer to the equator. Genetic risk factors may be involved, although these differ in separate ethnographic populations.48,49 Given the fact that PM is not strictly defined, epidemiological data on PM are lacking as well.

**Comorbidity**

PM and DM occur isolated or in connection with a CTD or with cancer.2,3 After the onset of PM, patients have a chance of about one in four to be diagnosed with an associated CTD such as scleroderma, systemic lupus erythematosus, Sjögren's syndrome, rheumatoid arthritis and mixed CTD.2 In a Dutch study, the cumulative risk of incident CTD was highest in subjects with non-specific myositis (clinical PM) and 33% at seven years.2 Depending on the classification criteria used, the frequency of myositis associated with CTD was 24 to 60% in an analysis of 100 French Canadian patients.3 The risk of several forms of cancer for adult patients with DM is increased, with a standardised incidence rate of 4.3 (95% CI 2.3 to 8.1), especially during the first three years after the diagnosis of DM is made.50 However, after five years an increased risk can still be detected.50 In a Scandinavian study, the risk of cancer was found to be increased before the diagnosis of DM was made (4.5 years (95% CI 2.8 to 8.7). In 71% of patients this occurred in a period less than two years before the diagnosis of DM.51 A French study found a cumulative incidence rate of malignancy of 21.4% and 28.5% one and five years after the diagnosis of DM, respectively.52 Cancer is not restricted to DM, but also occurs in patients with PM.5-50 However, accurate data are lacking due to the ill-defined entity of PM. In cases of immune-mediated necrotising myopathy a high rate of malignancy has been reported.5

PM and DM are serious diseases with a disease-related mortality of at least 10%. Mortality is mostly related to cancer, especially during the first years after onset of myositis. Furthermore, in the long term, myositis has a
chronic continuous or polycyclic disease course with major effects on perceived disability and quality of life, despite regained muscle strength.4

AUTOANTIBODIES

Detection of autoantibodies may be helpful in the differential diagnosis from other, non-autoimmune myopathies, and they are found in approximately 70% of the patients with PM or DM,53,54 as recently reviewed.55 These antibodies are classically divided into myositis-associated autoantibodies (MAAs), which can also be found in subjects with other CTDs, and myositis-specific autoantibodies (MSAs), which are primarily found in subjects with IIM.56 Autoantibodies against the histidyl-tRNA-synthetase (Jo-1) are the most common MSAs.57 They identify a group of patients with a unique clinical syndrome including myositis, interstitial lung disease (ILD), non-erosive arthritis, fever, and characteristic hyperkeratotic lesions along the radial and palmar aspects of the fingers known as ‘mechanic’s hands’.58,59 This constellation of symptoms has come to be known as the anti-synthetase syndrome. The presence of Jo-1 antibodies virtually excludes inclusion body myositis.60 Since the detection of Jo-1 antibodies, a number of additional aminoacyl-tRNA synthetases (ARS) have been identified, including those recognizing threonyl-tRNA synthetase (anti-PL-7),61 alanyl-tRNA synthetase (anti-PL-12),62 glycyrl-tRNA synthetase (anti-EJ),63 isoleucyl-tRNA synthetase (anti-OJ),64 asparaginyl-tRNA synthetase (anti-KS),64 tyrosyl-tRNA synthetase,65 and, most recently, anti-phenylalanyl synthetase (anti-Zo).66 Anti-Jo-1 is found in approximately 25 to 30% of myositis patients, and the other anti-ARS autoantibodies occur in 1 to 5% of myositis patients.57 The various anti-synthetase antibodies seem to be mutually exclusive in that individual patients usually do not produce more than one.58 The anti-synthetase autoantibodies may be found in patients with either PM or DM, and certain anti-synthetases may be more strongly associated with one or the other disease. However, different studies of the same anti-synthetase yielded very different results.69 Furthermore, although the relationship between anti-synthetase antibodies and myositis has been studied for almost 30 years, many questions remain about their pathological significance. Twenty to 30% of DM patients have Mi-2 antibodies, the autoantigen initially recognised as a nuclear protein. Using immunoprecipitation or immunodiffusion, few if any PM patients or normal controls produce Mi-2 autoantibodies.70–76 As mentioned before, some of the patients with an immune-mediated necrotising myopathy have autoantibodies targeting components of the SRP.69,77–81 however, the pathological relevance of these autoantibodies remains unclear.81 As previously mentioned, a novel autoantibody recognising 200-kd and 100-kd proteins was found to be associated with immune-mediated necrotising myopathy in subjects in whom prior statin use was a frequent observation.77 Novel highly specific MSAs, which are relatively common (13 to 21%) in DM patients and recognise 155-kd and 140-kd proteins, have recently been identified and may be associated with a higher rate of malignancy.52,53 The identity of the autoantigen recognised by these antibodies has not yet been established.53 Routine clinical tests for the most recent MSAs are lacking at present, but may prove to be of value in advancing our understanding of the pathogenesis of the diseases and the development of new treatments.

IMMUNOPATHOLOGY

Pathology

In most IIM, except for immune-mediated necrotising myopathy,84 there is prominent inflammation within the skeletal muscle tissue. However, the tissue constituents toward which the immune responses are directed differ. The primary target of the immune response in DM is the vascular endothelium of perimysial small arterioles and veins and perifascicular arterioles and capillaries. In contrast, the primary targets of the immune response in PM are the muscle fibres themselves. CD8+ cytotoxic T cells surround and invade non-necrotic myofibres. CD4+ helper T cells are found in large numbers within the endomysium. Plasmacytoid and myeloid dendritic cells are present in varying amounts within IIM muscle, often associating into nodular aggregates.55 A complex interregulated network of immunomodulators is involved in the recruitment, diapedesis and migration of these inflammatory cell subsets. Muscle cells actively participate in these immune reactions by expressing major histocompatibility complex I (MHC-I) antigens and costimulatory molecules on their sarcolemma.86 Blood vessel endothelial cells selectively recruit leucocytes, through upregulation of anchor proteins such as the adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1),57,58 allowing their transmigration from the circulation into the muscle tissue.

Cytokines

Cytokines are soluble chemical messengers that form an integrated signalling network regulating both innate and adaptive immune responses. The primary cellular sources of cytokines are the immune cells, but endothelial cells and muscle fibres can also express these immune regulators. The important role of the catabolic cytokine tumour necrosis factor (TNF) as a

Hak, et al. Dermatomyositis and polymyositis treatment targets.
regulator of the chronic inflammation associated with the IIM has previously been established. Lymphotoxins (LT), other members of the TNF superfamily, have been implicated in the cytotoxic response of CD8+ T cells towards non-necrotic muscle fibres in PM and are increased in DM patients, where they colocalise to intramuscular follicle-like structures that contain large numbers of T cells, B cells and plasmacytoid dendritic cells. Many other pro-inflammatory cytokines are regulators of inflammatory disease. Interleukin-1 beta (IL-1beta) is significantly upregulated in IIM and decreases when patients have been successfully treated with corticosteroids. In patients with DM and PM, serum levels of the related cytokine IL-18 are also increased. Recently, the overexpression of type I IFN in DM and PM has gained attention. Type I IFNs are cytokines expressed from multiple IFN genes and play a critical role in the regulation of the immune system. They appear to be part of the immunopathogenesis of DM and PM. The innate immune responses are characterised by plasmacytoid dendritic cell infiltration and IFN alpha/beta expression, and both features are associated with DM. Many IFN inducible genes are overexpressed in juvenile DM patients, and IFN-alpha expression correlates inversely with the duration of untreated disease.

Chemokines

Chemotactic cytokines termed chemokines regulate selective leucocyte activation and migration. The IFN-gamma inducible alpha-chemokines CXCL9, CXCL10 and CXCL11 are elevated in IIM patients. These chemokines have angiostatic properties and their expression correlates with the degree of capillary loss and inflammation in juvenile DM. CXCL9 positive fibres are found in areas with severe inflammation in PM, but are very rare in DM. Juvenile DM muscle contains significantly upregulated levels of CXCL13, a chemokine implicated in B cell organisation. Uregulation of the beta-chemokines CCL2, CCL3 and CCL4 has been described in IIM patients, and CCL2 in particular is viewed as one of the key disease regulators. Strong CCL2 expression localises to CD8+ T cells actively invading non-necrotic muscle fibres of PM, and to blood vessels. On the one hand, CCL2 is upregulated locally on the blood vessel endothelium near inflammatory foci of PM, which may direct specific receptive leucocytes to the endomyosial target sites and regulate the build-up of focal infiltrates. On the other hand, CCL2 is generally increased in blood vessels in DM tissues, also at sites remote from inflammatory cell collections. Its distribution mirrors other endotheliopathic changes that can be observed in DM blood vessels, which includes staining for TNF, ICAM-1 and membrane attack complex. CXCL9 and CCL2 are expressed by muscle fibres in PM tissues. The corresponding receptor CCR7 is present on inflammatory cells surrounding and invading non-necrotic muscle fibres and frequently these cells aggregate into nodules.

Current Treatment

Although there are data indicating that DM and PM can resolve spontaneously, the general opinion is that patients with muscle weakness need treatment. Some data indicate that a longer disease duration before the start of immunosuppressive treatment is prognostically unfavourable. However, sIBM was probably not well-identified and not excluded in these studies. Furthermore, there are also data showing no relationship between duration of illness until start of treatment and the occurrence or frequency of relapse. The goals of therapy are to improve the ability to carry out activities of daily living by increasing muscle strength and to ameliorate extramuscular manifestations (rash, dyspnoea, arthralgia, and fever).

Corticosteroids

Initial high-dose corticosteroids (1 to 1.5 mg/kg prednisolone per day for at least four weeks) followed by slow tapering to prevent relapses are the treatment of choice on an empirical basis; its effect has not been investigated in an RCT. In general, lack of high-quality RCTs assessing efficacy and toxicity of immunosuppressants characterises the field of IIM. A recent RCT compared oral dexamethasone pulse therapy (six cycles of 40 mg/day for four consecutive days at 28-day intervals) versus daily prednisolone (70 to 90 mg/day depending on the body weight) for 28 days, followed by a slow tapering regimen for 44 or 52 weeks (depending on the initial dose) in IIM. It was concluded that although pulsed high-dose oral dexamethasone is not superior to daily prednisolone as first-line treatment in IIM, it may be a good alternative by causing substantially fewer side effects in a broad range.

Second-line treatment

If treatment with corticosteroids fails (because of too small an effect, repeated relapses, or unacceptable side effects), various second-line treatments are in use. The small number of RCTs makes it difficult to decide which immunosuppressive agents are beneficial in PM and DM. Three studies compared immunosuppressants with placebo. A non-statistically significant effect on muscle strength was found for azathioprine compared with placebo in patients treated with prednisone. After three years of follow-up, those treated with the combination of prednisone plus azathioprine had improved more with respect to functional disability and required less.
prednisone for disease control, but differences were small and not ascertained blindly. A beneficial effect of intravenous immunoglobulins compared with placebo was found in patients with DM, although muscle weakness reappeared directly after stopping the infusion. These data were confirmed in a cross-over study among patients with DM. In PM, no controlled studies on intravenous immunoglobulins have been completed. No benefit was shown of plasma exchange and leucapheresis. One trial compared methotrexate with azathioprine in IIM, and found equivalent efficacy but better tolerance of methotrexate. Another trial compared cyclosporine with methotrexate in PM and DM, and found no statistically significant difference between the two groups. Intravenous methotrexate was compared with oral methotrexate plus azathioprine in a trial of refractory myositis, and although no statistically significant difference between the two treatments was found, a trend favouring combination therapy was detected. In a study with historical controls among children with DM, use of methotrexate in conjunction with an aggressively tapered course of prednisone was as effective as traditional long-term corticosteroid therapy, while decreasing the cumulative dose of corticosteroids.

In practice, most experience has been gathered with methotrexate (up to 25 mg/week) and azathioprine (up to 3 mg/kg/day). Intravenous immunoglobulins can have a short favourable effect in patients with DM. Other immunosuppressive drugs used in the treatment of PM and DM are cyclosporine (orally, 100 to 150 mg twice daily) which may also benefit childhood DM. Mycophenolate mofetil (2 gram per day) is emerging as a promising and well-tolerated drug. Cyclophosphamide (0.5 to 1.0 g/m²) intravenously has shown mixed results; it may be useful in case of ILD, but the evidence remains circumstantial. ILD can occur with DM and PM. There are no controlled studies on the best treatment for DM or PM complicated by ILD. In several case series favourable results are mentioned for treatment with corticosteroids with or without other immunosuppressants such as azathioprine, cyclophosphamide, cyclosporine and tacrolimus.

### FUTURE THERAPEUTIC PROSPECTS

Given the fact that use of chronic immunosuppressive therapy is associated with significant side effects, and many patients remain partially refractory to treatment, the discovery of novel therapeutic agents that are safe and effective for DM and PM is highly desirable. Improvements in treatment for IIM are hampered, however, by difficulties in the design of trials and the low incidence and prevalence of the disease. Furthermore, the disease-specific outcome measures used differ between studies, which impedes comparison of results.

### Anti-TNF therapy

Preliminary case reports show in general successful treatment of refractory DM and PM with anti-TNF treatment. In five juvenile refractory dermatomyositis patients, major clinical benefit was demonstrated after the initiation of anti-TNF therapy. However, an open-label study of TNF blockade combined with weekly methotrexate in six drug-naïve patients with DM and PM was terminated prematurely because of the low inclusion rate and high dropout rate due to disease progression and the occurrence of an infusion reaction. The two patients who did reach the primary endpoint showed improvement in all aspects studied. Furthermore, in an open pilot study, treatment with an TNF inhibitor was not effective in 13 patients with refractory IIM. In addition, onset of myositis has been reported in patients with arthritis during treatment with anti-TNF therapy. Lymphotoxins (LTs), cytokines related to TNF, represent other amenable targets for treating IIM. In this respect, soluble LT-betaR-Ig fusion protein, a potent blocker of the LT signalling pathway, might be worth exploring in the future.

### Anti-B-cell therapy

B-cells may play a pivotal role in the pathophysiology of IIM. As recently reviewed, early uncontrolled clinical experience indicates that B-cell depletion with rituximab (a monoclonal antibody targeting CD20 antigen on B lymphocytes) may be a valuable therapeutic approach for treatment of refractory IIM. A few case reports indicate beneficial effects of rituximab in patients with the anti-synthetase syndrome. Further investigation regarding the optimal dosing regimen, treatment length, and long-term safety profile of rituximab therapy for IIM is, however, warranted. In addition to rituximab, second- and third-generation anti-CD 20 monoclonal antibodies and agents that target B-cell growth factors, such as B cell activating factor belonging to the TNF family (BAFF) and a proliferation-inducing ligand (APRIL), could have promise for the treatment of myositis, and might be explored in this setting.

### Anti-IFN-alpha treatment

Another approach to new treatments for DM and PM would be the development of monoclonal antibodies that neutralise human IFN-alpha. Preliminary results using sifalimumab (an anti-IFN-alpha monoclonal antibody) in a single-dose phase-I trial among subjects with systemic lupus erythematosus showed sifalimumab-specific and dose-dependent inhibition of the overexpression of type I IFN-inducible mRNAs in the blood of treated subjects.
Other anti-cytokine therapies and anti-chemokine therapy

The prominent role played by other cytokines and chemokines, as mentioned before, makes them attractive targets for selective anti-inflammatory therapy. Chemokines with key roles in the immunopathogenesis could be neutralised by administering antibodies that abrogate chemokine-chemokine receptor interactions, inactivating chemokine analogues, or small molecule pharmaceuticals that antagonise receptor function. No chemokine-based strategies have been tested in IIM as yet.

OUTCOME MEASUREMENT

An international, interdisciplinary network, the International Myositis Assessment Clinical Study Group (IMACS), has proposed a core set of outcome measures to be used in clinical trials to assess three dimensions of myositis disease: disease activity (MYOACT), disease damage (MYODAM) and health-related quality of life (SF-36). Use of these outcome measures will help in validly comparing results of clinical trials among patients with IIM.

CONCLUSION

IIM have a presumed autoimmune pathogenesis. PM and DM may occur in isolation or in connection with a CTD or cancer. The current treatment of IIM consists of first-line high-dose steroids and various conventional second-line treatments. Improvements in treatment for IIM are hampered by difficulty in the design of trials and the low incidence and prevalence of the disease. Cytokines and chemokines are factors involved in the inflammatory process in IIM and are candidates for future therapeutic targets, as is anti-B-cell therapy. Outcome measures to be used in clinical trials in IIM include at present the core sets of outcome proposed by the IMACS.

REFERENCES

27. Tomasova Studynkova J, Charvat F, Jarosova K, Vencovsky J. The prominent role played by other cytokines and chemokines with key roles in the immunopathogenesis could be neutralised by administering antibodies that abrogate chemokine-chemokine receptor interactions, inactivating chemokine analogues, or small molecule pharmaceuticals that antagonise receptor function. No chemokine-based strategies have been tested in IIM as yet.


Dutch guidelines for diagnosis and treatment of chronic lymphocytic leukaemia 2011

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ABSTRACT

One of the principal responsibilities of the Chronic Lymphocytic Leukaemia (CLL) Working Party of the Dutch/Belgium Haemato-Oncology Foundation for Adults in the Netherlands (HOVON) is to create up-to-date guidelines for CLL. In this article, the revised guidelines for diagnosis and treatment are summarised. Despite recent expansion in treatment options for patients with CLL, the disease remains incurable in most cases and the optimal treatment approach for several subgroups of patients is still unclear. Therefore, it remains highly important to treat patients within clinical studies as much as possible. In this article, the current studies initiated by the HOVON CLL working party are emphasised.

KEYWORDS

Chronic lymphocytic leukaemia, guidelines, HOVON

INTRODUCTION

During the past ten years, significant progress has been made in both the diagnostics and treatment options for patients with chronic lymphocytic leukaemia (CLL). In 2005 the Dutch/Belgium Haemato-Oncology Foundation for Adults in the Netherlands (HOVON) founded a separate CLL working party. Besides initiation of clinical trials (currently five), the working party is responsible for formulating national guidelines for diagnosis and treatment of CLL. Based on novel data from large international phase II/III trials, the group recently revised the previous guidelines.

Where possible, these revised guidelines are based on published randomised trials. If such evidence is lacking, the guidelines reflect the expert opinion of the members of the group. Despite recent expansion in treatment options for patients with CLL, the disease remains incurable in most cases and the optimal treatment approach for several subgroups of patients is still unclear. Therefore, it remains highly important to treat patients within clinical studies as much as possible. Outside such trials, these guidelines provide recommendations how to treat patients in a uniform and rational manner.

The recommendations are divided into:

• Diagnosis
  - peripheral blood
  - additional tests
  - prognostic factors

• Treatment

• Treatment indications

• Treatment choices
  - first-line
  - relapse
  - refractory

• Actual treatment guidelines within HOVON studies / outside studies.

DIAGNOSIS

The guidelines for the diagnosis of CLL are primarily based on the recently revised guidelines of the International Workshop on Chronic Lymphocytic Leukaemia (IWCLL).
Blood
In case of clinical suspicion of CLL, it must be ruled out that the patient is suffering from another lymphoproliferative disease that can mimic CLL, such as hairy cell leukaemia, or leukaemic manifestations of mantle cell lymphoma, marginal zone lymphoma, splenic marginal zone lymphoma with circulating villous lymphocytes, or follicular lymphoma. Therefore, it is essential to evaluate both blood count and blood smear, and to perform immunophenotyping of circulating lymphoid cells. The diagnosis of CLL requires the presence of at least 5 x 10⁹/l clonal B lymphocytes (5000/μl) in the peripheral blood. The leukaemia cells found in the blood smear are characteristically small, mature-appearing lymphocytes with a narrow rim of cytoplasm and a dense nucleus lacking discernible nucleoli and having partially aggregated chromatin. Although these cells may be found admixed with larger or atypical cells, or pro-lymphocytes, the presence of more than 55% of such cells favours a diagnosis of pro-lymphocytic leukaemia (B-cell PLL). ‘Gumprecht schollen’ or smudge cells are characteristic morphological features of CLL.

CLL cells have a distinct surface marker expression pattern as compared with both normal B cells and other B-lymphoproliferative diseases. The four main immunophenotypical characteristics of CLL are:

- expression of B-cell associated antigens including CD19, and CD23;
- weak expression of CD20 and CD79b;
- expression of CD5, a T-cell associated antigen;
- low expression of membrane-bound immunoglobulin, which is usually either IgM or IgM combined with IgD. Each clone of leukaemia cells is restricted to expression of either kappa or lambda immunoglobulin light chains.

CLL cells are usually negative for CD10 and cyclin D1. FMC7 and CD22 are either negative or very weakly expressed. In 5 to 20% of otherwise healthy adults over the age of 40, an absolute increase of mononclonal CLL-like lymphocytes can be detected, yet with an absolute number (much) less than 5 x 10⁹/l. In the absence of lymphadenopathy or organomegaly (as defined by physical examination or CT scans), cytopenias, or disease-related symptoms, the presence of less than 5 x 10⁹/l B lymphocytes is defined as mononclonal B lymphocytosis (MBL). MBL may progress to frank CLL at a rate of 1 to 2% per year, closely resembling the risk of development of multiple myeloma in patients with monoclonal gammopathy of undetermined significance (MGUS).

Other investigations
Autoimmune haemolytic anaemia (Coombs positive and negative) and thrombocytopenia are frequently observed in CLL and might be hard to distinguish from cytopenias due to marrow infiltration. Therefore, in case of cytopenias, an active search for autoimmune features is needed. Although a bone marrow aspirate and biopsy are not strictly necessary for the diagnosis, they may be needed to differentiate bone marrow infiltration from autoimmune cytopenias. Before treatment initiation, active infections should be excluded. Because the clinical value of CT scans has not been demonstrated, CT scans are not recommended outside clinical trials. Table 1 shows the minimum tests for diagnosis, initial treatment and evaluation of treatment.

Prognostic factors
The disease is very heterogeneous. In less than 30% of all patients the disease has a very indolent course, with patients eventually dying from causes unrelated to CLL. About 15% of patients die rapidly – within two to three years from diagnosis – from CLL and/or treatment-related causes, whereas in the remaining proportion of CLL patients the disease has a relative indolent course during the first five to ten years, followed by a terminal phase lasting one to two years with considerable morbidity, both from the disease itself and from complications of therapy. Traditional clinical staging systems devised by Rai and Binet are the simplest and still best validated means of assessing prognosis for CLL patients. However, there is substantial heterogeneity in the course of the disease within defined stages. In recent years molecular and cellular markers have been correlated with disease aggressiveness: immunoglobulin VH (IgVH) gene segment usage (mutated or unmutated), and the proposed surrogate markers CD38 and ZAP-70 (reviewed by Kay et al.). Unfortunately these parameters thus far have only limited use in guiding when and how to treat and determining when and what type of therapy to use. Given the above, the working party recommends assessing IgVH mutational status or CD38 and ZAP-70 status only in the setting of clinical trials.

