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EDITORIAL

Hospital volume determines favourable outcome: probably also in internal medicine

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The very rapid development in molecular genetics and biotechnology, imaging technology, detailed insights into intricate mechanisms such as immunology, host defence, metabolism, tissue differentiation and cell regulation, and a host of new invasive and non-invasive techniques have resulted in revolutionary changes in medicine in the current era. Diagnostic management, therapeutic options, and preventive strategies are developing at a breath-holding pace while few physicians who practise day-to-day medicine seem to realise they are part of a highly exciting time in medicine, which is unparalleled by any other era in the preceding centuries. As these developments occur, in some situations medicine is getting increasingly complex, requiring highly specific and multifaceted infrastructure and demanding skills of medical and paramedical professionals. One of the responses of medical professionals is subspecialisation, which is not only widely present in internal medicine, but in virtually all medical specialisms.[1]

As the complexity of medicine increases, some specialities have come to the realisation that the outcome of medical treatment may be dependent on the number of patients that are treated within a given time interval. This association was hypothesised as early as in 1979 by Luft.[2] In recent years a large number of studies have been published demonstrating a clear relationship between hospital volume and clinically relevant outcome parameters, including survival. Initially, these studies were mainly done in surgical patients undergoing complex procedures, such as oesophageal resection, pancreaticoduodenectomy, or coronary artery bypass surgery.[3,4] However, similar associations have been demonstrated for complex urological procedures, such as cystectomy, and gynaecological oncology, including hysterectomy for uterine or cervical cancer.[5,6] Most of these studies show a near linear relationship between hospital volume and outcome and virtually all studies demonstrate a threshold below which the rate of complications and an unfavourable outcome steeply increases. As the awareness of the association between a minimal number of procedures and a favourable outcome of surgery increases, surgical societies have proposed a minimum of procedures as a quality indicator and in some situations regulatory bodies have adopted these minimum numbers. Interestingly, implementation of these minimum hospital volumes in the US or Canada has resulted in improving the outcome for major procedures.[7,8] In the Netherlands a similar trend has been demonstrated for major gastrointestinal oncological procedures.[9] For a long time it was assumed that the underlying mechanism that determines the relationship between hospital volume and patient outcome was the (surgical) skills of the doctor. However, it is becoming increasingly clear that other factors are at least as important and are doctor-independent. These factors include set up and organisation of preoperative and postoperative care and intensive care departments, experience of imaging and laboratory personnel, knowledge and skills of nurses and other paramedical disciplines, and familiarity of the entire institution with particularly complex patient groups, which for example determines the ability to quickly recognise complications at an early stage and act adequately in these situations.

However, if the relationship between hospital volume and patient outcome does not entirely depend on the skill of the operator, it may be hypothesised that a similar relationship may exist for complex non-surgical diagnoses. Indeed, some initial studies have shown such associations for the management of acute myocardial infarction, stroke, and even for common medical diagnoses including pulmonary embolism and peptic ulcer treatment.[10-13] Obviously, it is questionable whether these associations are universally translatable to other medical settings, for example in countries with a high level of medical care such as in the Netherlands. On the other hand, it is quite surprising that for medical specialities there is hardly any discussion on hospital volume as a determinant of patient outcome or even minimum volumes to achieve an acceptable outcome. While in surgical specialities there is intense debate on

this issue, it is awkwardly silent in societies of medical specialities in Europe and other parts of the Western world. However, it may safely be assumed that for many serious and highly complex, low-volume conditions in internal medicine a minimum volume of patients per year is required to achieve optimal patient outcome. Can we go on to treat severe antiphospholipid syndrome, Graves ophthalmopathy, acute renal failure, advanced chronic lymphatic leukaemia, hypertensive crises, Wegener's granulomatosis, chest syndrome in sickle cell disease, or cryptococcal meningitis in virtually all hospitals, even if the medical and paramedical staff are very rarely or hardly ever confronted with these problems and do not really know how precisely to handle these conditions and their associated complications?[14-18] Should we at least start some clinical studies to determine whether the care of patients with these conditions is up-to-date and achieves equal outcomes in (very) low-volume hospitals versus hospitals that see these patients on a more regular basis? It may be about time internal medicine and other medical specialities wake up and take the example of surgical colleagues and societies and start to think about adequate hospital volume as a determinant of patient outcome in low-volume complex medical disorders. Based on the results of these surveys it may well be that doctors need to suppress their (understandable) professional pride and face the reality that some patients may be better off in another clinical setting than under their care. And that has nothing to do with the individual knowledge and skills of doctors but merely depends on the clinical setting in which they work.