Cytogenetics using fluorescence in situ hybridisation (FISH) provides prognostic information, notably as to the probability of response to various therapeutic regimens. A deletion of chromosome 11q (11q22.3, the location of the ATM gene), and in particular deletion of chromosome 17p (17p13.1, the localisation of the p53 gene) is frequently associated with p53 dysfunction. Since most chemotherapeutic drugs currently used depend on p53 for their cytotoxic effect, such deletions strongly impair the efficacy of such treatments (reviewed by Kater and Tonino). Although both 11q deletion and 17p deletion are associated with rapid disease progression, such deletions can not be used to initiate therapy in the absence of the current treatment criteria. Cytogenetic studies are of importance to determine the probability of responses to
various treatment modalities. This is especially true in case of relapse. The value of cytogenetics for first-line treatment choices remains unclear. Despite the poor prognosis of 17p-deleted patients, it remains to be determined whether allogeneic stem cell transplantation in first remission improves survival. It will be clear that this group of patients should preferably be treated within clinical trials. Currently an international study in untreated symptomatic CLL patients harbouring a 17p deletion is being developed. Outside such a study, cytogenetics prior to first-line treatment is not strictly necessary. Because the percentage of patients with adverse cytogenetic abnormalities such as 17p deletion strongly increases with each subsequent treatment, it is advised to perform cytogenetics before each new treatment regimen, at least from the time of first relapse (table 1).

TREATMENT OF CLL

Indications for treatment
Because of the considerable heterogeneity in disease-related signs and symptoms and in the clinical course of the disease, along with the present lack of curative treatment modalities, the decision when and how to treat a CLL patient requires marked individualisation. Early treatment of asymptomatic patients resulted in a delay of disease progression but yielded no improved survival. Importantly there is some evidence that early treatment increases the risk of acute myeloid leukaemia. Table 2 shows the indications for treatment in daily practice. The situation is obviously different for clinical trials, and depends on the question and the inclusion criteria of the studies.

Key points in the choice of treatment
Because of the rapidly expanding treatment modalities the half-life of the current guidelines is limited. Important aspects in treatment choices are:

- age of the patient;
- performance status / comorbidity;
- cytogenetic risk profile (if known);
- response (duration) to previous therapy;
- toxicity of previous therapy;
- aim of treatment; palliative care or improved progression-free survival (PFS) and overall survival (OS).

Considerations as to first-line treatment
In 2006/2007 three phase III randomised trials were published, showing that the addition of cyclophosphamide to fludarabine (FC) significantly improves PFS of CLL.
patients. However, despite increased PFS the OS was not different. This obviously reflects the lower efficacy of salvage therapy, following intensified first-line treatment.

Considerations as to treatment of relapse
At proven relapse, the presence of a 17p deletion should be met:

Evidence of progressive marrow failure as manifested by the development of, or worsening of, anaemia and/or thrombocytopenia

Massive (i.e., at least 6 cm below the left costal margin) or progressive or symptomatic splenomegaly

Massive nodes (i.e., at least 10 cm in longest diameter) or progressive or symptomatic lymphadenopathy

Progressive lymphocytosis with an increase of more than 50% over a two-month period or lymphocyte doubling time (LDT) of less than six months. LDT can be obtained by linear regression extrapolation of absolute lymphocyte counts obtained at intervals of two weeks over an observation period of two to three months. In patients with initial blood lymphocyte counts of less than 30 x 10^9/l (30,000/μl), LDT should not be used as a single parameter to define a treatment indication. In addition, factors contributing to lymphocytosis or lymphadenopathy other than CLL (e.g., infections) should be excluded.

Autoimmune anaemia and/or thrombocytopenia that is poorly responsive to corticosteroids or other standard therapy

Constitutional symptoms, defined as any one or more of the following disease-related signs or symptoms:

- Unintentional weight loss of 10% or more within the previous six months
- Significant fatigue (i.e., ECOG PS 2 or worse; inability to work or perform usual activities)
- Fevers higher than 100.5°F or 38.0°C for two or more weeks without other evidence of infection, or
- Night sweats for more than one month without evidence of infection

- Night sweats for more than one month without evidence of infection

- Significant fatigue (i.e., ECOG PS 2 or worse; inability to work or perform usual activities)
- Fevers higher than 100.5°F or 38.0°C for two or more weeks without other evidence of infection, or
- Night sweats for more than one month without evidence of infection

The possible synergistic effect of (mild) chemotherapy, monoclonal antibody treatment and an agent that exerts its effect by influencing the interaction of the malignant cells with their microenvironment, without an increase in toxicity.

In an attempt to improve the response rate (which is associated with increased PFS) the CLL working party has recently initiated a phase I/II trial studying the effect of addition of lenalidomide to chlorambucil and rituximab: the HOVON 109 study. The rationale behind this study is the possible synergistic effect of (mild) chemotherapy, monoclonal antibody treatment and an agent that exerts its effect by influencing the interaction of the malignant cells with their microenvironment, without an increase in toxicity.

The first study in which an upfront treatment regimen not only resulted in improved PFS, but also in a beneficial effect on overall survival. A limitation of this trial is that the patients studied do not optimally reflect the ‘normal’ CLL patients: they were fit (CIRS score ≤6, (table 3) creatinine clearance >70 ml/min) and relatively young. Only 10% of patients were over the age of 70 years. Yet, FCR has become the worldwide standard first-line treatment for fit patients (i.e. patients who are suitable for fludarabine-containing therapy). Although FCR improved survival of patients with an 11q deletion, this was unfortunately not the case for patients with a 17p deletion. Several studies have shown that in the large group of, mostly less fit, elderly patients fludarabine-containing regimens have an unfavourable toxicity profile. A randomised study of the German CLL study group in patients above the age of 65 indicated an inferior overall survival with fludarabine monotherapy as compared with chlorambucil. To date, chlorambucil monotherapy is still widely used as first-line therapy in this population group. Despite obvious benefits of chlorambucil in the elderly and more vulnerable patients (such as low toxicity and oral administration) it is not very effective as monotherapy. In most studies, overall response rates (ORR) of chlorambucil are approximately 50% (31 to 72%) with virtually no complete remissions (CR), resulting in a PFS of less than 1.5 years (8.3 to 20 months). In recent trials the clinical value of combining the anti-CD20 antibodies rituximab or ofatumumab with chlorambucil has been studied. In 2010, Hillmen and colleagues presented the results of an English phase II study in 100 previously untreated patients with the combination of chlorambucil and rituximab. They found an ORR of 82% with 9% CR, and a PFS of 23.5 months. Although this seems slightly better than the results of chlorambucil monotherapy in the CLL4 trial (ORR 66%, 6% CR and 20 months PFS), these separate trials obviously cannot be compared.

Table 2. IWCLL/NCI treatment criteria

<table>
<thead>
<tr>
<th>Binet stage C or Rai stage III or Rai stage IV or Treatment of active/progressive disease*</th>
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<tbody>
<tr>
<td>* For progressive disease at least one of the following criteria should be met:</td>
</tr>
<tr>
<td>1 Evidence of progressive marrow failure as manifested by the development of, or worsening of, anaemia and/or thrombocytopenia</td>
</tr>
<tr>
<td>2 Massive (i.e., at least 6 cm below the left costal margin) or progressive or symptomatic splenomegaly</td>
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<tr>
<td>3 Massive nodes (i.e., at least 10 cm in longest diameter) or progressive or symptomatic lymphadenopathy</td>
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<td>4 Progressive lymphocytosis with an increase of more than 50% over a two-month period or lymphocyte doubling time (LDT) of less than six months. LDT can be obtained by linear regression extrapolation of absolute lymphocyte counts obtained at intervals of two weeks over an observation period of two to three months. In patients with initial blood lymphocyte counts of less than 30 x 10^9/l (30,000/μl), LDT should not be used as a single parameter to define a treatment indication. In addition, factors contributing to lymphocytosis or lymphadenopathy other than CLL (e.g., infections) should be excluded.</td>
</tr>
<tr>
<td>5 Autoimmune anaemia and/or thrombocytopenia that is poorly responsive to corticosteroids or other standard therapy</td>
</tr>
<tr>
<td>6 Constitutional symptoms, defined as any one or more of the following disease-related signs or symptoms:</td>
</tr>
<tr>
<td>- Unintentional weight loss of 10% or more within the previous six months</td>
</tr>
<tr>
<td>- Significant fatigue (i.e., ECOG PS 2 or worse; inability to work or perform usual activities)</td>
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<tr>
<td>- Fevers higher than 100.5°F or 38.0°C for two or more weeks without other evidence of infection, or</td>
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<tr>
<td>- Night sweats for more than one month without evidence of infection</td>
</tr>
<tr>
<td>Hypogammaglobulinaemia or monoclonal or oligoclonal paraproteinaemia does not constitute a basis for initiating therapy in itself. However, it is recommended to assess the change of these protein abnormalities if patients are treated.</td>
</tr>
</tbody>
</table>

1 Evidence of progressive marrow failure as manifested by the development of, or worsening of, anaemia and/or thrombocytopenia
2 Massive (i.e., at least 6 cm below the left costal margin) or progressive or symptomatic splenomegaly
3 Massive nodes (i.e., at least 10 cm in longest diameter) or progressive or symptomatic lymphadenopathy
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5 Autoimmune anaemia and/or thrombocytopenia that is poorly responsive to corticosteroids or other standard therapy
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- Significant fatigue (i.e., ECOG PS 2 or worse; inability to work or perform usual activities)
- Fevers higher than 100.5°F or 38.0°C for two or more weeks without other evidence of infection, or
- Night sweats for more than one month without evidence of infection

Patients with a 17p deletion should be treated with the same treatment regimen again or whether...
alternative regimens should be considered. An important issue for clinical decision making at relapse is whether the patient has relapsed or refractory disease. Relapse is defined by the IWCLL as development of progression following a period of at least six months of CR or PR after prior therapy. Refractory disease is defined by the IWCLL as either no response or progressive disease within six months after completion of previous therapy. Such a distinction between relapse and refractory disease is particularly relevant after treatment with fludarabine or chlorambucil monotherapy, since the majority of patients who develop progressive disease at least six to 12 months after these treatments can be treated successfully with the same regimen, or with immuno-chemotherapy. In patients with refractory disease, however, it is highly unlikely that responses will occur with immunochemotherapy.

If a patient is eligible for an allogeneic stem cell transplantation, a broader, EBMT-based definition of 'refractory' or more precisely high-risk disease is being used: relapse within one year after fludarabine-containing chemotherapy, or relapse within two years after fludarabine-rituximab containing immunochemotherapy or any relapse in patients with a 17p deletion.

**Treatment of relapsed CLL**

Currently, the optimal choice of treatment for patients with relapsed disease is unknown. In the absence of a 17p deletion patients can be successfully treated with either the same regimen as used previously, or by switching to other more potent treatment combinations, depending on the last remission duration.

In a randomised trial in CLL patients relapsed following monotherapy with fludarabine or chlorambucil, FCR induced a PFS that was ten months longer than with FC (30.6 vs 20.6 months). No difference in overall survival was observed. An important consideration is the fact that the median survival following relapse after FCR treatment is just 2.5 years for patients not eligible for allogeneic stem cell transplantation. Recently, a HOVON-led international trial was initiated in patients with a second or third relapse of their CLL (HOVON 101 or PROLONG) to study the value of maintenance therapy with ofatumumab (administered once every two months for up to two years), following a CR or PR obtained by any induction treatment.

**Treatment of high-risk relapsed/refractory CLL**

In fit younger patients (<70 years) with relapsed CLL within one year after fludarabine-based chemotherapy or within two years after fludarabine and rituximab-containing immunochemotherapy or with any relapse in patients with a 17p deletion (EBMT ‘high risk’ definition) a reduced-intensity stem cell transplantation (RIST) with an HLA-identical (family / MUD) donor should be considered, preferably in the context of a clinical trial (HOVON 88, see below). Response to induction treatment prior to RIST is found to be an important determinant of long-term outcome as patients with a high disease burden, particularly bulky lymphadenopathy at the time of transplantation or poor response to last treatment, have the tendency to relapse more often, whereas patients with progressive disease uniformly do badly. Currently, no optimal induction regimen, especially for patients with chemo-refractory disease, has been established. Options for induction treatment can be found below.

If patients do not qualify for a RIST, the therapeutic goal should be to induce responses resulting in improved quality of life. The following options are reported to be effective in patients with chemo-refractory disease:

- **Alemtuzumab (Campath-1H®)**, an anti-CD52 humanised monoclonal antibody, has been investigated extensively in CLL. It has significant anti-leukaemic activity, predominantly in the peripheral blood compartment, bone marrow, and spleen, whereas activity is lower in lymph nodes. Response rates of alemtuzumab in chemo-refractory patients are around 30% with a median response duration of approximately nine months. The efficacy of alemtuzumab is significantly reduced in patients with large lymph node masses (>5 cm diameter);
- **An alternative regimen is a combination of rituximab or alemtuzumab with high-dose steroids (dexamethasone or methylprednisolone), especially in patients with large lymph node masses.** Although the response seems better, the published phase II studies are rather small. Recently, an international forum of experts stressed that alemtuzumab-containing chemotherapy should only be applied within clinical trials because of very high risks of severe (opportunistic) infections;
- **Ofatumumab (HuMax CD20;Arzerra®)** is a fully human, high-affinity monoclonal antibody that binds to a different CD20 epitope to that targeted by rituximab. It induces complement-derived cytotoxicity more effectively than rituximab. A pivotal phase II study of ofatumumab in relapsed CLL patients showed impressive activity both in patients refractory to both fludarabine and alemtuzumab (double refractory or DR, n=59) and in patients with bulky lymphadenopathy refractory to fludarabine (bulky fludarabine refractory or BFR, n=79). ORR, time to next therapy, and OS were similar for the DR (51%, 9.0 months, 13.7 months) and BFR groups (44%, 7.9 months, 15.4 months). Based on these findings, ofatumumab has recently been registered for CLL patients who are refractory to fludarabine and alemtuzumab. In the Netherlands, the Health Care Insurance Board (CVZ) has advised to include ofatumumab on the list of expensive orphan drugs.
Guidelines for the treatment of CLL

Based on the above considerations, the Dutch CLL working party has formulated the following guidelines (see algorithm, figures 1 and 2).

First-line treatment of CLL (figure 1)

In clinical trials
- HOVON 68. This trial was closed on 11 September 2010; the first results are expected at the end of 2011;
- HOVON 109. Phase I/II study on the value of addition of lenalidomide to chlorambucil and rituximab. Inclusion criteria: Patients ≥65 years or <65 but not eligible for fludarabine (containing) therapy (CIRS score >6 (table 3)).

Outside clinical trials
- Fit patients (CIRS score ≤6 (table 3) creatinine clearance >70 ml/min, which will generally be patients ≤65 years): FCR (fludarabine, cyclophosphamide, rituximab, a maximum

Table 3. Cumulative Illness Rating Scale (CIRS)³

<table>
<thead>
<tr>
<th>Rating Strategy of Comorbidity</th>
<th>CIRS</th>
</tr>
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<tbody>
<tr>
<td>o No problem</td>
<td>0</td>
</tr>
<tr>
<td>1 Mild Illness/impairment with or without requirement of therapy, excellent prognosis, patient with normal activity.</td>
<td>1</td>
</tr>
<tr>
<td>2 Moderate Illness/impairment requiring therapy, good prognosis, compromised activity of patient.</td>
<td>2</td>
</tr>
<tr>
<td>3 Severe Illness/impairment with urgent requirement of therapy, prognosis unclear, marked restriction in activity.</td>
<td>3</td>
</tr>
<tr>
<td>4 Extremely severe Life threatening illness/impairment, emergency case of therapy, adverse prognosis.</td>
<td>4</td>
</tr>
</tbody>
</table>

Please take into account that CLL induced illness or organ damage are not included in this rating scale! The goal of this rating scale is to assess comorbidity other than CLL in the patient. If there are two or more illnesses/impairments of one organ system, the illness/impairment with the highest severity should be evaluated!

<table>
<thead>
<tr>
<th>Organ system</th>
<th>If illness/impairment present, please specify</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Heart</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>2. Blood pressure</td>
<td>☐</td>
<td>☐</td>
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<tr>
<td>3. Vascular</td>
<td>☐</td>
<td>☐</td>
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<tr>
<td>4. Respiratory</td>
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<td>☐</td>
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<tr>
<td>5. Ear/nose/throat</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>6. Upper gastrointestinal</td>
<td>☐</td>
<td>☐</td>
</tr>
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<td>7. Lower gastrointestinal</td>
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of six cycles). Fludarabine: 40 mg/m² orally, days 1-3; cyclophosphamide 250 mg/m² orally days 1-3; rituximab: the first infusion 375 mg/m², 500 mg/m² thereafter.

- Patients with comorbidities and older patients (>65 years): chlorambucil (for example 10 mg/m² daily for seven days, every four weeks, until maximum response, or x 12).

**Treatment of relapsed CLL (figure 2)**

**In clinical trial**
- HOVON 101 (PROLON): randomised phase III ofatumumab maintenance study. Inclusion criteria: Relapsed CLL second or third remission, and within three months after reaching the second or third CR/PR with any induction regimen.

**Outside clinical trials**
- Response duration following first line >1 year: repeat same treatment;
- In case of a response duration <1 year: second-line therapy, e.g. FCR;
- In both cases, then consider HOVON 101 (PROLON); see above.

In case of refractoriness to fludarabine, or relapse <1 year, following fludarabine-containing chemotherapy or relapse <2 years following fludarabine-containing immunotherapy, or in case of 17p deletion AND if the patient is eligible for an allogeneic allo-SCT:

**In clinical trial**
- HOVON 88 (R-DHAP followed by RIST). Inclusion criteria: <70 years and refractory or relapsed within one year after the fludarabine chemotherapy or two years after fludarabine-containing immunotherapy or relapse and 17p deletion.

**Outside clinical trial**
- Induction treatment as described in treatment options for refractory CLL (below), if possible followed by RIST.

In case of fludarabine refractoriness:

**In clinical trial**
- D’ACCORD study, dasatinib ± fludarabine. Inclusion criteria: refractory disease following fludarabine-containing (immuno) chemotherapy treatment or first signs of relapse within six months after an initial response on fludarabine-containing (immuno) chemotherapy treatment. The patient may have received additional treatments following fludarabine.

**Outside clinical trials**
- Alemtuzumab therapy (first week 3 mg, 10 mg, 30 mg followed by 30 mg three times a week for up to three months);
- In case of bulky disease or contraindications for alemtuzumab (high risk of infection): Consider:
  - Ofatumumab (2000 mg once a week for eight weeks, followed by 2000 mg once a month for four months);
  - Rituximab in combination with high-dose prednisone (375 mg/m² rituximab twice a week combined with HDMP 1 g/m² once a day for five days every four weeks up to a maximum of three cycles).

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New insights into the pathogenesis of chronic myeloid leukaemia: towards a path to cure

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KEYWORDS

Chronic myeloid leukaemia; leukaemic stem cells; molecular pathways; pathogenesis

INTRODUCTION

Chronic myeloid leukaemia (CML) is a myeloproliferative clonal disease arising at the level of a pluripotent stem cell. It is consistently associated with the presence of the Philadelphia (Ph) chromosome, which was first described by Peter Nowell and David Hungerford in 1960 and was named after the city where they discovered it.1 In 1973, it was Janet Rowley who discovered that the Ph chromosome results from a (9;22)(q34;q11) reciprocal translocation that juxtaposes the c-abl (ABL) oncogene on chromosome 9 with the breakpoint cluster region (BCR) on chromosome 22.2,3 The resulting mRNA molecules, encoded by this newly formed BCR-ABL gene, results in the formation of the BCR-ABL protein responsible for the disease entity CML. Depending on the breakpoint on the BCR gene, either a p210 fusion protein (M-bcr breakpoint) or a p190 fusion protein (m-bcr breakpoint) is generated. The p210 fusion protein is most common in CML, while the p190 fusion protein is mostly generated in acute lymphoblastic leukaemia.4 The BCR-ABL protein has constitutive kinase activity and is considered to be essential for the survival and growth of leukaemic cells.5 By triggering multiple downstream signalling pathways BCR-ABL promotes cell proliferation and transformation, suppresses apoptosis, alters cell adhesion to bone marrow stroma and induces genetic instability.5,6 Patients with CML may present with night sweats, fatigue, abdominal fullness, gout, leucocytosis and splenomegaly, but half of the patients are accidentally diagnosed by blood testing for other reasons. A massive accumulation of immature and mature myeloid cells is present in peripheral blood, bone marrow and spleen. In most cases, the disease initially presents in a relatively well-tolerated chronic phase, when functionally normal mature blood cells are produced. However, if inadequately treated or therapy resistant, CML evolves into an accelerated phase and will eventually progress to a rapidly fatal blast crisis, in which cell differentiation is blocked.7 In this phase, the disease either resembles acute myeloid (two thirds of cases) or acute lymphoblastic leukaemia (one third of cases).5 Before the introduction of the tyrosine kinase inhibitor (TKI) imatinib mesylate (IM), conventional treatment consisted of spleen irradiation, hydroxycarbamide and busulfan or interferon-alpha (IFN-alpha). Of these, only IFN-alpha was able to induce cytogenetic responses in around 20% of patients. Allogenic stem cell transplantation was (and still is) the only potentially curative treatment of the disease but, due to age restrictions and donor availability, only a minority of patients were eligible for this potentially dangerous treatment.8 The introduction of IM in the early 21st century revolutionised the treatment of CML. In the vast majority of patients, IM treatment induces cytogenetic and even molecular responses with very low or undetectable BCR-ABL transcript levels. These patients remain free from progression to blast crisis. However, IM does not cure the disease because it is unable to eradicate the leukaemic stem cells (LSCs), which therefore provides a potential reservoir for relapse.9 Likewise, the LSC is not affected by the recently introduced second-generation TKIs nilotinib and dasatinib.9 Because CML was the first malignant disease to be associated with a pathognomonic genetic abnormality, it is one of the most extensively investigated malignancies. Studying CML has not only unravelled the molecular pathogenesis of this disease but it also provided a framework for an increased understanding of molecular events involved in cancer initiation and progression of many other malignancies.7 In this review we present current insights into the pathogenesis of CML and discuss...
novel molecular targets for therapy, with the emphasis on elimination of CML stem cells.