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REVIEW

Myeloproliferative neoplasia: a review of clinical criteria and treatment

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ABSTRACT

Essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF) belong to the group of Philadelphia chromosome-negative myeloproliferative neoplasia (Ph- MPN). MPNs are clonal bone marrow stem cell disorders characterised by a proliferation of one or more of the myeloid, erythroid or megakaryocytic cell lines. Due to the different affected cell lines, MPNs show typical clinical and histological features. In 2005, a mutation in the JAK2 gene was discovered which generated more insight into the pathogenetic working mechanism of MPNs. However, the treatment of MPN patients is still mainly only palliative, although progress in reducing the symptoms of MPN patients has been made. This review will give a general overview of MPN patients for internal medicine physicians.

KEYWORDS

MPN, myeloproliferative neoplasia, essential thrombocythemia, polycythemia vera, primary myelofibrosis, treatment myeloproliferative neoplasia

HAEMATOPOIESIS

Haematopoiesis is the development of the cellular components of the blood. The formation and development of blood cells is initiated by the haematopoietic stem cells (HSCs). HSCs are primitive cells capable of self-renewal and differentiation. Due to the self-renewal capability, at least one of the daughter cells possesses the same HSC characteristics as the mother cell after cell division. During the entire life of an individual, the stem cell pool is maintained due to the self-renewal capability of the HSCs and supplies cells for multilineage haematopoiesis. [1,2]

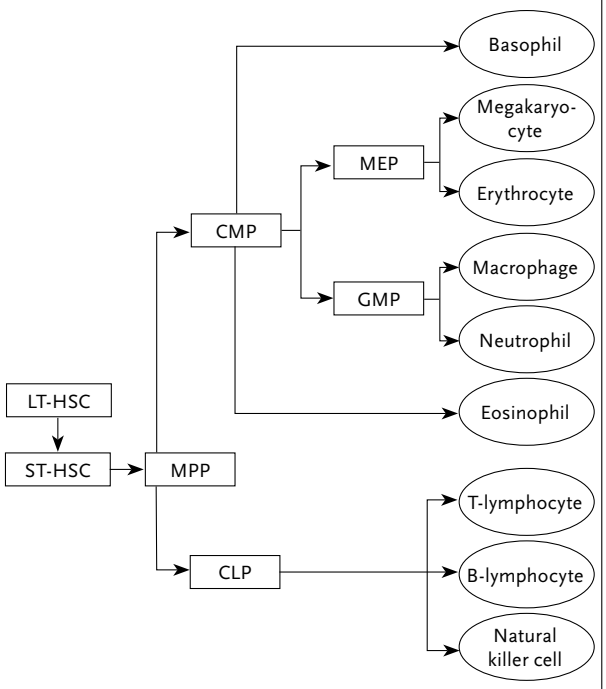
Currently it is considered that long-term repopulating HSCs (LT-HSC) differentiate into a short-term repopulating HSC (ST-HSC) and, as schematically shown in *figure 1*, they will differentiate further into multipotent progenitor cells (MPP) only capable of differentiating into the myeloid lineage or the lymphoid lineage. The common myeloid progenitors (CMP) give rise to megakaryocyte-erythroid progenitors (MEP), which differentiate into megakaryocytes and erythrocytes, and granulocyte-monocyte progenitors (GMP), which differentiate into macrophages and neutrophil granulocytes. The eosinophilic and basophilic granulocytes differentiate directly from the CMP. The common lymphoid progenitors (CLP) differentiate into T- and B-lymphoid cells and natural killer cells (*figure 1*). The progeny that arises from HSCs progressively loses its self-renewal capacity and gradually becomes more restricted to one lineage.[3,4]

HSCs require intrinsic and extrinsic factors for their activities provided by the stem cell niche. The interaction of HSCs with the stem cell niche determines whether the HSCs remain in a quiescent state or proliferate to progenitor cells and differentiate into mature blood cells.[5,6]

MYELOPROLIFERATIVE NEOPLASIA

Myeloproliferative neoplasia (MPNs) are clonal bone marrow stem cell disorders involving a multipotent haematopoietic stem cell, characterised by proliferation of one or more lineages of the myeloid, erythroid and megakaryocytic cell lines. This proliferation results in increased numbers of granulocytes, erythrocytes or platelets in the peripheral blood respectively.[7] William Dameshek was the first to introduce the term 'myeloproliferative disorders' in 1951 including essential