**PATHOGENESIS OF CHRONIC PHASE CML**

The first evidence that the Ph chromosome alone was sufficient to initiate chronic phase CML came from an experiment in which murine bone marrow was transplanted in lethally irradiated mice after infection with a retrovirus encoding *BCR-ABL*. The transplanted mice showed several haematological malignancies including a CML-like myeloproliferative disease.\(^\text{15}\) This was underscored in studies introducing forced *BCR-ABL* expression by viral gene transduction in human haematopoietic cells after transplantation in mice.\(^\text{16}\)

Although CML is considered to be a stem cell disease and the *BCR-ABL* translocation is presumed to be present in the leukaemic stem cell, CML cells differentiate down the myeloid lineages with almost all myeloid cells bearing the Philadelphia chromosome, while the lymphoid compartment is only partly affected. Only 50% of mature B cells are Ph\(^+\) while virtually no T or NK cells carry the Philadelphia chromosome.\(^\text{17}\) As an explanation, it was postulated that *BCR-ABL* induces a loss of differentiation beyond the T-lymphoid progenitor stage, while the Ph-negative progenitors produce the remaining lymphocytes.\(^\text{18}\)

The remarkable finding that very low *BCR-ABL* transcript levels are detected by ultrasensitive PCR techniques in the leucocytes of 30 to 75% of healthy adults and in several cell lines suggests that the generation of *BCR-ABL* translocations is a relatively frequent event, but also that additional genetic or epigenetic changes are required to generate clinical chronic phase CML. Other explanations for this phenomenon may either be that the chromosome translocation in healthy individuals occurs in more committed progenitor cells with long-term self-renewal capacity which are unable to form a clone with leukemic potential, or that the immune system in CML patients is unable to recognise and eliminate the *BCR-ABL* expressing cells while it is adequate in normal persons.\(^\text{19}\)

Why the *BCR* and *ABL* genes translocate is unknown but the relative closeness of non-homologous *ABL* and *BCR* genes in interphase nuclei of bone marrow cells could be an explanation.\(^\text{20}\)

**CML STEM CELLS**

Haematopoietic stem cells (HSCs) are defined by their two properties: they are capable to reproduce themselves, a property known as self-renewal, and they have the capability to give rise to all mature haematopoietic cell lineages throughout an individual’s lifetime. This means that one of the daughter cells retains its HSC identity, while the other daughter cell becomes a multipotent progenitor. Hereby, life-long haematopoiesis is provided.\(^\text{21}\)

More than half a century ago, in 1951, it was William Dameshek who first suggested that CML cells derive from an HSC and this concept is still considered to be correct. The *BCR-ABL* translocation alone is sufficient to initiate chronic phase CML as is evidenced by several mouse model experiments that either used transplantation with donor mice derived *BCR-ABL* transected bone marrow cells, or *BCR-ABL* transgenic animals. The *BCR-ABL* tyrosine kinase activity results in aberrant stem cell differentiation and survival with a subsequent expansion of the progenitor pool and their downstream progeny.\(^\text{10,18,19}\)

Although their progeny may be normally sensitive to both chemotherapy and TKIs, LSCs are quiescent, non-cycling cells that are inherently unsusceptible to chemotherapy and TKIs. Several mechanisms are responsible for this IM resistance. Firstly, a higher *BCR-ABL* mRNA and protein expression (up to 100 fold and 3-10 fold, respectively) was found in the most primitive compartment compared with more committed progenitor cells.\(^\text{22}\)

This may lead to insufficient inhibition of its kinase activity by IM and thereby to reduced cell killing. In more advanced CML, there is an increase in the average expression of *BCR-ABL*, while the large difference between the most primitive stem cells and the more committed progenitor cells remains. This high *BCR-ABL* expression parallels the autocrine production of IL-3, G/GM-CSF which only occurs in the most primitive stem cells.\(^\text{23}\) Possibly, this autocrine loop offers additional protection of stem cells against IM. Secondly, IM influx into the most primitive stem cells is hampered by their very low expression of their influx pump Oct-1; levels of *Oct-1* transcripts in more mature progenitor cells were more than 100-fold higher.\(^\text{24,25}\)

Thirdly, the mRNA levels of the *ABCB1* (P-glycoprotein) and *ABCG2* (BCRP) efflux pumps for which IM is a substrate were twofold higher in the most primitive cells compared with progenitors.\(^\text{26}\)

Fourthly, it has been demonstrated that the LSC compartment may already harbour several resistant *BCR-ABL* mutated stem cells before initiation of TKI therapy, which may thus confer a growth advantage after TKI treatment is started.\(^\text{27}\)

Lastly, in sharp contrast with the above-mentioned mechanisms, it was recently demonstrated by Corbin et al. that IM did abrogate tyrosine kinase activity in CML stem cells. However, CML stem cells could survive and proliferate even if *BCR-ABL* was inhibited, as long as cytokines were present. This implicates that LSCs may not be *BCR-ABL* independent and thus that non-TKI-based strategies for CML stem cell killing will be required.\(^\text{28}\)

Due to their persistence during and after therapy, LSCs form a potential reservoir for relapse, disease progression...
and resistance. This is supported by the work of Mahon et al. showing that 60% of IM-treated patients, who had had undetectable BCR-ABL transcripts for more than two years and who discontinued IM, rapidly relapsed, mostly within six months. Remarkably, almost 40% of patients did not relapse within one year, as will be discussed further below.

Stem cells can be distinguished from more committed progenitor cells by using long-term culture assays in which stem cells are considered those that are still able to form colonies after at least five weeks of culture; these are called long-term culture initiating cells (LTCICs). As treatment of CML restores normal haematopoiesis in the vast majority of patients, residual normal stem cells must be present at the time of diagnosis. Frequencies of both Ph- and Ph+ LTCICs are lower than in normal bone marrow, but the residual normal stem cells in CML have higher self-renewal capacity than the leukaemic stem cells. This is evidenced by long-term marrow cultures where the Ph population outgrows their malignant counterparts in many cases. Diminished self-renewal capacity of CML stem cells correlates with autocrine IL-3 and G/GM-CSF induced increased cycling. This in turn leads to a vastly enlarged pool of progenitor cells, resulting in a massive accumulation of myeloid cells in chronic phase CML, despite the reduction in leukaemic stem cell numbers.

Apart from discrimination of either leukaemic or normal stem cells through long-term cultures, LSCs can be distinguished from normal HSCs by their immunophenotypic properties. Both LSCs and HSCs reside in the CD34+CD38-Lin- population as is evidenced by in vivo transplantation experiments with irradiated, severely immunocompromised mice. Recently, we demonstrated that LSCs have higher CD34 and CD45 expression than normal HSCs and have different forward/sideward light scatter properties. Moreover, we showed that LSCs may have aberrant expression of CD7, CD11b and CD56, while these markers are never expressed by normal HSCs. Furthermore, LSCs express higher levels of Thy-1 (CD90) compared to residual normal HSCs.

Figure 1. Schematic representation of signalling pathways involved in BCR-ABL mediated leukaemogenesis. Inhibitory pathways are shown as — activating pathways as →. Dashed arrows represent translocation of proteins from cytoplasm to nucleus and vice versa. To improve readability of the figure, only the main intermediates of the signalling pathways are shown.
BCR-ABL ONCOPROTEIN AND ITS CRITICAL DOWNSTREAM MOLECULAR PATHWAYS

BCR-ABL has constitutive tyrosine kinase activity and drives several important downstream signalling pathways, necessitating a cooperative interplay to cause leukaemogenic potential. Eventually, most of them converge at the level of transcription factors, such as STAT proteins, c-Myc and Bcl-2 family, cooperating in leukaemogenesis and conferring a crucial role in the maintenance of LSCs (figure 1). In the next paragraphs we will highlight the most important pathways or proteins involved in CML and its transformation to blast crisis and describe the newest insights into the molecular pathology of CML.

Jak-STAT pathway

The Jak-STAT pathway is the principal signalling mechanism for a wide array of cytokines and growth factors, such as IL-3 and G/GM-CSF. Binding to their receptors results in Jak2 activation, leading to recruitment and activation of signal transducers and activators of transcription (STAT) factor families. Subsequently, STAT proteins migrate into the nucleus where they regulate transcription of genes that are involved in cell proliferation and survival, such as cyclin D1, cyclin D2, Bcl-X, c-Myc and NfkB (figure 1). In CML, Jak2 and STAT5 are constitutively activated.

There are two ways in which BCR-ABL affects the Jak-STAT pathway. Firstly, although IL-3 and G/GM-CSF production is very low in the quiescent leukaemic progenitors, they may spontaneously enter the cell cycle and at that moment start producing autocrine active IL-3 and G/GM-CSF, leading to STAT activation via the normal route. Secondly, BCR-ABL is also able to directly activate STAT5, thereby bypassing Jak2. In turn, Jak2 is also able to activate BCR-ABL. In this context, it is important in vitro that Jak2 knockout or Jak2 inhibition drastically reduced the BCR-ABL level and BCR-ABL activation, causing reduction of oncogenic signalling. In addition, inhibition of Jak2 can also overcome IM resistance in resistant cell lines, including T315I mutants (a highly resistant mutant) and blast crisis cells, paving the way for a role of Jak2 inhibitors in clinical CML.

Wnt/β-catenin

The Wnt/β-catenin signalling pathway is important for HSC self-renewal. Activation of this pathway is a hallmark for CML. Under normal conditions, β-catenin binds to the complex containing axin and the enzyme glycogen synthase kinase-3β (GSK3β). After serine/threonine phosphorylation it is degraded by the proteasome. Direct BCR-ABL mediated activation of β-catenin by phosphorylation of tyrosine residues Y86 and Y654 (Y phosphorylation) renders the free protein more stable and prevents its proteasomal degradation. Activated β-catenin then translocates to the nucleus where it interacts with lymphoid enhancer factor/T-cell factor (LEF/TCF) transcription factor, subsequently regulating the transcription of genes such as c-Myc and cyclin D1 (figure 1). In CML, especially in the accelerated phase and blast crisis, the granulocyte-macrophage progenitor pool has elevated levels of nuclear β-catenin compared with normal progenitors, for which a mutation in the β-catenin inactivating enzyme GSK3β seems to be responsible. Treatment with IM impairs Y phosphorylation and increases β-catenin binding affinity to the axin/GSK3β degradation machinery, resulting in degradation of β-catenin by the proteasome and thereby in normalisation of β-catenin levels. In vitro cell transduction with axin, a strong β-catenin antagonist, reduced the replating capacity of leukaemic cells. Altogether, β-catenin activation may play an important role in CML progression and IM resistance. Targeting β-catenin in synergy with IM might be of therapeutic value for CML patients, including IM-resistant patients.

ICSBP/Gas2

Another connection of CML with the Wnt-β-catenin pathway is via interferon consensus sequence binding protein (ICSBP), an interferon regulatory transcription factor. In CML, decreased expression of ICSBP is associated with poor prognosis, drug resistance and progression to blast crisis. Recently, the growth arrest specific 2 (Gas2) gene was identified as an ICSBP target. Gas2 expression, induced by ICSBP downregulation, inhibited calpain protease activity (a cysteine protease which modulates p53 levels), subsequently increasing stabilisation and activation of β-catenin (figure 1). This pathway may cooperate with the β-catenin inducing pathways described in the previous paragraph and suggests a still undetermined pro-leukaemic role of Gas2. Remarkably, we found high Gas2 levels in primary CD34+ cells of bone marrow and peripheral blood in chronic phase CML compared with normal CD34+ cells (unpublished data).

PP2A

Protein phosphatase 2A (PP2A) is a serine/threonine phosphatase with tumour suppressor activity. It acts by reversing kinase-induced phosphorylation of several key proteins involved in signal transduction pathways regulation, cell cycle progression, DNA replication, gene transcription and protein translation. BCR-ABL indirectly down-regulates PP2A via induction of the SET protein which is a physiological PP2A inhibitor (figure 1). IM treatment restores PP2A levels. The mutual antagonists

Thielen, et al. Pathogenesis of CML.

October 2011, Vol. 69, No 10

453
BCR-ABL and PP2A share several downstream targets essential for leukaemogenesis, among which are Rb, c-Myc, STAT3, ERK1/2, Akt, MAPK, BAD and Jak2.4 An essential part of PP2A tumour suppressor activity is the dephosphorylation of the serine-62 (S62) residue of the oncogenic transcription factor c-Myc by PP2A, resulting in an unstable c-Myc form. In this respect, it is quite interesting that fingolimod (FTY720), a PP2A activator, induced apoptosis and impaired clonogenicity of IM/dasatinib sensitive and resistant myeloid and lymphoid cell lines and in CML blast crisis and Ph+ALL progenitors, while normal CD34+ haematopoietic progenitors were not affected.45 Another recently described endogenous inhibitor of PP2A, cancerous inhibitor of PP2A (CIP2A), protects this c-Myc S62 residue from dephosphorylation by PP2A, thereby increasing c-Myc protein stability (figure 1).46 This appears relevant for CML, as was recently established by Lucas et al. They showed that CIP2A is a prognostic biomarker for CML progression under IM treatment: in patients who progressed to BC, CIP2A protein level at diagnosis was significantly higher than in good responders. Indeed, the probability of disease progression in these patients was 100% at 21 months.46 The above-mentioned studies suggest that CIP2A and PP2A qualify as therapeutic targets in CML.47

C-Myc
The upregulation of the proto-oncogene product c-Myc plays a central role in perturbing CML signalling. Although c-Myc activity sensitises cells towards apoptosis induction, it stimulates proliferation by induction of several cell cycle activation enzymes. Due to disabled apoptotic pathways, mainly caused by concomitant upregulation of several antiapoptotic proteins, as described below, the net effect in CML is pro-oncogenic.48,49 Next to the role of PP2A, β-catenin and the Jak/STAT pathway on c-Myc stability in CML, several other pathways affect c-Myc expression. Firstly, BCR-ABL binds to adaptor proteins, such as GRB2, SHC, CRKL and CBL, after which this complex recruits the nucleotide exchange factor SOS. SOS in turn activates RAS which further signals through RAF, MEK and MAPK, eventually leading to increased c-Myc expression.50 Secondly, BCR-ABL forms a multimeric complex with Jak2, Gab2, PI3K and Akt. This so-called BCR-ABL network downregulates the kinase activity of GSK3β eventually resulting in NFκB activation and subsequent enhancement of c-Myc expression (figure 1).51 Lastly, BCR-ABL inhibits the expression of thanatos-associated protein 11 (THAP11). THAP11 is a c-Myc transcription factor which mediates downregulation of c-Myc (figure 1). It was recently demonstrated that BCR-ABL suppresses THAP11 expression in CML cells, thereby promoting CML cell proliferation via upregulation of c-Myc. Silencing of BCR-ABL by kinase inhibitors or siRNA induced THAP11 expression and in turn repressed c-Myc expression.45

Pathways leading to apoptosis resistance
Deregulation of apoptosis allows LSCs to propagate. In CML decreased sensitivity towards apoptosis is a final consequence of BCR-ABL tyrosine kinase activity, and mainly involves the aberrant expression of the Bcl-2 family of apoptosis regulator proteins, such as the antiapoptotic members Bcl-2, Bcl-XL and Mcl-1 and the pro-apoptotic members Bad and Bim (figure 1).44,52,53 The antiapoptotic family members block translocation of cytochrome-c from mitochondria to the cytosol, thereby preventing execution of apoptosis via caspase activation.44,53 An important regulator of Bcl-2 superfamily-induced apoptosis in CML is the antiapoptotic PI3K/Akt pathway (see next sections). BCR-ABL induced PI3K/Akt activation results in Bad phosphorylation. Bad then dissociates from Bcl-2 and binds to the 14-3-3 adaptor protein, leaving less free Bad available for heterodimer formation with the antiapoptotic protein Bcl-XL (figure 1). Consequently, more antiapoptotic Bcl-2 and Bcl-XL remains in the cytoplasm, preventing cytochrome-c efflux from the mitochondria and subsequent apoptosis induction.54 Next, activation of STAT3 by BCR-ABL induces Bcl-XL expression, in turn contributing to apoptosis resistance of BCR-ABL expressing cells.54,55 Furthermore, the Bcl-2 inhibitor and antagonist Bim, an important downstream target supporting cell survival, is downregulated by BCR-ABL, which in turn upregulates Bcl-2, again preventing execution of apoptosis (figure 1).55,56

Pathways related to adhesion
Normal adhesion of HSCs and progenitor cells to the bone marrow microenvironment is regulated by interaction of integrins and other cell surface receptors with protein components of the extracellular matrix (ECM), such as fibronectin. This interaction not only aims to retain the cells in the bone marrow until they are mature, but also allows different intracellular signal pathways to modulate cellular functions, such as proliferation, migration and apoptosis.57 In CML, BCR-ABL induces altered adhesion to the ECM, resulting in release of immature cells into the peripheral blood, which is a characteristic feature.57 This relates to downregulation by BCR-ABL of L-selectin, ICAM-1 and CCR7, proteins that are implicated in cell adhesion and motility.58 Moreover, BCR-ABL, in a multimeric complex with adaptor proteins, binds several key proteins involved in β-integrin signalling, such as focal adhesion kinase, Pyk-2, vinculin, talin and paxillin, thereby making these proteins unavailable for normal β-integrin mediated signalling transduction.59 As signalling through β-integrin is essential for the reorganisation of F-actin fibres in the cytoskeleton and...
their linkage to the ECM, perturbation of this signalling path results in diminished adhesion of CML cells to the bone marrow microenvironment and in increased cellular motility.66,67

Perturbation of this process may also be important for another reason: normal β1-integrin signalling upregulates the cyclin dependent kinase inhibitor P27<sup>kip-1</sup>, which in turn inhibits cyclin-dependent kinase-2 (CDK-2), preventing cells from entering the cell cycle. Disrupted β1-integrin signalling thereby provides an additional mechanism by which BCR-ABL stimulates entry into the cell cycle (figure 1).52

**Pathways involved in stem cell maintenance**

**Hedgehog, BMI-1 and Notch**

Since the introduction of TKIs and the knowledge that LSCs can persist during treatment, efforts towards determining the molecular pathways that are critical for stem cell maintenance have been intensified. Recent studies showed that the developmental Hedgehog (Hh) pathway, known for its role in embryonic development, tissue regeneration and repair, plays a crucial role in governing the maintenance of leukaemia-initiating cells.63,64 Hh proteins mediate signal transduction in nearby and distant tissues by binding to their specific receptor Patched (PTC). PTC negatively regulates Smo, an Hh intermediate. After binding of Hh proteins to PTC, Smo is released from the inhibition of PTC, and is now able to activate the pathway, resulting in Gli transcription factor mediated transcription of target genes, such as *Ptch1*, *cyclin-D1* and *Bcl-2* (figure 1).63,65 Hh signalling is regulated by GSK3β, which may be important in view of recent data on this enzyme in advanced-phase CML.66 Noteworthy, the investigators found no significant effect of treatment with IM on the expression levels of the diverse proteins involved in the Hh pathway.66

A recent study showed that the expression of Hh ligand Sonic hedgehog (Shh), Smo and the transcription factor Gli1 were significantly higher in CML stem cells than in normal controls, with even higher levels in advanced-phase CML.66 Combination therapy of nilotinib and BMI-1-targeting drugs may be important in view of recent data on this enzyme in normal haematopoietic cells: in different malignancies, both oncogenic and tumour suppressor roles have been described.67 The role of Notch in CML is also controversial, since in one study, activated Notch signalling inhibited cell proliferation and reduced the ability of colony forming, suggesting an inhibitory effect on LSCs, while in another study activated Notch participated in the evolution from chronic phase CML to blast crisis.71,72 Clearly, further research is needed to establish the role of BMI-1 and Notch in CML.

**Promyelocytic leukaemia tumour suppressor protein**

Recently, Ito et al. showed for the first time that promyelocytic leukaemia tumour suppressor protein (PML) expression was high in CML CD<sub>34</sub><sup>+</sup> stem cells and was inversely associated with clinical outcome. PML acts as a repressor of the mammalian target of rapamycin (mTOR), which in turn plays a role in HSC maintenance and leukaemogenesis (figure 1).71 In vitro and in vivo data regarding inhibition with the PML inhibitor As<sub>2</sub>O<sub>3</sub> showed disruption of LSC maintenance, resulting in impaired quiescence and sensitisation of LSCs to pro-apoptotic stimuli, probably making As<sub>2</sub>O<sub>3</sub> a potential beneficial therapeutic agent in CML.71

**Pten pathway**

A gene recently identified by microarray analysis of LSCs in CML is *phosphatase and tensin homologue* (*Pten*). Compared with HSCs from healthy donors, decreased transcriptional activity was found in LSCs in CML patients.73 Pten dephosphorylates phosphatidylinositol
In pancreatic cancer cell lines it was shown that Pten PI3K activity and activation of Akt, eventually promoting inactivation decreases its phosphatase activity, favouring to suppress development of several tumours. Thus, Pten inhibited more differentiated leukaemia cells. Remarkably, and IM. The combination proved to be better than treatment of CML mice with the Alox-5 inhibitor zileuton effect on LSCs. 77 This led to the use of combination Most importantly, Alox5 deficiency had a specific inhibitory role on myelopoiesis and modulation of proliferation and inflammation and cancer, they also have a stimulating explanation why LSCs are insensitive to TKIs (see above). Specifically, this effect provides a further explanation why LSCs are insensitive to TKIs (see above). Anyhow, Alox5 seems pivotal in CML leukaemogenesis as BCR-ABL transduced Alox5−/− mice failed to develop CML. Most importantly, Alox5 deficiency had a specific inhibitory effect on LSCs.77 This led to the use of combination treatment of CML mice with the Alox-5 inhibitor zileuton and IM. The combination proved to be better than treatment with either drug alone in prolonging survival of CML in this model. Zileuton targeted the LSC while IM inhibited more differentiated leukaemia cells. Remarkably, loss of Alox5 also caused downregulation of β-catenin expression in LSCs (see above).77 These data indicate that targeting the Alox5 pathway might be a rational approach for optimising CML treatment.79 Phase 1 studies with the combination of imatinib and zileuton are already planned.

**FoxO pathway**

Lastly, a novel player in the field is the Forkhead-O (FoxO) subfamily of transcription factors. They play an important role in haematopoiesis and regulate diverse physiological processes, such as cell-cycle arrest, stress resistance, apoptosis and self-renewal capacity of HSCs. All four FoxO members (FoxO1, FoxO3, FoxO4 and FoxO6) act downstream of the PI3K/Akt pathway. They are phosphorylated by Akt upon growth factor stimulation or insulin, resulting in nuclear export and as a consequence FoxO is degraded in the cytoplasm (figure 1). In the absence of growth factors or insulin, unphosphorylated FoxO members reside in the nucleus and act as transcription factors, resulting in pro-apoptotic signalling. In mice, individual FoxO1 and FoxO4 knockout mice did not show an overt haematopoietic phenotype, but triple loss of FoxO members (FoxO1, FoxO3, FoxO4) caused defective long-term repopulation activity, correlating with increased cell cycling and apoptosis of HSC. In CML, BCR-ABL activates the PI3K/Akt pathway and this in turn suppresses the FoxOs, thereby supporting the proliferative and antiapoptotic properties of CML cells. Using mouse models, showed that particularly FoxO3a has a role in the maintenance of CML LSCs. Cells with nuclear FoxO3a and decreased Akt phosphorylation were enriched in the stem cell compartment. In addition, transforming growth factor-β (TGF-β) acts as a crucial regulator of Akt activation and controls FoxO3a localisation in CML LSCs. The combination of TGF-β inhibition and IM efficiently depleted LSCs and attenuated CML development.80 Also, degradation of FoxO3a by the proteasome plays an important role in suppression of FoxO. In line with that, treatment with bortezomib, a proteasome inhibitor, resulted in increased levels of FoxO and led to a complete molecular remission in a case of Ph+ acute lymphoblastic leukaemia.81

**MOLECULAR AND CELLULAR EVENTS INVOLVED IN TRANSFORMATION TO BLAST CRISIS**

In the eight-year follow-up of the IRIS trial, around one third of the patients discontinued IM due to primary or secondary resistance or IM intolerance. These patients are at risk for disease progression to accelerated phase or blast crisis.84 The processes responsible for this transformation
are still not fully understood although it is generally believed that unrestrained and increasing BCR-ABL activity causes genetic instability and ultimately promotes clonal evolution. BCR-ABL overexpression is considered to be the result of a multistep, time-dependent process, characterised by a multiplicity of genetic and epigenetic events. Genetic abnormalities include the presence of additional chromosomes, gene deletions and insertions, point mutations of which doubling of the Philadelphia chromosome, loss of 17p and trisomy 8 are the most common. It has been hypothesised that accelerated shortening of telomeres, particularly in the context of increased telomerase activity, might facilitate acquisition of these genetic aberrancies. On the molecular level, mutations of the tumour suppressor genes p53 and runt-related transcription factor gene 1 (RUNX1) are among the most common in myeloid blast crisis, while mutations at the cyclin-dependent kinase inhibitor 2A/2B (CDKN2A/B) and Ikaros transcription factor (IKZF1) are most common in lymphoid blast crisis. Relevant data concerning transformation of stem cells were demonstrated by Jamieson et al. Progression to blast crisis is associated with expansion of the myeloid progenitor compartment, which aberrantly requires self-renewal resulting in LSC generation and blast crisis transformation. This correlated with increased β-catenin levels and upregulation of β-catenin target genes expression, due to inactivating mutations in the GSK3β protein, as described above. Furthermore, progression to blast crisis was avoided in a β-catenin knockout mouse model of CML. Changes similar to those seen with β-catenin are seen for BCR-ABL; while increases of BCR-ABL levels due to amplification are seen in granulocyte-macrophage progenitors, they stay relatively constant in LSCs during progression. Epigenetic changes, such as increased methylation of several genes, have also been reported. Apparently, genomic instability is also a feature of the transformation process which may be related to the BCR-ABL induced increased production of reactive oxygen species and abnormal DNA repair mechanisms.

Finally, recent data show an important role of the RNA binding protein Musashi2 (Ms12) in CML progression. The expression of Ms12 is highly upregulated during human CML progression to blast crisis and is an indicator for poor prognosis. Ms12 represses Numb expression, a protein which drives commitment and differentiation and impairs development and propagation of blast crisis. Inhibition of Ms12 expression in mice restored Numb expression and prolonged survival.

### Table 1. Selection of inhibitors or activators of proteins involved in BCR-ABL induced signalling pathways. The list is incomplete and only restricted to molecules published in relation to CML

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Inhibitor</th>
<th>Activator</th>
<th>Substrate</th>
<th>Possible combinations</th>
<th>LSC/HSC specificity</th>
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<tr>
<td>JAK-STAT</td>
<td>Diverse Jak2 inhibitors</td>
<td>Jak2</td>
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<td>DTT388l3 and imatinib or dasatinib</td>
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<td>DTT388l3</td>
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<td></td>
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<td>FTY720</td>
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<tr>
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<td>Bortezomib/proteasome</td>
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<td></td>
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<td></td>
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<td>mTOR</td>
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<tr>
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<td>Zileuton</td>
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<td></td>
<td>ETYA (AA analogue)</td>
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<td>TGF-β-inhibitor and imatinib</td>
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PML = promyelocytic leukaemia tumour suppressor protein; LSC = leukaemic stem cells; HSC = haematopoietic stem cells; IM = imatinib mesylate.
CONCLUDING REMARKS

After the introduction of IM, CML has turned from an often fatal disease into a generally treatable chronic condition for most patients. Despite this great success, there are still problems that need to be addressed: around 45% of patients discontinue IM due to toxicity or unresponsiveness and approximately 8% of patients suffer from disease progression to the accelerated phase/blast crisis. The second-generation TKIs nilotinib and dasatinib are able to resolve side effects and to overcome resistance seen with some BCR-ABL mutations, but, like IM, are unable to eradicate the stem cell pool either.

CML LSCs are thought to be responsible for this TKI resistance and disease progression. They remain untouched by IM and other chemotherapy due to several mechanisms, described above. This allows LSCs to propagate and renders CML currently incurable. Searching for strategies to kill these LSCs and thereby achieving a cure of CML is the main focus of the current CML research.

A straightforward strategy for targeting LSC is through inhibition or activation of key molecules which lead to inhibition or restoration of signalling pathways, which play a role in regulation of both normal HSCs and LSCs. Examples of key molecules against which promising inhibitors or activators have already been developed are Jak2, PP2A, Hedgehog/Smo, 5-LO, PML, mTOR and FoxO (table 1). Combining several of these agents, possibly in combination with TKIs, may prove to be synergistic against CML stem cells as already suggested by in vitro and in vivo data. However, a potential problem of these strategies is that normal HSCs and LSCs share many common properties which means that signalling pathways determining the cell fate of LSCs also govern maintenance of normal HSCs. Thereby, normal HSCs may simultaneously be affected, leading to severe side effects. Accordingly, the next goal is to target genes that may simultaneously be affected, leading to severe side effects.

In conclusion, the complex pathogenesis of CML is slowly but steadily being unravelled. Although the introduction of TKIs has dramatically improved the prognosis of CML patients, many problems remain to be solved. Cure can still not be attained because TKIs are unable to eradicate the LSC compartment. Efforts towards determining the molecular pathways critical for the maintenance of these cells, as well as to develop better and faster techniques to differentiate the LSC from the normal HSC, have intensified the last decade. Using this knowledge will aid in development of new strategies to recognise and kill LSCs, hopefully progressing towards cure of CML.

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62. 439


Clinical effects of leucoreduction of blood transfusions

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ABSTRACT

For many years filtration for removal of leucocytes from red blood cell (RBC) and platelet transfusions was applied for selected patients to prevent cytomegalovirus (CMV) reactivation, HLA immunisation and recurrent febrile nonhaemolytic transfusion reactions (FNHTR). Since the 1980s, there was also growing concern about cancer recurrence and postoperative infections. In this review we discuss the studies on possible benefits of leucoreduction. In 2001 the Dutch Health Council decided that all blood products should undergo leucoreduction by filtration, as a precautionary measure to reduce possible transmission of variant Creutzfeld-Jacob disease (vCJD). The incidences of transfusion-transmitted CMV infection, HLA immunisation and FNHTR are decreased by universal leucoreduction. However, transfusion-related immunomodulation with presumed negative effects on cancer immunosurveillance, postoperative infections or aggravating organ failure, investigated in randomised controlled trials, revealed no support for extended indications for leucoreduction. An exception was seen in cardiac surgery where leucoreduction reduced short-term mortality by approximately 50%. The exact mechanism(s) for this effect is (are) not known. Pro-inflammatory cytokines induced by leucocyte-containing RBC transfusions in combination with the inflammatory response after cardiac surgery may aggravate morbidity and could lead to mortality.

In this review we discuss the evidence for the benefits of universal leucoreduction. Based on the available evidence, reversal to the use of buffy-coat depleted RBCs and restricted indications for leucoreduction by filtration (extended with open-heart surgery) is a safe option.

KEYWORDS

Blood transfusion, leucoreduction, clinical effects

INTRODUCTION

In the 1970s awareness grew that leucocytes present in blood components intended for transfusion could transmit cytomegalovirus (CMV), evoke human leucocyte antigen (HLA) antibodies and induce immunosuppression. Leucocyte antibodies can cause febrile transfusion reactions, platelet transfusion refractoriness and for dialysis patients prolong the waiting time for a suitable, cross-match negative, renal transplant. Seemingly in contrast, patients receiving a kidney allograft showed improved graft outcome after pretransplant blood transfusions. This observation caused concern for possible impairment of immune surveillance against cancer and susceptibility for postoperative infections. This initiated research on the role of allogeneic leucocytes in blood components to remove these leucocytes by centrifugation and later by filtration, with gradually increasing efficiency from 1-log up to 4-log reduction. The presumed adverse and beneficial effects of passenger leucocytes in blood transfusions are referred to as transfusion-related immunomodulation (TRIM).

Besides leucoreduction of platelet transfusions, indications for leucoreduced (LR) red blood cells (RBCs) were initially restricted to patients with a high risk for the sequels of HLA antibodies and CMV transmission, e.g. intrauterine transfusions, prematurely born infants, patients needing platelet transfusions and patients with or awaiting an organ transplant. In addition, patients who suffered twice from a febrile non-haemolytic transfusion reaction (FNHTR) further received LR blood components. In 2001, as a precautionary measure to reduce possible transmission of variant Creutzfeldt-Jakob disease (vCJD), the Dutch Minister of Health ordered that all blood products should be leucocyte depleted. Transmission of vCJD is, a decade later, no longer considered to be a serious transfusion risk to justify universal leucoreduction (ULR). In this review we discuss studies on other possible clinical benefits of leucoreduction.
After infection, CMV is latently present in mononuclear cells lifelong. When T lymphocyte mediated control is lost due to immune suppressive treatment, endogenous CMV replication can lead to CMV disease. CMV disease in immunocompromised patients is life-threatening. Foetuses and prematurely born infants and CMV-negative recipients of solid organs or haematopoietic stem cells of CMV-negative donors are at risk to acquire primary transfusion transmitted (TT)-CMV infection. Besides TT-CMV infection, another concern was that immunomodulation by allogeneic leucocytes in blood components would stimulate endogenous virus replication in CMV-seropositive recipients. To prevent TT-CMV infection, seronegative donors (approximately half of the donor population) can be selected. Leucoreduction, removing leucocytes harbouring latent CMV, is another option. A systematic review of studies in newborns on leucoreduction reported a clinically relevant but not significant (OR: 0.19; 95% CI: 0.01 to 3.41) possible reduction of TT-CMV infection. However, studies not conducted in the Western world often find no benefit of leucoreduction, attributed to a high level of community-acquired CMV infections in areas where CMV is more endemic. The question also arose whether leucoreduction and CMV-seronegative donor selection are equally safe. A systematic review of all available studies until 2005, mainly conducted in bone marrow transplant (BMT) recipients, showed that compared with non-CMV screened and non-LR transfusions both approaches showed a huge and significant 92 to 93% reduction of TT-CMV infection. After transfusion of blood from CMV-seronegative donors an incidence of TT-CMV infection was observed in 1.45% of 829 recipients (11 studies) and after LR transfusions in 2.73% of 878 recipients (12 studies). Three controlled studies compared selection of CMV-seronegative donors with leucoreduction by filtration in BMT patients. A meta-analysis of the three studies revealed that CMV-seronegative transfusions compared with LR transfusions was associated with a 58% reduction in CMV risk (OR: 0.42; 95% CI: 0.22 to 0.79). Recent prospective studies, using CMV-PCR, even observed a higher CMV conversion rate. Of 46 haemato-oncological patients, three (6.5%) became CMV-PCR positive. They had received 1316 blood products of which 450 derived from CMV-seropositive donors, suggesting a transmission rate of 0.65%/product. Because community-acquired CMV could have occurred during the study period spanning two years, the incidence of CMV transmission may be less, but this study illustrates that leucoreduction does not provide complete elimination of CMV transmission. Besides technical failures, non-cell bound CMV in plasma that can be detected in donors up to a year after primo-infection might be a cause. The VATS (Viral Activation Transfusion Study), a randomised controlled trial in human immunodeficiency virus (HIV) positive anaemic patients, showed that the viral replication rate of CMV and HIV was not enhanced after standard, non-LR RBC compared with LR-RBC transfusions, offering no support that allogeneic leucocytes lead to stimulation of endogenous CMV activation. A recent survey of current practice in the United States on the prevention of TT-CMV infection showed that 65% of responding institutions considered both methods to be equally safe. In the Netherlands, a coupled transfusion strategy (seronegative donor selection and leucoreduction) is advised for very high-risk patients, such as patients needing intrauterine transfusions and very low birth weight newborns, while transplant patients receive only LR blood components. Although leucoreduction significantly reduces TT-CMV infection the question whether it is as safe as selection of CMV-seronegative donors has not been solved.

**STUDIES ON CYTOMEGALOVIRUS TRANSMISSION**

Febrile non-haemolytic transfusion reactions (FNHTR) are the most common transfusion reactions. FNHTR results from leucocytes in transfused blood destroyed by antibodies in the recipient, generating pyrogens in vivo or by pyrogenic cytokines such as IL-6, IL-8, TNF-α, IL-1β and CD40L, which are released during storage by contaminating leucocytes and platelets. In a multivariate analysis the storage duration of RBCs before transfusion was identified as a more significant factor associated with FNHTR than leucocyte contamination. In a randomised controlled trial (RCT) the supernatants of stored non-LR platelets and not the platelets themselves caused febrile reactions. RCTs on the incidence of FNHTR after LR components as compared with standard products are scarce. In an RCT using FNHTR after platelet transfusions as primary endpoint a modest absolute decrease of 11.7% by leucoreduction was found. Two large RCTs in multi-transfused patients evaluating transfusion reactions as secondary endpoint, the VATS and the TRAP study (Trial to Reduce Alloimmunization to Platelets) did not find a decrease of FNHTR, either for leucoreduction of red cells or for leucoreduction of platelets.

Because FNHTR in incidentally transfused patients is a rarer event, comparative studies would require large patient numbers. Such studies were mainly conducted as ‘before-and-after ULR’ retrospective studies. In the John Hopkins Hospital (Baltimore, Maryland, USA) a study was conducted comparing the year 1994 (before) with 2001 (after) evaluating more than 35,000 RBC transfusions. This study reports a reduction of FNHTR from 0.37 to 0.19% (p=0.0008). A similar retrospective analysis from Canada comprising over 140,000 RBC and over 57,000 platelet units, reported a reduction of 0.33 to 0.11% in favour of TT-CMV infection.3 However, studies not conducted in areas where CMV is more endemic.4
of LR-RBC and 0.45 to 0.19% for LR platelets (p<0.001).22 The introduction of ULR more than halved FNHTR in these studies, but it should be noted that in both of these large surveys the non-LR transfusions were not buffy-coat reduced (removing approximately 60% of the leucocytes and 90% of the platelets) as was standing practice in most European countries, including the Netherlands. Residual FNHTR to LR blood components may arise from small amounts of residual leucocytes in case of heavily immunised patients with strong antibodies or from soluble factors (IL-8, sCD40-ligand) released by leucocytes and platelets in the hours prior to filtration or during storage of platelets.16,23,24 In case of platelet transfusions, which are leucoreduced to prevent alloimmunisation, reduction of storage interval or even washing before transfusion may limit FNHTR and other adverse events.

**STUDIES ON TRANSFUSION-RELATED LUNG INJURY**

Transfusion-related lung injury (TRALI) is a life-threatening transfusion reaction with an estimated incidence of 1:1000 to 5000 plasma-containing blood transfusions. TRALI is defined as non-cardiogenic lung oedema presenting within six hours after completion of transfusion. Although strong leucocyte-reactive antibodies in donor plasma can cause TRALI, the syndrome is more often the result of two or multi-hit events. Endogenous neutrophil priming associated with the patient’s underlying illness, combined with biological response modifiers (BRMs) in blood products, result in neutrophil-induced pulmonary endothelial damage leading to capillary leakage. Besides leucocyte-reactive antibodies present in donor plasma, soluble factors accumulating during storage of red cells and platelet products have been associated with TRALI.14–17 Although the presence of leucocytes contributes to the generation of BRMs during storage enhancing accumulation of lipid-priming agents and lysophosphatidylcholines (lyso-PC) as neutrophil-priming factors, sCD40L and other cytokines released by platelets may play a key role in endothelial activation causing TRALI.18–20 To further reduce FNHTR and TRALI, removal of plasma or washing of blood components before transfusion has been proposed.26,31

**STUDIES ON PLATELET TRANSFUSION REFRACTORINESS**

Patients who receive platelet transfusions can develop refractoriness, defined as lack of post-transfusion increment and mostly resulting from clinical causes increasing platelet turn-over. Anti-HLA class I antibodies are the major cause of immunological platelet transfusion refractoriness. Platelets strongly express HLA class I antigens and depending on the strength of allo-antibodies, transfused incompatible platelets are immediately or more slowly destroyed. The immune response to foreign HLA antigens differs from an immune response against all other antigens, which is dependent on the indirect pathway. In the indirect pathway, foreign antigen is processed to peptides and presented by self HLA class II antigens on antigen presenting cells (APCs) and activates self CD4+ T-cells. Through the direct pathway, foreign donor HLA class II expressing APCs directly stimulate recipient CD4+ cells approximately 100 times more efficiently than by the indirect pathway.23 In peripheral blood, dendritic cells, monocytes and B-cells but not platelets constitutionally express HLA class II antigens. Removal of class II bearing white cells virtually abolishes HLA class I immunisation by platelets. The mechanism has not been completely unravelled, but an active process is presumed requiring even a low number of donor leucocytes.33 Besides foreign HLA antigens (signal 1) also co-stimulatory molecules are necessary (signal 2) for an effective APC-CD4+ T-cell interaction. After approximately two weeks of storage, leucocytes lose expression of co-stimulatory molecules associated with impaired immunogenicity.34,35 Experiments in the mouse confirmed that, dose-dependently, addition of viable leucocytes but not non-viable leucocyte (fragments) to a platelet suspension, strongly enhanced antibodies against HLA class I antigens.36

After an initial observational study,37 from 1983 to 1995, six (five small European) RCTs with a sample size of seven to 46 patients and in total comprising 295 patients (140 after prior exposure to pregnancy and/or transfusions and 155 naive patients) compared leucocyte-reduced with standard platelet transfusions. A meta-analysis of these early studies concluded on a 68% reduction of risk for platelet refractoriness (95% CI: 0.18 to 0.36) by LR platelet transfusions.19 In 1997 the results of a larger study from the US, the TRAP study,38 comprising 400 patients, came to a similar outcome of a highly significant 74% reduction in platelet refractoriness. In both the combined European studies and the US TRAP study immunological naive patients benefited most from leucoreduction (>85% reduction in refractoriness) as compared with patients with prior pregnancies (circa 50% reduction).38 Since leucoreduction of platelet transfusions, refractoriness towards random platelet transfusions due to HLA antibodies affects less than 5% of the patients.39

**STUDIES ON TRANSFUSION-INDUCED ALLOIMMUNISATION**

Few studies investigated HLA immunisation after LR RBC transfusions. An observational before-and-after ULR study in RBC-transfused dialysis patients found no reduction in...
HLA antibodies.\textsuperscript{47} An RCT comparing buffy-coat depleted versus LR RBC in cardiac surgery patients observed not only a similar incidence of HLA antibodies in both groups, but also of RBC antibodies.\textsuperscript{47}

Red blood cells carry very few HLA class I antigens, but maybe their longer survival time creates a better opportunity for indirect presentation compared with short-living platelets. The presence of allogeneic leucocytes in RBC transfusion was also presumed to enhance antibodies against RBC antigens. Data in animal studies suggested that a concomitant inflammatory reaction as danger signal, as could be given by transfusion of allogeneic leucocytes, would enhance RBC alloimmunisation.\textsuperscript{44} Observational studies on this subject are not equivocal.\textsuperscript{43} A large Dutch before-and-after ULR study comparing buffy-coat depleted RBC and LR RBC found no difference in RBC alloimmunisation.\textsuperscript{44}

**STUDIES ON PRETRANSPLANTATION BLOOD TRANSFUSIONS**

Pretransplantation third-party blood transfusion reducing kidney graft rejection has been investigated in only three RCTs of different designs.\textsuperscript{45-47} One study in 52 patients compared the effect of standard unmodified RBCs with buffy-coat-poor or washed RBC on the development of HLA antibodies and graft survival. No difference in outcome was observed, but the leucoreduced products did not meet the standards (<10$^5$ leucocytes/unit) and all products may have been equally effective.\textsuperscript{45} In a multicentre randomised study in 423 prospective cadaver kidney transplantation patients, a better one-year (90\% vs 82\%; \textit{p}=0.02) and five-year (79\% vs 70\%; \textit{p}=0.023) graft survival was observed after three random pretransplantation transfusions of unmodified RBCs compared with no transfusions.\textsuperscript{46} Also severe rejections were significantly reduced in patients receiving RBCs. In a third multicentre study, 144 patients were randomly assigned to one HLA-DR shared transfusion (\textit{n}=49), one HLA-DR mismatched transfusion (\textit{n}=48) or no transfusion (\textit{n}=47). Blood transfusion consisted of unmodified RBCs stored for less than 72 hours. There was no difference in graft survival at one year (90, 92 and 92\%) or at five years (79, 84 and 80\%) respectively. The incidence of acute rejections in patients who had received an HLA-DR shared transfusion was lower than observed in the other two groups (19 vs 33\%), but this was not statistically significant in this small study.\textsuperscript{47} The three studies do not allow a combined analysis because of heterogeneity in design and the different immunosuppressive protocols and blood products used. Although the largest study found a protective effect of blood transfusion on renal graft survival,\textsuperscript{46} the smaller study designed on the presumed mechanism of induction of allograft tolerance by HLA-DR sharing blood transfusions was not supportive.\textsuperscript{47} Lacking more confirmatory studies an evidence-based conclusion on graft-tolerising effect by pretransplant allogeneic leucocytes in blood products is as yet not possible.