Figure 1. *Development of haematopoietic stem cells, a schematic view*



HSC = haematopoietic stem cells; LT-HSC = long-term repopulating HSC; ST-HSC = short-term repopulating HSC; MPP = multipotent progenitor; CMP = common myeloid progenitor; MEP = megakaryocyte-erythroid progenitor; GMP = granulocyte-macrophage progenitor; CLP = common lymphoid progenitor.

thrombocythemia (ET), polycythemia vera (PV), primary myelofibrosis (PMF), chronic myelogenous leukaemia (CML) and erythroleukaemia (Di Guglielmo syndrome). These disorders were grouped together based on their similarities in clinical phenotype and the belief that there was an underlying undiscovered stimulus responsible for the proliferative activity of bone marrow cells in these myeloproliferative disorders.[8]

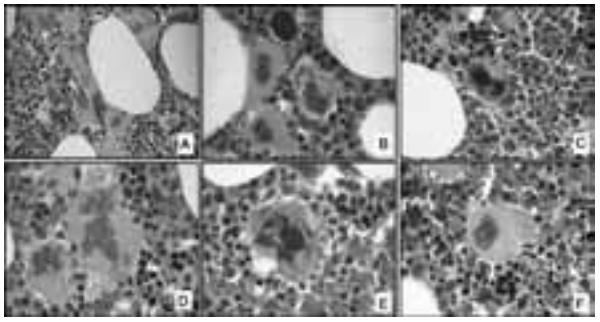
According to the World Health Organization (WHO) 2008 criteria, MPNs are now divided in classical MPNs which carry the Philadelphia (Ph+) chromosome (chronic myeloid leukaemia) and classical MPNs which do not carry the Philadelphia (Ph-) chromosome, including ET, PV and PMF. The Philadelphia chromosome is a result of t(9:22) with the *BCR-ABL1* fusion gene.[9] In this article the classical Ph- MPNs are highlighted.

Clinical and histological criteria of MPN

The typical features of ET are thrombotic and haemorrhagic complications, although most patients are asymptomatic. Transient ischaemic attacks, erythromelalgia and Budd-Chiari syndrome are complications which can occur in ET patients or can develop before the diagnosis of ET is apparent. Bleeding complications are a result of an extremely high platelet count resulting in an acquired von Willebrand disease; von Willebrand factor will be proteolysed with increasing platelet counts.[10] Histomorphological findings in the bone marrow of ET patients are loose clusters of predominant large to giant megakaryocytes. The megakaryocytes exhibit a normal maturation with hyperlobulated and staghorn-like nuclei (*figure 2*). No marked left-shifting of the erythroid or myeloid cell line is apparent. The presence of reticulin is extremely rare in ET patients at presentation and very few patients (<10%) develop myelofibrosis during their disease course, known as post-ET myelofibrosis. ET patients have a risk of approximately 2% to develop acute myeloid leukaemia (AML).[11,12]

Polycythemia vera is characterised by a trilineage proliferation of the erythroid, myeloid and megakaryocytic cell line, usually resulting in mainly increased erythrocytes and often also leucocytes and blood platelets. Patients also display a persistently raised haemoglobin and haematocrit level. The clinical features of PV patients are vascular occlusive events, enlarged spleen, aquagenic pruritus (intense itching after a hot bath or shower) and haemorrhagic complications after injuries and surgery. In about 30% of the patients PV will develop to myelofibrosis, known as post-PV myelofibrosis, and leukaemic transformation will occur in about 10% of the PV patients.[12] The bone marrow of PV patients displays panmyelosis and therefore an increase in cellularity. The megakaryocytes reveal a range from small to giant megakaryocytes without maturation defects of nuclei and cytoplasm and are arranged in loose clusters (*figure 2*). There is always a proliferation and often a left-shifting of the myeloid cell lineage and especially of the erythroid precursor cells. Slightly increased reticulin fibrosis can be seen in the bone marrow.[11]

Figure 2. *Examples of morphological features in megakaryocytes*



A. Dense clustering (HE, 630x) B. Loose clustering (HE, 1000x) C. Dysmorphic nucleus (HE, 1000x) D. Hyperlobulated nucleus (HE, 1000x) E. Staghorn nucleus (HE, 1000x) F. Cloud-like nucleus (HE, 1000x)