**STUDIES ON CANCER RECURRENCE**

After the finding that blood transfusions could improve renal transplant outcome,\textsuperscript{1} concern was expressed of a deleterious effect of (leucocyte-containing) transfusions on cancer immune surveillance.\textsuperscript{9} This initiative over 100 retrospective and observational clinical studies, not resulting in conclusive data.\textsuperscript{48} In contrast few RCTs in colorectal cancer surgery with curative intent have been conducted to compare leucocyte-containing (buffy-coat depleted) with LR RBCs. Local and distant cancer recurrence was similar in both groups at short-term and at five-year follow-up.\textsuperscript{49-52} It may be noted that colorectal cancer is a weakly immunogenic tumour and that the malignant cells can downregulate HLA expression and co-stimulatory molecules allowing tumour cells to escape from immune attack, whether or not the immune response is suppressed by transfusions.\textsuperscript{31} An immunosuppressive effect of leucocyte-containing RBC transfusions on cancer immune surveillance has been shown to be absent for colorectal cancer; it has never been shown in other malignancies either, although because of lack of studies it can still not be excluded.

**STUDIES ON POST-TRANSFUSION INFECTIONS**

Another concern of the immunosuppressive effect of leucocyte-containing RBC transfusions was susceptibility for infections, in particular in the postoperative period. Sixteen RCTs, conducted in various clinical settings, evaluated post-transfusion infections as primary or secondary endpoint after LR RBC transfusions; six in colorectal surgery,\textsuperscript{49,50,54-57} six in open heart surgery\textsuperscript{58-63} and four in miscellaneous conditions.\textsuperscript{11,64-66} These studies varied as to single or multiple centre design, clinical diagnosis, methods to document infections and proportion of transfused patients ranging from 14 to 95\% and revealed different outcomes (table 1). Several meta-analyses were performed but these came to different conclusions. Meta-analyses using intention-to-treat analyses seldom found associations between LR transfusions and postoperative infections.\textsuperscript{57} The other meta-analyses, restricted to transfused patients only, thereby excluding 36\% of the study population, reported up to almost 60\% reduction in postoperative infection after transfusion of LR RBC.\textsuperscript{58} A role of leucocyte-containing RBCs on the increase
The development of HLA-antibodies and postoperative infections is not proven beyond reasonable doubt. However, only a few studies adjusted for the number of administered RBC, which may be an important factor as observed in cardiac surgery patients.

**STUDIES ON MORTALITY AFTER SURGERY**

A Dutch randomised controlled trial, aimed to investigate development of HLA-antibodies and postoperative infections after RBC transfusions in cardiac surgery, found surprisingly a higher mortality rate in patients receiving leucocyte-containing RBC transfusions. Mortality due to multi-organ dysfunction syndrome (MODS) was the major cause of excess deaths after non-LR transfusions. In this study patients were randomised to three different blood products: buffy-coat-depleted (BCD) RBCs were compared with two filtered RBCs: either fresh filtered RBCs (FF) or stored filtered RBCs (SF). Between the two types of filtered RBCs the mortality rate was not different. This suggests that soluble mediators, still present in the SF products, caused no more adverse effects than FF RBCs, lacking leucocyte-derived soluble mediators.

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### Table 1. Characteristics of patients participating in RCTs comparing leucodepleted versus leucocyte-containing RBCs

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<th>Author; year</th>
<th>No. patients/ transfused (%</th>
<th>Clinical setting</th>
<th>Transfused patients</th>
<th>No. RBCs mean±SD or median (range)</th>
<th>Transfused patients with &gt;4 RBC (%</th>
<th>Main endpoints</th>
<th>Results (LD vs BCD)</th>
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<td>LD 24 (7-10)</td>
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<td>0.2 vs 23%&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>LD 24 (7-10)</td>
<td>BCD 9 (3-9)</td>
<td>ND</td>
<td>Infections</td>
<td>3.0 vs 23%&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Tartier et al.; 1998</td>
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<td>Colorectal surgery</td>
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<td>ND</td>
<td>Mortality</td>
<td>3.4 vs 2.8%&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Titlestad et al.; 2001</td>
<td>279/112 (45)</td>
<td>Colorectal surgery</td>
<td>LD 24 (7-10)</td>
<td>BCD 9 (3-9)</td>
<td>ND</td>
<td>Infections</td>
<td>15 vs 44%&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>van Hillen et al.; 2004</td>
<td>1055/545 (52)</td>
<td>Colorectal cancer surgery and aortic aneurism</td>
<td>LD 26 (7-10)</td>
<td>BCD 9 (3-9)</td>
<td>ND</td>
<td>Infections</td>
<td>43 vs 37%</td>
</tr>
<tr>
<td>Skanberg et al.; 2005</td>
<td>642/298 (46)</td>
<td>Colorectal cancer</td>
<td>LD 24 (7-10)</td>
<td>BCD 9 (3-9)</td>
<td>ND</td>
<td>Respiratory support</td>
<td>7.6 vs 8.1%&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nathens et al.; 2006</td>
<td>1864/268 (14)</td>
<td>Trauma patients</td>
<td>LD 24 (7-10)</td>
<td>BCD 9 (3-9)</td>
<td>ND</td>
<td>Infections</td>
<td>7.6 vs 5.3 vs 3.9%&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>van de Watering et al.; 2006</td>
<td>914/866 (93)</td>
<td>CABG ± valve surgery</td>
<td>FF 28 (5-10)</td>
<td>SF 28 (5-10)</td>
<td>ND</td>
<td>Infections</td>
<td>17 vs 18 vs 23%</td>
</tr>
<tr>
<td>Beacey et al.; 2009</td>
<td>357/295 (83)</td>
<td>CABG ± valve surgery</td>
<td>LD 24 (7-10)</td>
<td>BCD 9 (3-9)</td>
<td>ND</td>
<td>Infections</td>
<td>7.8%&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Wallis et al.; 2009</td>
<td>597/409 (69)</td>
<td>CABG ± valve surgery</td>
<td>LD 24 (7-10)</td>
<td>BCD 9 (3-9)</td>
<td>ND</td>
<td>Infections</td>
<td>49 vs 38 vs 35%</td>
</tr>
<tr>
<td>Bilgin et al.; 2004</td>
<td>474/ 432 (91)</td>
<td>Valve surgery ± CABG</td>
<td>LD 24 (7-10)</td>
<td>BCD 9 (3-9)</td>
<td>ND</td>
<td>Infections</td>
<td>5.9 vs 2.9 vs 2.5%&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Connery et al.; 2004</td>
<td>98/ 69 (70)</td>
<td>Primary CABG</td>
<td>LD 24 (7-10)</td>
<td>BCD 9 (3-9)</td>
<td>ND</td>
<td>Infections</td>
<td>23 vs 32%&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Boshkov et al.; 2005</td>
<td>1227/ 562 (46)</td>
<td>CABG ± valve surgery</td>
<td>LD 24 (7-10)</td>
<td>BCD 9 (3-9)</td>
<td>ND</td>
<td>Infections</td>
<td>8.4 vs 12.7%</td>
</tr>
<tr>
<td>Dzik et al.; 2006</td>
<td>2780/100 (100)</td>
<td>All patients</td>
<td>LD 24 (7-10)</td>
<td>BCD 9 (3-9)</td>
<td>ND</td>
<td>Infections</td>
<td>3.4 vs 2.8%</td>
</tr>
<tr>
<td>Collier et al.; 2001</td>
<td>531/524 (99)</td>
<td>HIV-positive</td>
<td>LD 24 (7-10)</td>
<td>BCD 9 (3-9)</td>
<td>ND</td>
<td>Infections</td>
<td>5.8% vs 53%&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Data on ALI were reanalyzed and presented in another publication<sup>10</sup> than the initial publication.<sup>14</sup><sup>1</sup>Statistically significant (p<0.05) between BCD and LD (SF+FF).<sup>2</sup>*This RCT was interrupted early. ALI = acute lung injury; BCC = buffy-coat-containing RBCs; BCD = buffy-coat-depleted RBCs; FF = fresh filtered RBCs; LD = leucodepleted RBCs; MODS = multi-organ dysfunction syndrome; ND = not documented; ns = not significant; PR = plasma-reduced RBCs; PTI = pulmonary tract infections; SF = stored filtered RBCs; WB = whole blood; WBF = white blood cell filtered.

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<sup>1</sup>Deaths in cardio-surgical patients in ALI were reanalyzed and presented in another publication<sup>10</sup> vs the initial publication<sup>14</sup>.<sup>1</sup>Statistically significant (p<0.05) between BCD and LD (SF+FF).
factors. A subsequent Dutch RCT conducted in high-risk cardiac surgery (anticipating higher transfusion needs) investigated the effect of leucoreduction on the incidence of MODS, but found no difference in incidence (circa 20%) after leucocyte-containing RBCs or LR RBCs. However, MODS as a cause of death occurred more often in patients who received BCD RBC. Subgroup analysis showed that only patients who received more than three RBC units suffered higher mortality in the group receiving BCD RBC.

In total 12 RCTs investigated mortality in different clinical settings: six in cardiac surgery and six in other settings. Overall, no adverse effect of leucocyte-containing transfusions on short-term mortality has been found (OR: 1.14; 95% CI: 0.89 to 1.45). This meta-analysis identified an exception for cardiac surgery (OR: 1.72; 95% CI: 1.05 to 2.81) (figure 1). The observation that it is not the soluble mediators released by leucocytes during storage but rather the number of units transfused that entails the worse outcome, suggests that more complex surgical patients requiring more RBC transfusions are more susceptible to TRIM. We analysed in more detail the causes of death in our two RCTs in cardiac surgery. This revealed an excessive mortality rate in patients who received standard BCD RBCs, compared with before storage filtered LD RBCs; these patients died from a combination of MODS and the presence of infections in the postoperative period (OR: 2.92; 95% CI: 1.22 to 6.97; p=0.02). All other causes of short-term mortality, such as bleeding, cardiac causes, surgical complications, postoperative infections alone and MODS without infections, were equal in both transfusion arms.

Comparison of long-term mortality after transfusions of BCD RBCs or LR RBCs has only been investigated after colorectal cancer surgery, which observed no difference in survival. Although in cardiac surgery the long-term survival is negatively influenced after perioperative allogeneic blood transfusions as compared with nontransfused patients, the long-term effect of allogeneic leucocytes in RBCs after cardiac surgery is not known.

<table>
<thead>
<tr>
<th>Figure 1. Short-term mortality in RCTs comparing filtered RBCs and non-filtered RBCs (OR, 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-cardiac surgery Cardiac surgery RCTs</td>
</tr>
<tr>
<td>Favours non-filtered RBCs</td>
</tr>
<tr>
<td>Non-cardiac surgery</td>
</tr>
<tr>
<td>Jensen'96</td>
</tr>
<tr>
<td>Tiffler'don</td>
</tr>
<tr>
<td>v.Hilten'04</td>
</tr>
<tr>
<td>Combined, all</td>
</tr>
</tbody>
</table>

### POSSIBLE MECHANISMS OF TRANSFUSION-RELATED IMMUNOMODULATION

From the mentioned RCTs, a negative effect of allogeneic leucocytes in RBC transfusions was only found in open heart surgery showing (transfusion dose related) excess mortality from postoperative infections in patients who had developed MODS, indicative for a two- or multiple-hit event. This suggests a synergistic effect between transfusion-related immunomodulation and excessive tissue damage and/or with extracorporeal bypass. In cardiac surgery blood is exposed to the extracorporeal circuit, hypothermia and ischaemia/reperfusion injury. Tissue damage generates products and exposes structures of degraded tissue (e.g. heat-shock proteins, proteases) interacting with sensors (Toll-like receptors) on macrophages leading to immediate release of stress.
hormones, inflammatory cytokines and chemokines.\textsuperscript{77} Besides release of cortisol, serotonin, TNF-\(\alpha\), IL-1-\(\alpha\), IL-6 and IL-8, the coagulation and complement systems are activated.\textsuperscript{77}

Cardiopulmonary bypass surgery always leads to a systemic inflammatory response syndrome (SIRS). SIRS is characterised by two or more of the following criteria: hypothermia (temperature less than 36\(^\circ\)C) or fever above 38\(^\circ\)C, tachycardia more than 90 beats/min, tachypnoea (more than 20/min) or pCO\(_2\) less than 4.4 kPa (32 mm Hg), leucopenia below 4 x 10\(^9\)/l or leucocytosis above 12 x 10\(^9\)/l. SIRS reflects a cytokine storm with an abnormal regulation of cytokines and is immediately counteracted by a compensatory anti-inflammatory response syndrome (CARS).\textsuperscript{78} CARS has an immune paralysing effect and is characterised by anti-inflammatory cytokines, such as TGF-\(\beta\), IL-4 and IL-10 and inhibition of the IL-12-IFN-\(\alpha\) pathway, impairing natural defence against invading micro-organisms.\textsuperscript{79} SIRS usually resolves with adequate supportive therapy and most of the patients recover. However, overwhelming SIRS can dominate CARS and progress to MODS, which may lead to mortality. Cytokine profiles have been extensively investigated during and after cardiac surgery. A study that evaluated the cytokine pattern up to 48 hours after CABG surgery in 24 patients recovering uneventfully from SIRS shows that cardiac surgery immediately evokes a biphasic cytokine response.\textsuperscript{80}

In patients participating in an RCT comparing LR RBCs with BCD RBCs the pro- and anti-inflammatory cytokine profiles were investigated.\textsuperscript{81} The analyses revealed that patients who would develop infections had higher IL-6 and patients who would develop MODS higher IL-12 concentrations in the group that received more than three units of leucocyte-containing RBCs. These findings support that leucocyte-containing blood transfusions amplify an inflammatory response in addition to an ongoing SIRS induced by cardiac surgery. This may lead to a more profound CARS associated with enhanced susceptibility for postoperative infections. Leucocyte-containing RBC transfusions to patients with an activated inflammatory response seems to imbalance the postoperative SIRS-CARS equilibrium by initially aggravating SIRS. The findings that fresh-filtered RBCs and after storage-filtered RBCs both reduced postoperative complications compared with leucocyte-containing RBCs suggests that not the soluble mediators accumulated in stored RBCs but the allogeneic leucocytes are the culprit in these clinical effects of transfusion-related immunomodulation.\textsuperscript{82} The possible mechanisms leading to mortality in association with allogeneic (leucocyte-containing) RBCs after cardiac surgery are shown in figure 2.

In addition to the role of leucocyte-containing RBC transfusions to postoperative mortality after cardiac surgery, we found an independent role for platelet transfusions enhancing mortality.\textsuperscript{83} Platelets expressing CD40L upon activation (in the extracorporeal bypass circuit as well as during storage of platelet products) are presumed to play a vital link between coagulation and inflammation and may enhance microthrombi and venous thromboembolism, in particular under changing rheological conditions.\textsuperscript{84-92} Both thrombi and infection play a pivotal role in the development of MODS and mortality.\textsuperscript{87,88}

**CONCLUSIONS**

We have reviewed the state of evidence for other benefits of universal leucoreduction. We conclude that: 1) For prevention of TT-CMV infection, in addition to leucoreduction, selection of CMV-seronegative donors is still applied for high-risk patients. 2) Leucoreduction of platelet transfusions is significantly associated with a large reduction of HLA-antibody formation and refractoriness to random donor platelet transfusions; however, ULR does not seem to prevent HLA antibodies after RBC transfusions and does not influence alloimmunisation against RBC antigens. 3) Universal leucoreduction halves the incidence of FNHTR, but cytokines and chemokines accumulating during storage of cellular blood products are responsible for residual FNHTR and TRALI. 4) Transfusion-related
immunomodulation with presumed negative effects on cancer immunosurveillance, postoperative infections or aggravating organ failure, investigated in randomised controlled trials, revealed no support for extended indications for leucoreduction except in cardiac surgery using extracorporeal bypass circulation where leucoreduction of RBCs reduced short-term mortality by approximately 50%, resulting from a combination of MODS and infections. This likely represents a multi-hit synergy between pro-inflammatory cytokines induced by leucocyte-containing transfusions, deepening of compensatory immunosuppression enhancing infections, in combination with activated platelets that may aggravate micro-thrombosis leading to MODS mortality.

Recently, abolishment of universal leucoreduction and going back to leucoreduction for the classical indications, adjusted with open heart surgery has been questioned. Based on the available evidence, restriction of indications for leucoreduction and reversal to the use of buffy-coat depleted RBCs is a safe option.

REFERENCES


43. Blumberg N, Heal JM, Gettings KF. WBC reduction of RBC transfusions is associated with a decreased incidence of RBC alloimmunization. Transfusion. 2005;45(7):945-52.


A 62-year-old woman presented at the emergency department because of progressive throat pain and pain on swallowing for two days. She noticed a red painful area on the ventral side of her neck, spreading out in all directions. There was no fever and no shortness of breath. A few days before becoming ill, she visited her grandson who at the time was feverish with blisters on the face. Physical examination revealed a mildly ill patient without stridor, normal oxygen saturation, respiration rate 24/min, body temperature 36.3 °C, and a normal blood pressure and heart rate. Mouth inspection revealed some pharyngeal redness. The trachea was slightly displaced. There were signs of inflammation (heat, pain, redness and swelling) in the prethyroidal region (figure 1A) and multiple enlarged and slightly tender lymph nodes in the neck. Laboratory analysis showed a C-reactive protein of 405 mg/l and severe leucocytosis (31.7*10⁹ /l). A CT scan of the neck was performed (figure 1B).

**WHAT IS YOUR DIAGNOSIS?**

See page 474 for the answer to this photo quiz.
A 25-year-old HIV-positive female was admitted to our hospital with fever and pain in the groin region. She had been HIV positive since 2004 with poor compliance. Highly active antiretroviral therapy (HAART) was reintroduced two months prior to admittance. Further medical history mentioned skin lesions of the pubic mound for one year. On admission physical examination showed profound redness, tenderness and lymphadenopathy of the groin, along with the known skin lesions of the pubic mound (figure 1). Recent laboratory results showed a CD4 count of $80 \times 10^6/ \text{mm}^3$.

WHAT IS YOUR DIAGNOSIS?

See page 475 for the answer to this photo quiz.
A 65-year-old woman, without relevant medical history, presented at the emergency department complaining of acute abdominal pain in the left lower quadrant. The pain increased on acute movements and coughing. She did not experience nausea, vomiting or fever. Physical examination revealed tenderness to palpation in the left lower abdominal quadrant with rebound tenderness and guarding. Further physical examination was unremarkable. Laboratory results showed slight leucocytosis (16 x 10^9/l) with a normal urinalysis. Under the suspicion of diverticulitis we performed contrast-enhanced computer tomography (CT) of the abdomen. This revealed a fat lobula surrounded by an enhanced rim with infiltration of the adjacent adipose tissue (figures 1 and 2).

**What is your diagnosis?**

See page 476 for the answer to this photo quiz.
A 56-year-old male was referred for fever without shivers (38.7 °C) with a daily spike in the evening and a non-productive cough for six weeks. The symptoms had started two weeks after returning from Boston, USA. In the past year he stayed in Singapore, Cyprus and China. Except of some mild dyspnoea during exercise there were no other complaints and no history of recent diarrhoea. He had already been treated with budesonide, amoxicillin-clavulanic acid and clarithromycin without any effect before referral to our hospital. Medical history showed an inguinal hernia correction and malaria 35 years ago. On physical examination he appeared well. His blood pressure was 122/80 mmHg, the pulse 88 beats/min, and temperature 37.0 °C. Further examination revealed no abnormalities.

Laboratory findings showed an elevated C-reactive protein of 133 mg/l (normal <5 mg/l), elevated leucocytes of 13 x 10⁹/l (normal 4-10 x 10⁹/l) and mild anaemia (haemoglobin 7.4 mmol/l). Cholestatic liver enzymes were elevated: alkaline phosphatase 222 U/l, gamma-glutamyltransferase 205 U/l (normal ≤120 and ≤55 respectively), with minor elevation of alanine aminotransferase (ALAT 72 U/l) and normal bilirubin values. Blood gas analysis (without additional oxygen): pH 7.47, pCO₂ 4.4 kPa, pO₂ 11.6 kPa, saturation 97%, and HCO₃ 24 mmol/l. Chest X-ray was unremarkable apart from a slight elevation of the right hemidiaphragm.

The blood test for malaria was negative. Serology for B. pertussis, L. pneumophila, C. burnetii, syphilis, Epstein-Barr virus and human immunodeficiency virus were negative. Test results for antibodies to cytomegalovirus (CMV) were positive for IgG and IgM. Since IgM antibodies can be falsely positive or may remain positive for a long time and because there was no lymphadenopathy or a mononuclear shift in his white blood cells, we did not consider an acute CMV infection as the cause of his fever. Blood cultures remained negative.

Because analysis in the outpatient clinic had not revealed a diagnosis, the case could be considered fever of unknown origin. An ultrasound of the abdomen and a ¹⁸-Fluoro-deoxyglucose (FDG) PET scan were ordered. Because of logistics, the FDG-PET scan results were available before the ultrasound was performed.

WHAT IS YOUR DIAGNOSIS?

See page 464 for the answer to this photo quiz.
Equations estimating renal function in patients with diabetes

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KEYWORDS
Chronic kidney disease, CKD-EPI, diabetes, glomerular filtration rate, MDRD

INTRODUCTION
Renal function testing is routinely performed in various patient populations with a wide range of renal function. Impaired renal function is an independent risk factor for (premature) cardiovascular disease.1 Several traditional (diabetes mellitus (DM) and hypertension) and non-traditional (including endothelial dysfunction and oxidative stress) risk factors seem to play an attributable role, but exact mechanisms and interactions remain to be elucidated.1 Currently, the glomerular filtration rate (GFR) is considered to be the best overall indicator of renal function.2

Gold standards for assessing GFR, such as renal inulin clearance or isotopic methods,3,4 are cumbersome and costly and therefore reserved for research settings. A less costly and less complex method to measure renal function is the 24-hour creatinine clearance (CrCl). This is the most frequently applied method to assess renal function in daily practice, although collecting 24-hour urine samples is time consuming, and the reliability of the outcome is highly dependent on the accuracy of the urine collection.5

Several prediction formulas for estimating renal function have been developed. The four-variable Modification of Diet in Renal Disease equation (MDRD) is the prediction formula that is most frequently used.6-8 Its advantages and disadvantages have been extensively debated.8,9 Its major disadvantages include its imprecision and systematic underestimation of GFR in patients with normal to high normal serum creatinine levels, and the underestimation in women and young people.8,9

To overcome the aforementioned disadvantages, a new prediction equation, the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI), was developed.10 This formula was developed in a population with predominantly young and middle-aged people (87% ≤65 years) with an average GFR of 68 ml/min/1.73m2; 43% were female.10 Potential complementary covariates such as renal transplant, diabetes and weight were considered, but the final equation used the same variables as the MDRD equation.10 Therefore, it is not clear whether the CKD-EPI can be applied in all populations. Since an accurate estimate of renal function is important and renal function is frequently assessed in diabetic patients, we wanted to evaluate the performance of the CKD-EPI and the MDRD equations in a large, anthropometrically diverse cohort of diabetic patients.

MATERIALS AND METHODS
This retrospective observational cross-sectional study was conducted at the diabetes outpatient clinic of the Maxima Medical Centre in Eindhoven, the Netherlands. A total of 1997 serum creatinine concentration results from adults, previously diagnosed with type 1 or type 2 DM, were collected. An anonymous database was created, using data from the ‘Chipsoft Electronisch Zorg Informatie Systeem’ [Chipsoft Electronic Care Information System] (CS-EZIS), the computerised medical record system used at the
Maxima Medical Centre. Data collected included 24-hour urinary creatinine (mmol/l), serum creatinine (μmol/l), HbA1c (mmol/mmol), weight (kg), height (centimetres), age (years), and gender, all data being collected on the same day, except for the 24-hour urine collection, which was collected in the 24 hours prior to the other measurements. The body mass index (BMI) of each patient was calculated (BMI = weight (kilograms) / height (meters)²) and added to the database. Ultimately, 916 patients remained eligible for inclusion. Two subjects younger than 18 years and three subjects with an CrCl >250 ml/min were excluded, since the MDRD has not been validated in these patient groups. In 176 cases, subjects had collected two 24-hour urine samples during the indicated period. In these cases, the mean of the two 24-hour CrCl’s was used.

Medication details and information on comorbidities were not available. Since no information on race was available, all patients were considered to be Caucasian. No formal approval from the Medical Ethics Committee was required, as our data included only anonymous patient characteristics and laboratory data.

Renal function measurements and definitions

The serum creatinine concentration was measured by an enzymatic technique (Modular PA, Roche), and validated by isotope dilution mass spectrometry (IDMS). Renal function was estimated by two different eGFR equations, the MDRD and the CKD-EPI (table 1). Twenty-four hour CrCl corrected for body surface area (BSA) was calculated (table 1). The Dubois formula was used to calculate the BSA,¹⁰

<table>
<thead>
<tr>
<th>Table 1. Renal function prediction equations</th>
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<tbody>
<tr>
<td><strong>Equation</strong></td>
</tr>
<tr>
<td>CKD-EPI</td>
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<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>MDRD</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Creatinine clearance</td>
</tr>
</tbody>
</table>

CKD-EPI = chronic kidney disease epidemiology equation; MDRD = modification of diet in renal disease formula; BSA = body surface area; IDMS = isotope dilution mass spectrometry.

**Statistical analysis**

Analyses were performed using SPSS 16.0 (SPSS, Chicago, IL). Q-Q plots and histograms were used to assess normality. Continuous variables are represented as mean (± standard deviation) for the normally distributed values and as a median (interquartile range) for the non-normally distributed variables.

Spearman’s coefficient of correlation was calculated to determine the correlation between the CrCl and the eGFR calculated by the MDRD and the CKD-EPI formulas. Bland-Altman plots were created showing the mean of two measurement methods (i.e. CrCl and the MDRD / CKD-EPI) against the absolute difference between these two methods. Krippendorff’s coefficient, an aggregate measure for method concordance, was calculated (see textbox; 1 meaning perfect concordance and -1 meaning perfect discordance between the two methods), since neither maximum correlation nor agreement in accuracy and precision alone will suffice to prove concordance and thus sufficient reproducibility among methods; this requires μ 1, σ 1, σ 2, and ρ = +1 (μ being the population mean of the CrCl, μ being the population mean of the MDRD or the CKD-EPI, σ 1 and σ 2 being the standard deviation of μ 1 and μ 2 respectively, ρ being the Pearson’s correlation coefficient between the CrCl and the MDRD or the CKD-EPI. Krippendorff’s coefficient corresponds to the Bland-Altman plot in a similar way as ρ corresponds to the simple scattergram,¹¹ see textbox.

The bias and precision (see textbox) of both formulas were determined.

Ultimately we evaluated the classification of patients according to the CKD-EPI or the MDRD equation compared with when CrCl is used to classify patients. Moreover, the prevalence of stage III-V CKD in this diabetic population was evaluated per age group.

**RESULTS**

The patient characteristics are presented in table 2. Age ranged from 18 to 92 years with 53.6% of the population aged under 65 years. The population represented a wide range of renal function (CrCl 11 to 250 ml/min/1.73m²). Of the subjects, 71% had a CrCl >60 ml/min/1.73m².
The correlation and Krippendorff’s coefficient

The correlation was 0.75 and 0.76 between the MDRD and the CKD-EPI, respectively, and the CrCl. Figure 1 shows the Bland-Altman plots that evaluate the extent of agreement between the CrCl and both GFR estimating equations. Krippendorff’s coefficient, demonstrating the method concordance between both GFR prediction equations and the CrCl, was almost equally large for the MDRD and the CKD-EPI: 0.54 and 0.57, respectively.

Bias and precision

The results for the bias and the precision are presented in Table 3. The bias of the MDRD and the CKD-EPI compared with the CrCl was -22 (±26) and -20 (±26) ml/min/1.73m², respectively (p<0.01 for both). Both the MDRD and the CKD-EPI showed a large bias and imprecision in all CrCl categories, which was most prominent in people with a CrCl >90 ml/min/1.73m²: -53.4 (±35.2) and -51.4 (±34.8) ml/min/1.73m² for the MDRD and the CKD-EPI, respectively.

eGFR prediction formulas and staging

Figures 2A and 2B represent the eGFR values for both formulas by age and gender. For both the CKD-EPI and the MDRD a steep decline in eGFR was observed with ageing. When compared with the MDRD-4, the CKD-EPI gave higher estimates of GFR at young age (≤65 years). At older age, MDRD-4 and CKD-EPI gave a similar estimation of GFR. The influence on CKD staging using the CKD-EPI or MDRD formula is illustrated in Tables 4A and 4B, for

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All (n=916)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
<td>916</td>
</tr>
<tr>
<td>Sex, male (%)</td>
<td>55.3</td>
</tr>
<tr>
<td>Age (year)</td>
<td>63 [53, 72]</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>50 [42, 60]</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28 [25, 32]</td>
</tr>
<tr>
<td>Creatinine (μmol/l)</td>
<td>79 [67, 97]</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min/1.73m²)</td>
<td>96 [70, 123]</td>
</tr>
<tr>
<td>MDRD (ml/min/1.73m²)</td>
<td>77 ± 25</td>
</tr>
<tr>
<td>CKD-EPI (ml/min/1.73m²)</td>
<td>79 ± 24</td>
</tr>
</tbody>
</table>

Data are presented as number (%) or median [interquartile range].

Table 3. Precision of eGFR prediction equations

<table>
<thead>
<tr>
<th>Creatinine clearance (ml/min/1.73m²)</th>
<th>All (n=916)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bias (ml/min/1.73m²)</td>
<td>36.2</td>
</tr>
<tr>
<td>Precision (ml/min/1.73m²)</td>
<td>35.3</td>
</tr>
</tbody>
</table>

Precision (ml/min/1.73m²), defined as the standard deviation of the mean difference between the estimated glomerular filtration rate (estimated by the modification of diet in renal disease formula (MDRD) and the chronic kidney disease epidemiology collaboration equation (CKD-EPI)) and the creatinine clearance, is shown per creatinine clearance stage. CI=confidence interval.

Figure 1. Bland-Altman plots comparing the creatinine clearance and the estimated glomerular filtration rate, calculated by the Modification of Diet in Renal Disease formula or the Chronic Kidney Disease Epidemiology Collaboration equations. The upper and lower horizontal lines represent the upper (+2 SD) and lower (-2 SD) limits of agreement. The horizontal line in the middle represents the mean difference between the creatinine clearance and the GFR estimating equations.
men and women respectively. Smaller stages than those given in the KDOQI guidelines are used to provide more detailed insight. These tables clearly demonstrate that the CKD-EPI provides higher eGFR values than the MDRD, specifically at higher levels of eGFR and in women (along the total range of renal function). Of the women 26.4% were categorised in a lower CKD stage using the CKD-EPI.

Figure 3 presents the consequence of the introduction of the CKD-EPI on the prevalence of stage III-V CKD. A decline in the number of young people (<65 years) diagnosed with stage III-V is observed, from 12.6 to 10.7%. In the elderly patient category, the numbers of diagnosed patients remains similar using the CKD-EPI or the MDRD.

**Table 4A. Estimated GFR stage for males using the CKD-EPI or MDRD formula**

<table>
<thead>
<tr>
<th>CKD-EPI (ml/min/1.73m²)</th>
<th>MDRD (ml/min/1.73m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30</td>
<td>10</td>
</tr>
<tr>
<td>30-44</td>
<td>34</td>
</tr>
<tr>
<td>45-59</td>
<td>56</td>
</tr>
<tr>
<td>60-74</td>
<td>64</td>
</tr>
<tr>
<td>75-89</td>
<td>114</td>
</tr>
<tr>
<td>&gt;90</td>
<td>203</td>
</tr>
<tr>
<td>Total</td>
<td>507</td>
</tr>
</tbody>
</table>

Numbers represent absolute numbers. Blank cells have no observations. CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration Equation; MDRD = Modification of Diet in Renal Disease formula (MDRD).

**Table 4B. Estimated GFR stage for females using the CKD-EPI or MDRD formula**

<table>
<thead>
<tr>
<th>CKD-EPI (ml/min/1.73m²)</th>
<th>MDRD (ml/min/1.73m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30</td>
<td>12</td>
</tr>
<tr>
<td>30-44</td>
<td>11</td>
</tr>
<tr>
<td>45-59</td>
<td>12</td>
</tr>
<tr>
<td>60-74</td>
<td>64</td>
</tr>
<tr>
<td>75-89</td>
<td>95</td>
</tr>
<tr>
<td>&gt;90</td>
<td>97</td>
</tr>
<tr>
<td>Total</td>
<td>409</td>
</tr>
</tbody>
</table>

Numbers represent absolute numbers. Blank cells have no observations. CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration Equation; MDRD = Modification of Diet in Renal Disease formula (MDRD).
DISCUSSION

In this study, we evaluated the performance of the CKD-EPI as a new method of estimating renal function in diabetic patients with a wide range of renal function. When using CrCl as the comparator and using correlation, bias and precision as tools to evaluate the performance of formulas estimating renal function, the CKD-EPI did not show any additional value compared with the MDRD for use in clinical practice. Bias was comparably high for both MDRD and CKD-EPI and both prediction equations had an equal lack of precision: a lack of precision that increased with deteriorating renal function.

The CKD-EPI was developed to overcome the deficiencies of the MDRD equation, such as the lower accuracy at measured GFR >60 ml/min/1.73m², and underestimation of eGFR in women and healthy young white men. The proposal by Levey et al. to replace the MDRD with the CKD-EPI formula for routine clinical testing because of its superior accuracy can be disputed, for instance in the group of diabetic patients. Although the CKD-EPI performed better than the MDRD in the validation dataset when the GFR was >60 ml/min/1.73m², its precision remained limited. As this imprecision was seen in all groups of the validation dataset, transplant status, diabetes, and weight were selected as predictor variables. The performance of the CKD-EPI did not improve significantly as a result of these attempts to improve the precision of the formula. In spite of these findings, this formula is also recommended to be used in diabetic patients. This lack of precision and the presence of bias has consequences for the correct classification of CKD.

The performance of the CKD-EPI compared with the MDRD has been sparsely assessed in diabetic patients. In these two recent studies, in which diabetic patients with a good renal function or an impaired renal function, respectively, were assessed (mean measured GFR 102±24 ml/min/1.73m² using 51CR-EDTA and 55.4±29 ml/min/1.73m² using inulin), it was demonstrated that the CKD-EPI had a substantially greater bias than the MDRD. The first study, evaluating the consequences of the bias and imprecision on CKD staging found that 16% of the study population was misclassified as having CKD. Unfortunately, the authors of the first study did not mention the characteristics of the subgroup that was misclassified.

From studies of the general population with middle-aged people it was shown that using the CKD-EPI equation to estimate GFR reduces the number of patients categorised in CKD stage III-V (eGFR <60 ml/min/1.73m²). People who had an MDRD-eGFR <60 ml/min/1.73m² but were reclassified to ‘normal’ (no CKD) using the CKD-EPI, had a cardiovascular risk profile similar to the diabetic population. Recent studies have emphasised the importance of careful calibration of serum creatinine measurements. The fact that a traceable enzymatic serum

without evidence of CKD and had no greater expectation of mortality during follow-up. In both studies the individuals who were reclassified were more often white, women and younger. Those who remained in stage 3a (eGFR <60 and 245 ml/min/1.73m²) had a significantly greater burden of diabetes, higher fasting plasma glucose, and higher HbA1c levels.

Based on the results of our study, it can be suggested that the CKD-EPI might lead to underdiagnosing of kidney disease in younger subjects; overall 19.8% is categorised in a lower stage when the CKD-EPI is used. Although the number of patients included in this study is small, there is a trend for young and especially female patients to be re-categorised in a lower CKD stage when the CKD-EPI is used to estimate GFR. Differences between estimated GFR using the CKD-EPI and MDRD were largest in the age categories <65 years. The fact that the bias of the CKD-EPI and the MDRD is influenced by age was found previously in a group of potential kidney donors and adult patients who underwent a GFR measurement for clinical reasons, using 125I-iothalamate. It was shown that absolute bias was larger in the younger patient group. From previous studies we know that younger people (18-64 years) have an increased risk of mortality and end-stage renal disease at similar levels of GFR estimated by the MDRD. Such a finding in relatively young persons requires further evaluation of the patient. The sooner these people are diagnosed as having a reduced renal function, the sooner they can be treated.

Apart from creatinine-based renal function prediction equations, cystatin C is also increasingly mentioned as a biomarker that can be used in formulas to predict GFR. Various studies found cystatin C to be a better predictor of GFR than creatinine although other studies found no difference. Particularly in patients with muscle loss and in populations where rapid detection of small changes in GFR is important, cystatin C may provide a more accurate estimate of kidney function than serum creatinine. In patients with DM, cystatin C appears to be more sensitive than creatinine for the detection of mild reduction in kidney function. However, whether cystatin C improves medical decision making, leading to more favourable patient outcomes, remains to be evaluated in future research.

Strengths and limitations

This is one of the few studies evaluating the effect of the CKD-EPI on the classification of CKD in a diabetic cohort. Due to the wide range of renal function of the included patients, this study gives a good representation of the precision of both GFR-estimating equations in a diabetic population. Recent studies have emphasised the importance of careful calibration of serum creatinine measurements. The fact that a traceable enzymatic serum
The classification of CKD in diabetic patients and the related risk of complications (i.e., cardiovascular morbidity and mortality, acute kidney injury, end-stage renal disease) can be facilitated by GFR estimations, as long as one recognises that the precision of both the MDRD and the CKD-EPI equations is limited. Compared with the MDRD equation, the CKD-EPI equation gives higher estimates of GFR in young diabetic people, leading to a lower prevalence of CKD on population level. The performance of the CKD-EPI equation in diabetic patients with normal renal function has to be determined in a study in which a gold standard to measure renal function is used as comparator.

CONCLUSION

The classification of CKD in diabetic patients and the related risk of complications (i.e., cardiovascular morbidity and mortality, acute kidney injury, end-stage renal disease) can be facilitated by GFR estimations, as long as one recognises that the precision of both the MDRD and the CKD-EPI equations is limited. Compared with the MDRD equation, the CKD-EPI equation gives higher estimates of GFR in young diabetic people, leading to a lower prevalence of CKD on population level. The performance of the CKD-EPI equation in diabetic patients with normal renal function has to be determined in a study in which a gold standard to measure renal function is used as comparator.

REFERENCES


Listeria peritonitis in patients on peritoneal dialysis: two cases and a review of the literature

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\textbf{ABSTRACT}

Two cases are reported of patients on continuous ambulatory peritoneal dialysis who presented with peritonitis caused by \textit{Listeria monocytogenes}. They were successfully treated with intraperitoneal and intravenous administration of amoxicillin. In patients on peritoneal dialysis, \textit{Listeria monocytogenes} is a very rare cause of peritonitis, with only 11 cases reported to date, and mainly occurring in immunocompromised patients. In contrast to the majority of the reported cases, neither of our patients had received immunosuppressive drugs. To our knowledge, these are the first two cases of \textit{Listeria} peritonitis reported in the Netherlands.

\textbf{KEYWORDS}

Peritoneal dialysis, \textit{Listeria monocytogenes}, peritonitis

\textbf{INTRODUCTION}

\textit{Listeria monocytogenes} (LM) is a food-borne pathogen which may cause serious systemic infections with meningitis, septicaemia and endocarditis. The majority of human cases are attributable to contaminated foods such as milk, other dairy products and meat.\textsuperscript{1} In patients on peritoneal dialysis, \textit{Listeria monocytogenes} is a very rare cause of peritonitis. Data from Al-Wali\textsuperscript{2} as well as a repeated PubMed search yielded only 11 cases reported in the English language.

We present the first two cases in the Netherlands of \textit{Listeria} peritonitis in patients receiving continuous ambulatory peritoneal dialysis (CAPD). They were successfully treated with amoxicillin.

\textbf{CASE REPORT}

Patient A is a 62-year-old man with renal failure due to chronic glomerulonephritis (histologically not examined), for which he had been treated with CAPD in the past two years. His medical history included hypertension and replacement of a stenotic aortic valve. He had never experienced an episode of peritonitis. His medication included furosemide, metoprolol, pantoprazole, phenprocoumon and sevelamer. He presented with fever of almost 39 °C and abdominal discomfort. He did not complain of diarrhoea or vomiting, but he had noticed a cloudy PD effluent. On physical examination there was some tenderness in the lower abdomen without abnormalities in the PD catheter tract. Laboratory findings were unremarkable except for a white blood cell (WBC) count of 11.4 x 10\textsuperscript{9}/l (normal 4.0 to 10.0 x 10\textsuperscript{9}/l). Examination of the PD fluid revealed a WBC of 352 x 10\textsuperscript{6}/l (normal <100 x 10\textsuperscript{6}/l), 97% of which were granulocytes. A diagnosis of PD-associated peritonitis was made, and the patient was started on an empirical antibiotic regime with cefuroxim and gentamicin intraperitoneally. Yet, the fever increased as did the abdominal pain, while the PD-fluid leucocyte count rose to 3520 x 10\textsuperscript{6}/l. Gram’s staining of the PD fluid revealed the presence of Gram-positive rods, suggestive for infection with \textit{Listeria}. Amoxicillin 1000 mg thrice daily intravenously was added to the antibiotic regimen. A CT scan of the abdomen did not show signs of perforation or intra-abdominal abscesses. Subsequent cultures of PD fluid obtained on two consecutive days confirmed infection with \textit{Listeria monocytogenes}. Antibiotic treatment was changed to amoxicillin intraperitoneally at a dose of 150 mg/l with each PD exchange. Within two days the symptoms improved, as did the PD effluent cell count. Blood cultures obtained
on admission remained sterile. The antibiotic treatment was continued for three weeks after normalisation of the PD cell count. When obtaining a more detailed history, the patient denied ingestion of possible contaminated foods.

Patient B is a 71-year-old man diagnosed with end-stage renal disease due to hypertension and resection of his right kidney in 1952 for tuberculosis. He had been on CAPD for four years at the time of presentation. Further relevant medical history included carcinoid of the lung for which a lobectomy had been performed three years previously. One year before presentation he had had peritonitis with *Streptococcus parasanguinis* and *Clostridium* species. He presented with fever and a decrease in appetite without abdominal discomfort and with a cloudless dialysate, without diarrhoea or vomiting. His medication included folic acid, iron sulphate, erythropoietin, acenocoumarol, multivitamin, doxazosine, pantoprazole and sevelamer. At presentation his temperature was 39.0 °C and physical examination showed peritoneal tenderness. Laboratory findings revealed a dialysate WBC count of 290 x 10^6/l of which 58% were granulocytes. Empirical treatment was started with intraperitoneal vancomycin and gentamicin according to the local protocol. In the next days, abdominal discomfort continued and his body temperature remained subfebrile. Dialysate WBC count showed a decrease to 103 x 10^6/l on the second day of admission.

Gram’s staining of the PD effluent revealed Gram-positive bacilli, which were later identified by culture as *Listeria monocytogenes*. Amoxicillin 2000 mg twice daily intravenously was added to the regimen. Within the next four days, the symptoms resolved and the patient was discharged after 14 days. Intravenous antibiotic therapy was continued via a central venous catheter for a total of six weeks. Further history taking disclosed the ingestion of cheese from non-pasteurised milk two weeks before hospital admission.

**DISCUSSION**

LM is a food-borne pathogen that may cause severe infections in pregnant and Immunocompromised individuals. It is a facultatively anaerobic, nonsporulating Gram-positive, facultative intracellular rod that grows over a broad range of temperatures. Infections follow ingestion of contaminated food containing the bacteria in high concentrations. The essential determinant of pathogenesis is the transcriptional activator PrfA, which activates the majority of genes required for cell entry and intracellular parasitism. LM induces its own internalisation by cells that are not usually phagocytic, which is mediated by host surface proteins. The bacteria grow and subsequently spread from cell to cell.

Listerial infections can present as several clinical syndromes of which meningitis and septicaemia are the most common. Peritonitis is a much less common consequence of infection by *Listeria monocytogenes*. Spontaneous bacterial peritonitis due to LM has been well described in patients with cirrhosis of the liver and appears to be caused by transmigration of the organism through the intestinal wall into the systemic circulation. *Listeria* peritonitis in patients on peritoneal dialysis might have a similar pathogenesis. PD-associated *Listeria* peritonitis is an exceedingly rare occurrence with only 11 cases reported earlier in the English-language literature (table 1). The two patients reported here were not connected in any way and were treated in two different dialysis centres.

PD-associated peritonitis caused by *Listeria* does not present differently from that caused by the usual causative micro-organisms, with progressive abdominal pain, cloudy PD effluent and possibly fever, sometimes preceded by nausea, vomiting and diarrhoea. No late sequelae such as development of encapsulating peritonitis were described in this very small sample of patients. Of note, the majority of patients received immunosuppressive drugs or were in a poor general health (table 1). Cell-mediated immunity, in which interferon γ and tumour necrosis factor (TNF) play important roles, is essential in controlling LM infection. In immunocompromised patients, as well as pregnant women, these defence mechanisms are depressed causing an increased number of *Listeria* infections. The two patients in the present report did not appear to have obviously compromised immunity other than chronic renal failure (and their age, which in itself has an influence on innate and adaptive immunity). This raises the question whether PD patients should receive a strict advice to refrain from consuming products from non-pasteurised milk; given the rare incidence thus far of LM in PD patients not on immunosuppressants such an advice does not seem appropriate.