In primary myelofibrosis the patient's complaints and symptoms depend mainly on the degree of anaemia and splenomegaly. The typical early symptoms are fatigue, weight loss, night sweating and fever. These constitutional symptoms are believed to be mediated by the abnormal release of cytokines from clonal megakaryocytes as a result of emperipolesis. When the fibrosis is in an advanced stage, the complaints are, apart from the constitutional symptoms, paleness due to anaemia, hepatosplenomegaly, spleen infarct and osteosclerosis. Budd-Chiari syndrome can be a feature of early-phase disease and can be the presenting symptom.[12,13] In the bone marrow of prefibrotic PMF an overall hypercellularity is evident including prominent growth of abnormally differentiated and giant megakaryocytes. The megakaryocytes reveal hypolobulated, cloud-like and hyperchromatic nuclei and demonstrate dense clustering (*figure 2*), often accompanied by left-shifted granulocyte proliferation. In the prefibrotic PMF reticulin fibrosis may be absent, but during the disease course reticulin fibrosis increases, finally resulting in collagen fibrosis with osteosclerosis. Leukaemic transformation occurs in about 10% of the PMF patients.[14] However, the symptoms listed above are not strictly limited to ET or PV or PMF patients, in fact they can occur in all three classical Ph- MPN, such as bleeding complications (spontaneous or after surgery), thrombosis and fatigue. MPN patients may even be asymptomatic in the early phases of the disease and it may be a coincidence that an MPN disease is discovered by abnormal blood counts or by diseases which are features of early-phase MPN, such as Budd-Chiari syndrome, heart attack, cerebral vascular accident, pulmonary thrombus and deep venous thrombosis. An important factor in thromboembolic events is the *JAK2*^{V617F} mutation. No differences in thromboembolic events were seen between heterozygous and homozygous *JAK2*^{V617F} PV patients, in contrast to homozygous ET patients, who show increased risk of cardiovascular events compared with heterozygous and wild-type ET patients. It was also shown that ET and PV patients with a higher allele burden have a higher risk of thrombotic events.[15] This indicates an important risk factor for the *JAK2*^{V617F} mutation in the development of thrombosis. The *JAK2*^{V617F} occurrence rate in patients with thrombosis of the deep veins (DVT) and pulmonary embolism (PE) is low, therefore a general *JAK2*^{V617F} screening is not recommended among patients with spontaneous DVT and PE. This is in contrast to patients who present with splanchnic and intrahepatic vein thrombosis; these patients show a high prevalence of the *JAK2*^{V617F} mutation and a diagnosis of ET or PV should be kept in mind.[16,17]

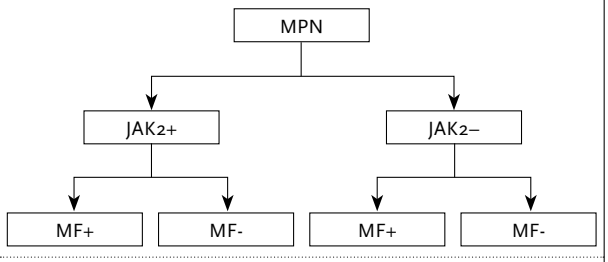
The Polycythemia Vera Study Group (PVSG) made the first attempt to establish diagnostic criteria for the Ph-MPNs in 1967. The diagnostic criteria were updated several times during the following decades and are even now widely used by haematologists. However, the appropriate use of bone marrow biopsy (BMB) histology as a diagnostic tool was neglected. To stress the relevance of a BMB, the WHO added a set of histological diagnostic criteria in 2001. The recent discovery of the *JAK2*^{V617F} mutation and the recognition of pre-fibrotic PMF resulted in the 2008 WHO classification of MPNs.[18-20] However, the early phases of ET, PV and PMF are difficult to distinguish on morphology alone as they share many morphological characteristics. It was shown by Wilkins *et al.* that some of the histological criteria as described in the WHO classification were difficult to reproduce.[21] Nevertheless, it is very important to distinguish these three MPN subtypes reliably in the early phase, because of a different risk of thromboembolic complications of PV and the worse survival rate of PMF patients compared with ET patients, who have a normal life expectancy.[21,22]

Although Ph- MPNs are divided into three clinically distinct entities, the use of three distinct diagnoses can also be questioned; ET, PV and PMF show a great abundance of overlap in their morphological characteristics, clinical signs and symptoms and can also share the same molecular mutation (*JAK2*^{V617F}). A proposed simplistic model for revision of the MPN classification is shown in *figure 3*. It might be more reasonable to divide the MPNs into JAK2 positive and negative diseases and subdivide them into patients with and without myelofibrosis.[23]