No randomised clinical trials have compared antimicrobial agents for the treatment of LM infections. Ampicillin is regarded as the antibiotic of choice. The clinical outcome of patients with LM-associated CAPD peritonitis, as reported in the various cases, was uniformly favourable with good responses to intraperitoneal (IP) or intravenous (IV) ampicillin therapy and continuation of CAPD without needing to remove the PD catheter (table 1). The use of vancomycin, which is included in many empirical regimens for treating PD-related peritonitis, was associated with a high incidence of treatment failure (table 1), despite

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**Table 1**

<table>
<thead>
<tr>
<th>Treatment Regimens</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraperitoneal vancomycin</td>
<td>Cure</td>
</tr>
<tr>
<td>Intravenous gentamicin</td>
<td>Cure</td>
</tr>
<tr>
<td>Amoxicillin 2000 mg</td>
<td>Cure</td>
</tr>
</tbody>
</table>

---

**Table 2**

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Comorbidities</th>
<th>Response to Treatment</th>
<th>Treatment Regimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>71</td>
<td>Male</td>
<td>Chronic renal failure</td>
<td>Cure</td>
<td>Intraperitoneal vancomycin</td>
</tr>
<tr>
<td>52</td>
<td>Female</td>
<td>Chronic respiratory disease</td>
<td>Cure</td>
<td>Intravenous gentamicin</td>
</tr>
</tbody>
</table>

---

**Table 3**

<table>
<thead>
<tr>
<th>Side Effects</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea</td>
<td>Antiemetics</td>
</tr>
<tr>
<td>Vomiting</td>
<td>Antidote medications</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>Antibiotics</td>
</tr>
</tbody>
</table>

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**Table 4**

<table>
<thead>
<tr>
<th>Pathogenesis</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell entry</td>
<td>Listeria induces its own internalisation</td>
</tr>
<tr>
<td>Intracellular growth</td>
<td>Mediated by host surface proteins</td>
</tr>
</tbody>
</table>

---

**Figure 1**

- [Diagram of Listeria pathogenesis]
good sensitivity in vitro. This is due to the fact that LM is an intracellular pathogen; vancomycin has less efficacy in such types of infection considering its mechanism of action.

Dosage and duration of treatment with ampicillin varied among the reported cases. Our patients were treated with prolonged courses and high doses of amoxicillin, in parallel with the guidelines for treatment of other serious Listeria infections. Antibiotic treatment of PD peritonitis can be delivered by either the IV or IP route; IP treatment is generally preferred as it results in higher local levels of antibiotics and can be easily administrated at home once initial clinical improvement has occurred. Most authors treated for not less than three weeks, although some cases also reported good responses to shorter treatment regimens.

CONCLUSION

Listeria monocytogenes (LM) is a very rare cause of peritonitis in patients on peritoneal dialysis (PD), usually occurring in patients with compromised immune function due to medication, disease and/or old age. Successful treatment can be achieved with amoxicillin or ampicillin, either IP or IV; treatment with vancomycin is not recommended for this infection. When treated early and with the appropriate antibiotics, the prognosis is favourable. On the basis of the current limited experience, no advice can be given on the duration of treatment, but three weeks appears to be adequate.

REFERENCES


Table 1. Summary of reported cases with Listeria peritonitis on peritoneal dialysis

<table>
<thead>
<tr>
<th>Reference</th>
<th>Age</th>
<th>Underlying disease</th>
<th>Immunosuppressive drugs</th>
<th>Therapy</th>
<th>Route</th>
<th>Duration</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myers et al. 1983</td>
<td>71</td>
<td>ITP</td>
<td>Prednisone</td>
<td>Erythromycin (penicillin allergy)</td>
<td>IP and IV</td>
<td>2 weeks</td>
<td></td>
</tr>
<tr>
<td>Allais et al. 1989</td>
<td>31</td>
<td>SLE</td>
<td>Prednisone</td>
<td>Vancomycin and ampicillin</td>
<td>IV</td>
<td>4 weeks</td>
<td>Failure on vancomycin</td>
</tr>
<tr>
<td>Al Walli et al. 1990</td>
<td>53</td>
<td>Wegener's granulomatosis</td>
<td>Cyclophosphamide</td>
<td>Vancomycin, aztreonam and ampicillin</td>
<td>IP</td>
<td>3 weeks</td>
<td>Failure on vancomycin</td>
</tr>
<tr>
<td>Dryden et al. 1997</td>
<td>60</td>
<td>CLL</td>
<td>Prednisone</td>
<td>Vancomycin, amoxicillin and gentamycin</td>
<td>IV</td>
<td>5 days</td>
<td>Failure on vancomycin</td>
</tr>
<tr>
<td>Hart et al. 1997</td>
<td>67</td>
<td>Severe cardiac failure, alcoholism</td>
<td>None</td>
<td>Amoxicillin</td>
<td>IP</td>
<td>2.5 weeks</td>
<td></td>
</tr>
<tr>
<td>Lunde et al. 1994</td>
<td>38</td>
<td>Chronic glomerulonephritis</td>
<td>None</td>
<td>Amoxicillin and tobramycin</td>
<td>IP</td>
<td>2 weeks</td>
<td></td>
</tr>
<tr>
<td>Banjeri et al. 2003</td>
<td>66</td>
<td>Polymyositis</td>
<td>Prednisone</td>
<td>Vancomycin, ampicillin and gentamicin</td>
<td>IP</td>
<td>4 weeks</td>
<td>Failure on vancomycin</td>
</tr>
<tr>
<td>Tse et al. 2005</td>
<td>37</td>
<td>SLE</td>
<td>Prednisone and azathioprine</td>
<td>Amoxicillin and amikacin</td>
<td>IV</td>
<td>4 weeks</td>
<td>Septic shock, catheter removed</td>
</tr>
<tr>
<td>Ahmad et al. 2008</td>
<td>28</td>
<td>SLE</td>
<td>Prednisone</td>
<td>Amoxicillin, cefazolin and ceftazidim</td>
<td>IP</td>
<td>3 weeks</td>
<td></td>
</tr>
<tr>
<td>Stylianou et al. 2008</td>
<td>68</td>
<td>Cardiac pathology</td>
<td>None</td>
<td>Vancomycin and netilmicin</td>
<td>IP</td>
<td>6 weeks</td>
<td>Died of heart failure Resolution of peritonitis</td>
</tr>
<tr>
<td>Present report</td>
<td>62</td>
<td>Chronic glomerulonephritis</td>
<td>None</td>
<td>Amoxicillin</td>
<td>IP</td>
<td>3 weeks</td>
<td></td>
</tr>
<tr>
<td>Present report</td>
<td>71</td>
<td>Hypertension</td>
<td>None</td>
<td>Amoxicillin</td>
<td>IV</td>
<td>6 weeks</td>
<td></td>
</tr>
</tbody>
</table>

CLL = chronic lymphatic leukaemia; IP = intraperitoneal; ITP = idiopathic thrombocytopenic purpura; IV = intravenous; SLE = systemic lupus erythematosus.


**Diagnosis**

The PET scan showed a large ‘cold’ lesion (diameter: 13 cm) in the right liver lobe with slight activity around the border lines. These results suggested a liver abscess. Ultrasound examination of the liver showed a large abscess. To rule out a pyogenic liver abscess aspiration of the abscess was performed. Bacterial cultures were negative; however, polymerase chain reaction on drainage material was positive for *Entamoeba histolytica*. Serology for *E. histolytica* (IgG) was positive as well (titre: 1:3200). He was treated with oral metronidazole 750 mg three times a day for ten days, followed by paromomycin 500 mg three times a day for ten days. He made a full recovery. This is the first report using FDG-PET scan for the diagnosis of an amoebic liver abscess of the liver. The pathology of an amoebic liver abscess is a central area of necrotic hepatocytes with no inflammatory component, surrounded by a vascular oedematous zone with compression of the liver parenchyma and a rim of connective tissue, with an inflammatory infiltrate and amoebic trophozoites. Persisting cough can be a sign of amoebic liver abscess in patients with fever of unknown origin caused by diaphragm stimulation. It is important to consider *E. histolytica* in travellers returning from countries where *E. histolytica* is endemic.

**References**


Needlestick injuries and infectious patients in a major academic medical centre from 2003 to 2010

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ABSTRACT

To implement adequate preventive measures in a hospital, the number and nature of occupational exposures to blood must be known. In the Amsterdam Academic Medical Centre a standardised procedure was used to assess all reported occupational exposures to blood from 2003 to 2010.

1601 incidents were reported of which 66% were needlestick accidents. Thirty-five percent of the incidents concerned persons in training and 27% concerned experienced nurses. Twenty-nine percent of accidents occurred during cleaning up after a medical procedure, including the recapping of needles in 6%. In 8% of the accidents the patient was known or found to be infected with hepatitis B or C virus or HIV and in 86% of accidents the personnel were immune to HBV. One case of HCV transmission occurred.

The number and nature of the occupational exposures indicate that preventive measures must focus on the replacement of needles by safety devices and on awareness training of experienced nurses and of persons in training.

KEYWORDS

Needlestick injury, sharps injury, HBV, HCV, HIV

INTRODUCTION

To some degree it is inevitable that healthcare workers sustain injuries from sharp objects such as needles, scalpels and splintered bone. In addition, the employee’s mucosa may be exposed to droplets or splashes of blood, saliva and urine. Patients showing erratic behaviour may inflict bite and scratch wounds. These incidents, hereinafter referred to as ‘occupational exposure’, carry the risk of transmission of infectious agents of which hepatitis B and C virus (HBV, HCV) and human immunodeficiency virus (HIV) are the most relevant.

The number of occupational infections is determined by the prevalence of infectious agents among the patients, the transmissibility of the agents, the incidence of occupational exposures, and the efficacy of preventive measures after exposure. By definition, the prevalence of HBV, HCV or HIV infection approaches 100% in patients visiting specific outpatient clinics for the treatment of HBV, HCV or HIV infection. To estimate the prevalence of HBV, HCV and HIV infection in other patients, one often resorts to test results obtained by the local screening of pregnant woman or first-time blood donors, although patients often belong to high-risk groups. The reported incidence of needlestick injuries varies widely in different groups of medical workers. In a British study, senior surgeons reported 29 needlestick injuries in two years,¹ while 59% of 311 German medical students recalled at least one needlestick injury during their medical study.² To improve the prevention of occupational exposures, better information is needed on the incidence of sharps injuries.³ The Academic Medical Centre (AMC) in Amsterdam facilitates the training of more than 2000 medical students, medical specialists and nurses, who can be assumed to be a major source of occupational exposure to infectious agents. To monitor, manage and prevent occupational exposures to HBV, HCV and...
HIV in the AMC, the Occupational Health and Safety Department devised a two-staged project. The first stage of the project aims at defining the problem by analysing the number and nature of occupational exposures. In the second stage preventive measures will be selected, based on the epidemiology of occupational exposures. Since 2003 a standardised procedure is in use at the AMC for the detailed registration and follow-up of each reported occupational exposure. Here we report on the number and nature of occupational exposures that occurred in the Academic Medical Centre in Amsterdam in the years 2003 to 2010 and on the infection status of the patients involved.

**Methods**

Since 2003, each reported incident in the Academic Medical Centre in Amsterdam, involving exposure of personnel to blood or secreta of a patient, is managed and recorded according to a protocol by the Occupational Health and Safety Department of the hospital. Following a standardised questionnaire, the circumstances of each accident, including possible risk factors of the source, are investigated and stored in a database. Access to the database is strictly limited to the safety manager and to the occupational health physicians and nurses. Following the protocol, information on the HIV, HBV and HCV infection status of the source patient was categorised and managed as follows. If determined to be relevant by the occupational health physician in charge, the source patient was asked for permission to determine his or her infection status. If the patient could not be reached, or refused testing, or could not be identified (for example in accidents involving anonymous needles hidden in waste or laundry), the infection status was determined to be ‘unknown, not available’. In other cases it was decided that the infection status of the source was not relevant. For example: the HBV infection status of the source is irrelevant if the exposed person is immune for hepatitis B. In such cases the infection status of the source was determined to be ‘unknown, not relevant’.

**Results**

During the study period (2003 to 2010) 1601 occupational exposures were reported, which amounts to an average of 0.55 reported exposures per day. The most common exposure involved needlestick injuries in 66% of cases, followed by cut wounds (17%) and splashes (12%), as reported in table 1. Table 2 describes the professional background of the personnel involved. Most accidents occurred among experienced nurses (27%). Table 3 provides an overview of the nature of the activities during which occupational exposures occurred. Cleaning up after a medical procedure was the most important cause of accidents (23%), followed by injuries during surgery and stitching (20%).

Regarding the infection status of the source patients, in 34% of the accidents the source patient tested negative for HBV, HCV and HIV. In 126 (8%) accidents the patient tested positive for one or more of the blood-borne viruses (60 HIV; 33 HBV and 53 HCV infections), including 19 patients with a double or triple infection. In 39% of cases the infection status of the patient was unknown and considered not relevant, while in 10% of accidents the infection status of the source patient was considered relevant but could not be obtained.

Regarding the immune status of the personnel, pre-existing immunity to HBV was documented in 86% of the healthcare workers involved; 4% were not vaccinated or had showed insufficient response to vaccination; and in 10% the HBV immune status was unknown, including persons with an undocumented, oral report of immunity.

**Discussion**

During an eight-year observation period, the number of reported needlestick injuries and other exposures to potentially infectious material in the Academic Medical Centre in Amsterdam remained fairly constant at a rate of 0.5 reported incidents per day. The number of unreported accidents is unknown. Probably ‘self-counselling’

<table>
<thead>
<tr>
<th>Table 1. Number and nature of reported occupational exposures to potentially infectious material in the Academic Medical Centre in Amsterdam</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reported exposures</strong></td>
</tr>
<tr>
<td>Needlestick</td>
</tr>
<tr>
<td>Cut</td>
</tr>
<tr>
<td>Splash</td>
</tr>
<tr>
<td>Bite wound</td>
</tr>
<tr>
<td>Other</td>
</tr>
</tbody>
</table>
frequently takes place, meaning that healthcare workers, with or without consulting their colleagues, decide not to report an accident because the risk involved is considered to be low. At first sight, nurses were most frequently involved in occupational exposures, namely in 27% of reported cases. However, if one takes all persons in training together (physicians in training, senior and junior medical students, and nurses in training), it appears that trainees account for 35% of the reported incidents. By far the most frequent type of accident was a needlestick injury. Surprisingly, accidents tend to occur during tidying-up after the ‘real’ work is done: cleaning-up after medical procedures and recapping of needles accounted for 29% of incidents. Some of the injuries acquired during cleaning-up can be attributed to colleagues who leave the removal of contaminated sharp objects to others.

Apart from the nature of occupational exposures, the risk of transmission of HBV, HCV or HIV from patient to personnel also depends on the prevalence of these infections among the patients. Recently a representative number of random Amsterdam citizens, aged 18 years or older, were tested for HBV and HCV infection, revealing the presence of HBV infection in 0.41%, while HCV infection was found in 0.63% of the adult population in Amsterdam. The prevalence of HIV in the general population of Amsterdam is unknown; an indicator is

---

### Table 2. Professional background of medical personnel involved in occupational exposure to potentially infectious material

<table>
<thead>
<tr>
<th>Profession</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nurse</td>
<td>54</td>
<td>50</td>
<td>47</td>
<td>46</td>
<td>50</td>
<td>64</td>
<td>69</td>
<td>47</td>
<td>427</td>
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<tr>
<td>Medical student</td>
<td>20</td>
<td>25</td>
<td>21</td>
<td>45</td>
<td>44</td>
<td>52</td>
<td>41</td>
<td>37</td>
<td>285</td>
</tr>
<tr>
<td>Junior physician, in training</td>
<td>22</td>
<td>25</td>
<td>26</td>
<td>32</td>
<td>27</td>
<td>51</td>
<td>35</td>
<td>25</td>
<td>243</td>
</tr>
<tr>
<td>Senior physician (specialist)</td>
<td>6</td>
<td>7</td>
<td>13</td>
<td>17</td>
<td>21</td>
<td>22</td>
<td>19</td>
<td>12</td>
<td>117</td>
</tr>
<tr>
<td>Laboratory technician</td>
<td>15</td>
<td>8</td>
<td>6</td>
<td>14</td>
<td>21</td>
<td>11</td>
<td>21</td>
<td>9</td>
<td>105</td>
</tr>
<tr>
<td>OR assistant</td>
<td>14</td>
<td>17</td>
<td>9</td>
<td>8</td>
<td>12</td>
<td>12</td>
<td>10</td>
<td>13</td>
<td>95</td>
</tr>
<tr>
<td>Doctor’s assistant</td>
<td>8</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>8</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>64</td>
</tr>
<tr>
<td>Junior physician, not in training</td>
<td>10</td>
<td>13</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>7</td>
<td>45</td>
</tr>
<tr>
<td>Nurse in training</td>
<td>2</td>
<td>7</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>33</td>
</tr>
<tr>
<td>Radiology technician</td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>3</td>
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<td>2</td>
<td>18</td>
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<tr>
<td>Cleaner</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>Anaesthesiology assistant</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>Sterilisation</td>
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<td>3</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>5</td>
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<tr>
<td>Other profession</td>
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<td>3</td>
<td>13</td>
<td>15</td>
<td>18</td>
<td>117</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>167</td>
<td>186</td>
<td>173</td>
<td>204</td>
<td>197</td>
<td>247</td>
<td>236</td>
<td>191</td>
<td>1601</td>
</tr>
</tbody>
</table>

*In 2006 teachers in anatomy started the reporting of cutting accidents acquired by medical students during dissection lessons.

---

### Table 3. Nature of 1601 occupational exposures in a large academic hospital

<table>
<thead>
<tr>
<th>Activity</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Cleaning up after medical procedure</td>
<td>41</td>
<td>45</td>
<td>52</td>
<td>52</td>
<td>48</td>
<td>40</td>
<td>40</td>
<td>42</td>
<td>360</td>
</tr>
<tr>
<td>Operation, stitching</td>
<td>31</td>
<td>42</td>
<td>33</td>
<td>36</td>
<td>41</td>
<td>47</td>
<td>51</td>
<td>35</td>
<td>318</td>
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<tr>
<td>Blood sampling</td>
<td>17</td>
<td>24</td>
<td>18</td>
<td>29</td>
<td>31</td>
<td>29</td>
<td>32</td>
<td>24</td>
<td>207</td>
</tr>
<tr>
<td>Handling of iv drip</td>
<td>19</td>
<td>18</td>
<td>10</td>
<td>16</td>
<td>20</td>
<td>21</td>
<td>24</td>
<td>10</td>
<td>138</td>
</tr>
<tr>
<td>Laboratory activities</td>
<td>11</td>
<td>14</td>
<td>10</td>
<td>14</td>
<td>11</td>
<td>11</td>
<td>12</td>
<td>12</td>
<td>95</td>
</tr>
<tr>
<td>Injection</td>
<td>16</td>
<td>11</td>
<td>12</td>
<td>7</td>
<td>12</td>
<td>13</td>
<td>15</td>
<td>14</td>
<td>100</td>
</tr>
<tr>
<td>Recapping</td>
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<td>13</td>
<td>8</td>
<td>10</td>
<td>8</td>
<td>21</td>
<td>13</td>
<td>8</td>
<td>92</td>
</tr>
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<td>5</td>
<td>6</td>
<td>11</td>
<td>5</td>
<td>5</td>
<td>17</td>
<td>13</td>
<td>13</td>
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<tr>
<td>Assistance during operation</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>9</td>
<td>16</td>
<td>15</td>
<td>23</td>
<td>84</td>
</tr>
<tr>
<td>Handling of catheter or drain</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>5</td>
<td>3</td>
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</tr>
<tr>
<td>Other activities</td>
<td>7</td>
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<td>11</td>
<td>23</td>
<td>10</td>
<td>27</td>
<td>18</td>
<td>9</td>
<td>110</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>167</td>
<td>186</td>
<td>173</td>
<td>204</td>
<td>197</td>
<td>247</td>
<td>236</td>
<td>191</td>
<td>1601</td>
</tr>
</tbody>
</table>
the 1.4% prevalence of HIV among pregnant women in Amsterdam.5 Not surprisingly, the prevalence of HBV, HCV and HIV infection among patients involved in occupational exposures was much higher: 8% of the source patients were infected with HBV, HCV or HIV. An unknown proportion of the 19% of relevant source patients with unknown infection status must be added to this figure.

Transmission of HBV can be prevented largely by pre- and post-exposure immunisation of personnel. Transmission of HIV can be prevented by post-exposure prophylaxis.6 Immunisation against HIV or HCV is not available, but fortunately the risk of transmission of HIV or HCV by hollow needlestick injuries is low, 0.3% and 1.8% respectively.7 Considering that in addition, the majority of source patients were not infected, the a priori chance of any transmission to personnel is low. Indeed in the study period no transmission of HBV or HIV was found. Only one transmission of HCV was observed, caused by a needlestick injury. In addition, in the year before the study period a case of HCV transmission occurred, also caused by needlestick injury.8 Therefore the main benefit of improved preventive measures is the reduction of the number of labour-intensive post-exposure procedures, which are costly for the hospital and a burden for the workers who experienced an accident.

The lack of vaccination and post-exposure measures against HCV infection, the higher transmissibility of HCV as compared with HIV, and the prevalence of HCV among patients explain why transmissions of HCV occurred. In the near future the registration of two orally available HCV protease inhibitors is expected (telaprevir and boceprevir). It may be possible to use these drugs as post-exposure prophylaxis after occupational exposure to HCV.

We conclude that in the Academic Medical Centre in Amsterdam, two-thirds of reported occupational exposures are needlestick injuries. In roughly two-thirds of the cases trainees and nurses are involved and one third of the cases occur during cleaning-up and recapping after medical procedures. Several studies document a substantial reduction of the number of percutaneous injuries after the introduction of safety devices, although many studies do not account for confounding factors such as simultaneous implementation of other interventions.9,10 Nevertheless it seems appropriate to focus preventive measures on the replacement of needles by safety devices and on awareness training of experienced nurses and trainees.

REFERENCES

Clinical pathological conference
A non-Hodgkin’s lymphoma patient with persistent anaemia after chemotherapy

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INTRODUCTION

A clinical pathology conference is held each trimester at the Department of Internal Medicine of the Academic Medical Centre Amsterdam. Some weeks before the conference, a senior resident is presented with a ‘paper’ case to be solved. The resident is provided with some but not all details on the case, including clinical, laboratory and radiological data, and in the current case (presented by a resident in Internal Medicine and Haematology) bone marrow cytology slides, but no reports. Based on this information, the resident puts a case together with a focus on clinical reasoning, leading to a provisional diagnosis. Afterwards, the clinician who provided the case reveals the actual diagnosis and clinical course. Below, a recent case on persistent anaemia following chemotherapy in a non-Hodgkin’s lymphoma patient is presented.

KEYWORDS
Anaemia, non-Hodgkin’s lymphoma, reticulocytes, parvovirus B19

THE CASE

A 75-year-old man presented at the outpatient haematology clinic for a routine visit. Two months earlier, he had finished six cycles of chemotherapy for a non-Hodgkin’s lymphoma. At diagnosis, six months earlier, he had presented with fatigue, night sweats and lymphadenopathy at both sides of the diaphragm. Histological examination of a lymph node biopsy showed large CD20-positive lymphocytes with prominent nucleoli and abundant cytoplasm together with smaller lymphocytes and histiocytes. The bone marrow was not involved. At that time (2002), these pathological findings fitted with the World Heath Organisation (WHO) classification of diffuse large B cell lymphoma (DLBCL) stage IIIb. Interestingly, lymphoma cells were EBER- (EBV) positive and a subpopulation of the malignant cells showed plasma cell differentiation with intracytoplasmic expression of IgA kappa. In the serum an IgA kappa paraprotein was found. According to the revised WHO classification of Tumours and Haematopoietic and Lymphoid Tissues in 2008, this lymphoma would now be classified as an EBV-positive diffuse large B cell lymphoma of the elderly. It occurs in patients >50 years with no known history of immunodeficiency, in contrast to other EBV-driven lymphoproliferative diseases such as post-transplantation lymphoproliferative disorders. Its occurrence may be associated with the physiological age-related immunological deterioration characterised by a decline in T lymphocyte repertoire, numbers and function. Median age at diagnosis is 71 years, and the prognosis of EBV+ DLBCL is generally poor, with a median survival of two years.1

The medical history of the patient revealed paroxysmal atrial fibrillation for which he was taking flecainide and acetylsalicylic acid. Two years earlier he had undergone a cholecystectomy because of cholecystolithiasis and at the age of 55, he had been diagnosed with Sjögren’s syndrome. He was married and had two sons. He had been sailing the world as a chief engineer officer until his retirement ten...
years ago. He had never smoked or used illicit drugs and reported drinking one or two beers a day.

After the diagnosis of DLBCL, the patient was included in the HOVON (Haemato-Oncology Foundation for Adults in the Netherlands) 46 trial, which was recruiting at that time. In this protocol, the added value of the anti-CD20 monoclonal antibody rituximab (Mabthera) to CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone) chemotherapy was compared with CHOP chemotherapy alone in elderly patients (>65 years of age) with CD20-positive DLBCL. The patient was randomised to receive standard therapy (CHOP without rituximab). Treatment was complicated by cystitis due to temporary urinary retention, urticaria following adriamycin infusion, axonal sensomotoric polyneuropathy, and recurrent anaemia for which the patient received red cell transfusions. After the third cycle, complete metabolic remission, assessed by gallium imaging, was obtained. Computed tomography (CT) scanning of neck, thorax and abdomen after the sixth cycle confirmed complete remission.

At the current visit, the patient reported no specific complaints, except for fatigue. At physical examination an irregular pulse was noted. No lymphadenopathy or hepatosplenomegaly were found, and the remainder of the examination was also normal. Routine laboratory tests showed a haemoglobin level of 5.1 mmol/l (8.2 g/dl) with a mean corpuscular volume (MCV) of 89 fl, but normal platelet count (342 x 10⁹/l) and leucocyte count (5.0 x 10⁹/l). Creatinine and lactate dehydrogenase (LDH) were normal at 56 μmol/l and 165 U/l, respectively. The differential leucocyte count showed 44% neutrophils, 7% eosinophils, 0% basophils, 25% lymphocytes, 8.4% monocytes and no reticulocytes.

**Clinical Reasoning**

This patient presented with mild fatigue and a persistent normocytic anaemia two months after finishing six courses of CHOP chemotherapy for non-Hodgkin’s lymphoma.

Classification of anaemia: mean corpuscular volume and reticulocytes

The work-up of anaemia is often guided by additional parameters such as the mean red blood cell volume or corpuscular volume (MCV) of red cells. Typically, this allows classification of anaemia into microcytic (low MCV, the result of insufficient haemoglobin-production); normocytic (normal MCV, reflecting bone marrow production failure); or macrocytic (high MCV, caused by defective DNA synthesis) (table 1). Another helpful and perhaps more simple parameter in the differential diagnosis of anaemia is the reticulocyte count. Reticulocytes are immature red blood cells with ribosomal RNA remnants in the cytoplasm that usually compose about 1 to 2% of circulating red blood cells. The reticulocyte count reflects the activity of bone marrow erythrocyte production: it is elevated in case of increased red cell production (to compensate for blood loss or haemolysis) and reduced in case of bone marrow disease (figure 1).

<table>
<thead>
<tr>
<th>Classification of anaemia based on mean corpuscular volume (MCV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low MCV (microcytic)</td>
</tr>
<tr>
<td>Iron deficiency</td>
</tr>
<tr>
<td>Bone marrow disease</td>
</tr>
<tr>
<td>Hereditary haemoglobinopathies (α, γ, δ)</td>
</tr>
<tr>
<td>Combined nutrient deficiency (γ)</td>
</tr>
<tr>
<td>Chronic renal insufficiency (γ)</td>
</tr>
<tr>
<td>Erythropoetin resistance (δ)</td>
</tr>
<tr>
<td>Haemolysis</td>
</tr>
<tr>
<td>Normal MCV (normocytic)</td>
</tr>
<tr>
<td>Vitamin B12 deficiency</td>
</tr>
<tr>
<td>Folate deficiency</td>
</tr>
<tr>
<td>High MCV (macrocytic)</td>
</tr>
<tr>
<td>Myelodysplastic syndrome</td>
</tr>
<tr>
<td>Alcohol</td>
</tr>
<tr>
<td>Medication</td>
</tr>
</tbody>
</table>

1. Haemoglobin C, D, E, sickle cell anaemia, and alpha/beta thalassemia; 2. haematological malignancy or solid tumour metastases; 3. iron and folate and/or vitamin B12 deficiency; 4. reduced renal erythropoietin production; 5. anaemia of chronic disease; 6. e.g. methotrexate (anti-folate), hydroxycarbamide.

**Figure 1. Classification of anaemia based on reticulocyte counts**

Reduced production (reticulocytes low):
- Renal: - Low erythropoietin
- Bone marrow: - Erythropoetin resistance: chronic inflammation
- - Bone marrow disease: leukaemia, MDS, infiltration
- - Toxic: medication
- - Deficiencies: vitamin B12, folic acid, iron
- - Infection: EBV, CMV, HIV, Hepatitis, parvovirus B19

Increased loss (reticulocytes high):
- Haemolysis:
- - Hereditary
- - Auto-immune
- - TTP/MHA
- - TTP/MHA
- - TTP/MHA

Red cell loss leads to compensatory increased red cell production reflected by high reticulocyte counts; suppressed red cell production is associated with low or absent reticulocytes. MDS = myelodysplastic syndrome; EBV = Epstein-Barr virus; CMV = cytomegalovirus; HIV = human immunodeficiency virus; TTP/MHA = thrombotic thrombocytopenic purpura/microangiopathic haemolytic anaemia.
Impaired production versus increased destruction or loss

The disadvantage of using the MCV in the work-up of anaemia is that one has to be familiar with the characteristics of the different forms of anaemia. The reticulocyte count provides a more mechanistic approach, based on the pathophysiology of anaemia.

Our patient presented with a normocytic anaemia and absent reticulocytes in the peripheral blood. This suggests impaired red blood cell production by the bone marrow, the causes of which have been outlined above and in Table 1. Deficient erythropoietin production seems unlikely in our patient as his renal function is normal. There are quite a few medications that have been described to have myelosuppressive side effects, but flecainide, the antiarrhythmic that he had been taking for years, is not one of them. Infiltration of bone marrow with relapsed lymphoma at this early time point, only affecting red blood cell production but not platelet or leucocyte production, seems improbable. In fact, only two months earlier, CT scanning of neck, thorax and abdomen had shown complete remission. As for nutritional deficiencies, chemotherapy could theoretically lead to a suboptimal nutritional status. In practice, however, chemotherapy treatment in the outpatient setting rarely results in clinically significant nutritional deficiencies. Finally, when a lymphoma patient presents with anaemia, autoimmune haemolysis should be excluded, even when there is no overt paraproteinaemia present. In fact, in about 20% of initially unexplained (idiopathic or primary) autoimmune haemolytic anaemia’s a non-Hodgkin’s lymphoma turns out to be the underlying cause.4 Usually haemolytic anaemia is accompanied by a compensatory increase in reticulocyte numbers. The absence of reticulocytes makes haemolysis less likely, but this should be confirmed by a normal LDH and haptoglobin, because in rare cases, haemolytic autoantibodies can be directed against erythrocyte progenitors.3 In the present case autoimmune haemolytic anaemia seems unlikely given the normal LDH and low reticulocyte number. A normal LDH makes less frequent causes of bone marrow failure, such as EBV-related haemophagocytosis, also unlikely.4

Interestingly, when reviewing the medical records of the patient, it was noted that during the six cycles of CHOP chemotherapy, the patient had received over 15 units of red blood cells. At initial presentation, his red blood cell count had been normal. CHOP chemotherapy does have myelosuppressive side effects, with a temporal reduction in haemoglobin, leucocyte and platelet counts as a result. Whereas in the majority of patients this requires support at some point, it is highly unusual to have such a high red cell transfusion dependency. This is related to the fact that CHOP-related myelosuppression is only transient and limited in time, while the median survival of red blood cells is 100 to 120 days. Normally, temporal reductions in red blood cell production are masked by the longevity of previously produced red blood cells, and bone marrow haematopoiesis usually recovers before significant anaemia requiring repeated blood transfusions develops.

In addition, leucocyte and platelet counts were not affected in this patient, an observation that warrants further attention. There are only a few possible explanations for an isolated deficiency of red blood cell production in the bone marrow. First, primary bone marrow disease such as myelodysplastic syndrome (MDS) that predominantly affects erythrocyte production has to be excluded, since MDS may be induced by chemotherapy, even though the time between chemotherapy and the development of anaemia in this case was very short and MDS is typically characterised by a high MCV. Secondly, viral infection of bone marrow, in particular with human parvovirus B19, can lead to ‘pure red cell aplasia’, bone marrow failure to produce red blood cells. Occasionally, other viral infections of bone marrow such as infection with cytomegalovirus (CMV) or human immunodeficiency virus (HIV) can lead to isolated anaemia. More often, however, CMV and HIV infection result in mild pancytopenia.

DIFFERENTIAL DIAGNOSIS

At this point, myelodysplastic syndrome or viral infection of the bone marrow seem the most likely explanation for this patient’s anaemia. Nutrient deficiencies and lymphoma relapse should be excluded.

ADDITIONAL TESTING

Guided by the clinical reasoning outlined above, additional laboratory testing and a bone marrow biopsy and aspirate were performed. Laboratory results showed normal iron, folate and vitamin B12 levels. In addition to the normal LDH, also bilirubin and haptoglobin were found to be normal, excluding haemolysis as the cause for his anaemia. Serology of hepatitis B, C, HIV-1, HIV-2 and human parvovirus B19 performed eight months earlier, at the time of lymphoma diagnosis, was negative, while EBV and CMV serology (IgG but not IgM) was positive.

BONE MARROW ASPIRATE AND BIOPSY

Bone marrow aspirate and biopsy are shown in Figure 2. The bone marrow showed normal cellularity, and normal maturation of the myeloid and megakaryocyte lineages was
confirmed. In the erythroid lineage, however, a complete maturation stop was seen at the pro-erythroblast stadium, and pro-erythroblasts were unusually large (figure 2B). This finding is pathognomonic of human parvovirus B19 infection of the bone marrow, and infection was confirmed by anti-parvovirus B19 immunohistochemistry (figure 2C). In addition, no clusters of lymphocytes that would suggest lymphoma relapse were seen, nor signs of myelodysplastic syndrome. In fact, apart from giant pro-erythroblasts and absence of normal red cell maturation, the bone marrow showed an entirely normal morphology.

CLINICAL DIAGNOSIS

Aplastic anaemia caused by chronic human parvovirus B19 infection of the bone marrow.

DISCUSSION OF DIAGNOSIS AND PATHOPHYSIOLOGY

Parvovirus B19 infection is common with a seroprevalence rate exceeding 80% among the elderly. The clinical spectrum of infection is broad. In a large proportion of healthy individuals B19 infection is asymptomatic, and if symptomatic, it most commonly presents as erythema infectiosum or ‘fifth disease’, in particular in young children. In a minority of cases it may cause arthropathy, hydrops foetalis and possibly myocarditis and autoimmune diseases, which are mostly observed in adults. Acute B19 infection temporarily hampers erythropoiesis, which does not normally lead to significant anaemia, as the infection is cleared within 14 days in the immunocompetent host. Normal erythropoiesis then resumes long before circulating mature red cells have reached the end of their life spans (100 to 120 days). However, in individuals who depend on high levels of red cell production, for example patients with haemoglobinopathies (i.e. sickle cell disease) or other forms of chronic haemolysis, parvovirus B19 infection can induce transient, but sometimes lethal, aplastic crises. In these patients, the life span of red cells is too short to overcome a temporal reduction in erythropoiesis.

In hindsight, human parvovirus B19 serology was negative in our patient at the time of the diagnosis of lymphoma. It is therefore assumed that he became infected through transfusion of a high-level B19 viraemic blood product (although a primo infection through normal transmission in the community cannot be excluded). About 0.006% of Dutch blood bank donors have such high-level (DNA >10⁶ IU/ml) parvovirus B19 viraemia. In immunocompetent individuals, transmission of B19 virus through highly viraemic blood products is clinically irrelevant, as the virus is rapidly cleared through pre-existing immunity. Neonates, pregnant women, allogeneic stem cell transplantation recipients in the first year after transplantation and patients with acquired or congenital haemolytic anaemia routinely receive parvovirus B19 negative blood products, as parvo B19 infection in these patients can cause severe complications.

Our patient had no previously acquired immunity against parvovirus B19, putting him at risk for primary infection. In addition, he had been diagnosed with EBV-positive lymphoma which is known to be associated with immune deficiency in general and of the elderly in particular. Chemotherapy, even without rituximab or another form of immunotherapy, may have compromised his immunity even further, and this combination might have been the reason for his inability to clear the virus. In a very similar case an elderly patient with a Hodgkin’s lymphoma developed anaemia due to chronic parvovirus infection after polychemotherapy.

Today, it is still under debate whether B19 seronegative patients receiving chemotherapy should receive
B19-negative blood products only. With the high B19 seroprevalence rate in patients and the low incidence of high-level viraemia in blood bank donors, cost-effectiveness studies in this group of patients are not feasible. This case demonstrates, however, how parvovirus B19 infection in a parvovirus B19 seronegative elderly patient on chemotherapy may lead to chronic parvovirus B19 infection, resulting in prolonged anaemia with high transfusion dependency.

EPILOGUE

Active human parvovirus B19 infection in this patient was confirmed by PCR on bone marrow and peripheral blood. Indeed, parvovirus B19 selectively infects erythroid progenitor cells in the bone marrow (figure 2C). These progenitor cells express globoside (also known as the blood group P antigen), the receptor for B19, as well as α5β2 integrin, the co-receptor for B19. Infection of erythroid progenitor cells induces cell-cycle arrest and apoptosis (rather than red cell lysis which would increase LDH levels, such as in vitamin B12 deficiency-induced anaemia), typically leaving the bone marrow with pro-erythroblasts, the earliest progenitors of the red cell line, without mature erythroid cells (figure 2B). In addition, proteins that are produced by the virus (such as NS1) can lead to thrombocytopenia and neutropenia, although much less frequently.

Treatment of transiently anaemic patients due to parvovirus B19 infection is primarily supportive, when necessary with red cell transfusions. In immunocompromised patients with chronic infection and persistent anaemia, clearance of the virus can be supported by intravenous immunoglobulin (IVIG). Data on the efficacy of IVIG are based on case reports and small series of patients with a variety of underlying conditions such as solid organ transplantation, advanced HIV infection and indeed treatment with (immuno)chemotherapy. Large clinical trials are lacking. However, from the data available it seems that IVIG treatment of persistent parvovirus B19-induced anaemia is beneficial in the vast majority of cases (reviewed in Mouthon et al.9). The patient was treated with IVIG after which parvovirus B19 in the serum became undetectable and the anaemia resolved. Three years later, however, the lymphoma relapsed. He declined further chemotherapy and died of pneumonia shortly thereafter.

REFERENCES

DIAGNOSIS

The differential diagnosis focused initially on infectious conditions that may arise in the neck region, including an abscess, cellulitis, thyroiditis or pharyngolaryngitis. The CT showed a large abscess in the right tonsil region, parapharyngeal, retropharyngeal and supraglottic intralaryngeal spreading out towards the superior mediastinum (figure 1B). Blood cultures and an abscess puncture delivered a group A beta-haemolytic streptococcus (GABHS) confirming the diagnosis of a retropharyngeal abscess (RPA) with GABHS. Because of a history of penicillin allergy, treatment was started with intravenous clindamycin and ceftriaxone. The patient was intubated to prevent airway obstruction, admitted to the ICU and the abscess was surgically drained. Recovery was uneventful with detubation after one week and discharge after two weeks.

RPA is a rare entity in adults, with only 51 cases reported between 1975 to 1995. However, an increasing incidence per decade has been suggested, possibly resulting from improved diagnostic techniques (i.e. CT scanning). Interestingly, the incidence of RPA due to GABHS has increased in the last decade, with some reports indicating a frequency of 54%. Intriguingly, 30% of RPA in children presents without fever and 21% have no subjective complaints of upper airway obstruction, despite severe CT abnormalities (as was the case in our patient). Although GABHS is sensitive to penicillin and clindamycin, the initial treatment of RPA should also cover possible Gram-negative bacteria. It is important to stay alert to the development of toxic shock and possible post-streptococcal complications, i.e. glomerulonephritis and reactive arthritis. According to the national guidelines, family members should receive chemoprophylaxis when there is evidence of a streptococcal septic shock syndrome or necrotising fasciitis. In conclusion, RPA is a severe life-threatening infection that can be missed easily because of the absence of fever and subjective respiratory problems. A CT scan may prove helpful for both formulating the diagnosis and the further therapeutic approach. Appropriate treatment with antibiotics and surgical drainage is normally followed by complete recovery and prevents further local, respiratory and systemic complications.

REFERENCES

Our diagnosis was cellulitis of the groin region secondary to the skin lesions. The differential diagnosis of the skin lesions included molluscum contagiosum, verruca vulgaris, keratoacanthoma, syringoma or condylomata accuminata. In addition, cutaneous manifestations of opportunistic infections such as histoplasmosis, cryptococcosis and coccidiodomycosis were considered. A dermatologist with experience in HIV-related skin disorders confirmed the clinical diagnosis of an ‘agminated’ form of molluscum contagiosum, based on the combination of the central plaque on the pubic mound and the peripheral lesions on the lower abdomen and thighs, which presented as dome-shaped, round to oval papules with a pale pink waxy surface and central umbilication.

Molluscum contagiosum is caused by a pox-like DNA virus, usually seen in children as a benign self-limiting disease. In adults the incidence is growing and it can be considered a sexually transmitted disease. Up to 8% of HIV patients are infected with the molluscum contagiosum virus and in patients with a reduced cellular immunity, molluscum contagiosum can manifest for several years, presenting with giant lesions (>1 cm) as well as agminated clusters of over a hundred lesions.

Treatment of molluscum contagiosum in HIV patients is challenging since conventional methods are often refractory. Initiation of HAART has shown improvement, but can also result in molluscum contagiosum as part of an immune reconstitution inflammatory syndrome (IRIS), as reported in one HIV patient. Pharmacological treatment with cidofovir demonstrates promising effects.

Cidofovir is a nucleotide analogue of deoxycytidine monophosphate that has shown broad antiviral activity against DNA viruses, including molluscum contagiosum. Cidofovir has shown effectiveness in HIV patients, both intravenously and topically. Our patient was admitted and treated for cellulitis with fluoxacillin intravenously with good clinical response. HAART was continued. IRIS seemed unlikely since the skin lesions already existed prior to HAART and no apparent worsening had been noted. Topical cidofovir therapy was started to maximise treatment benefit. Although the patient discontinued both HAART and cidofovir treatment after several weeks, the mollusca contagiosa improved dramatically over the course of several months leaving only scar tissue.

REFERENCES

deskundige geregistreerde Samenvatting van de Productkenmerken. Te verkrijgen bij Novartis Pharma B.V., Postbus 241, 6800 LZ Arnhem, 026-3782111, of via www.novartis.nl

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The diagnosis is epiploic appendagitis. This is a clinical entity that can be easily misdiagnosed as acute appendicitis or diverticulitis. It is mainly caused by torsion of the epiploic appendages, resulting in ischaemia and subsequently irritation of the peritoneum. The reason for the torsion remains unknown. The sigmoid colon and the caecum are the predominant physiological sites of appendageal occurrence. Therefore the pain is usually located in the left or right lower abdominal quadrant, mimicking appendicitis or diverticulitis. The abdominal pain is often rapid in onset and very localised. Coughing, deep breathing and acute movements may exacerbate the pain. Localised tenderness and guarding of the abdomen is usually found on physical examination. The leucocyte count can be normal or slightly elevated.

The diagnosis of epiploic appendagitis is difficult due to the nonspecific presenting symptoms and physical examination and the lack of a pathognomonic clinical feature. Additional ultrasound or abdominal CT scan are necessary to establish the diagnosis. Pathognomonic CT findings are a 1 to 4 cm oval-shaped fat density lesion surrounded by inflammatory changes. Thickening of the parietal peritoneum wall can sometimes be observed. In contrast to diverticulitis, the diameter of the colonic wall is mostly regular without signs of thickening.

Epiploic appendagitis is a self-limiting disorder. The symptoms usually resolve spontaneously within five to ten days. Conservative management is indicated when an accurate radiological diagnosis is established. As we expected, the symptoms resolved after seven days in our patient.

Concluding, in patients with localised, acute abdominal pain which is not associated with other symptoms such as nausea, vomiting, fever or typical laboratory findings, the diagnosis of epiploic appendagitis should be considered as a rare differential diagnosis to sigmoid diverticulitis and appendicitis.

ACKNOWLEDGEMENTS

We thank L.C.W. de Jonge, radiologist, for providing us with the CT images.

REFERENCES