THE JAK2 MUTATION AND MPN

In 2005, several groups identified a mutation in the tyrosine kinase domain of JAK2 in MPN patients, resulting in a substitution of valine for phenylalanine at position 617 of JAK2 (*JAK2*^{V617F}). The first genetic step is an acquired point mutation and results in a heterozygous

Figure 3. *Proposed model for reconsidering the classification of MPNs*



MPN = myeloproliferative neoplasia; JAK2+ = positive for the JAK2V617F mutation; JAK2- = wild-type JAK2; MF+ = myelofibrosis present in the bone marrow; MF- = myelofibrosis absent in the bone marrow.

mutational status. The homozygous *JAK2*^{V617F} mutation is the result of mitotic recombination between homologous chromosomes 9p and results in loss of heterogeneity of 9p (LOH) and is a second genetic step in the aetiology of the MPNs.[24-28] The *JAK2*^{V617F} mutation is present in granulocytes, erythroblasts and myeloblasts and in all erythropoietin (EPO)-independent erythroid colonies. The erythroid colonies with the *JAK2*^{V617F} mutation are able to grow in the absence of EPO. Therefore, the *JAK2*^{V617F} mutation also results in factor-independent growth of various haematopoietic cell lines.[29] Further, the receptors of bone marrow progenitor cells are hypersensitive to thrombopoietin (TPO, stimulates proliferation and differentiation of megakaryocytes), EPO (stimulates erythroblasts), stem cell factor (SCF, induces proliferation and self-renewal of multipotent haematopoietic progenitors) and granulocyte-stimulating factor (GSF, stimulates proliferation and differentiation of granulocytes). The hypersensitivity for these cytokines results in specific stimulation of the megakaryopoiesis, erythropoiesis and granulopoiesis.[30]

The *JAK2*^{V617F} mutation is present in >95% of the PV patients and in approximately 50% of the ET and PMF patients.[15,31] The *JAK2*^{V617F} mutation deregulates the JAK2 kinase activity. The mutation is located in the JH2 domain of the JAK2 gene, which negatively regulates the activity of the kinase domain, JH1. Valine 617 and cysteine 618 both maintain the kinase domain of JAK2 in an inactive state. Substitution of valine 617 for phenylalanine destabilises this inhibitory interaction, resulting in increased JAK2 kinase activity. Altogether, this suggests that there is a sustained JAK2 activation, while the feedback mechanism has been destroyed with a growth factor independent activation.[24] PV patients without the *JAK2*^{V617F} mutation virtually all have a *JAK2*^{V617F} exon 12 mutation.

Also, more early genetic abnormalities are currently being defined and related with disease development.

TREATMENT OF MPN

The current treatment of MPN patients is mostly supportive, while standard therapy has not been defined firmly. The treatment of ET and PV patients should be done according to their risk stratification for the occurrence of thromboembolic processes (*table 1* and *table 2*) as evaluated in a large prospective study of the European Collaboration on Low-dose Aspirin in Polycythemia (ECLAP).[32] Age greater than 60 years and a previous history of thrombosis were found to be risk factors for thrombosis in both ET and PV. Is one of these two criteria present the ET and PV patient is at high risk, whereas if none of the criteria are present ET and PV patients are at low risk. ET and PV patients who have platelets >1000 x 10^9/l are of intermediate risk to develop thrombosis or if they have any of the following risk factors: hypertension, hypercholesterolaemia, smoking and diabetes mellitus (*table 1*). These are generic cardiovascular risk factors, and their role is still controversial. Other possible risk factors, which have to be validated in prospective studies, might be leukocytosis and the presence of the *JAK2*^{V617F} mutation, although the latter is controversial.

Table 1. *Risk stratification of patients with ET and PV for the occurrence of thrombosis*

Risk category	Age >60 years or history of thrombosis	Generic cardiovascular risk factors
Low	No/No	No
Intermediate	Platelets >1000 x 10^9/l	Yes
High	Yes/No or No/Yes	Irrelevant

Table 2. *Treatment of ET and PV according to their risk stratification*

Risk category	ET	PV
Low	Low-dose aspirin* if microvascular disturbances are present	Phlebotomy + low dose aspirin*
Intermediate	Low-dose aspirin* if microvascular disturbances are present	Phlebotomy + low dose aspirin*
High	Low-dose aspirin* if microvascular disturbances are present + hydroxyurea^	Phlebotomy + low dose aspirin* + hydroxyurea^

***In the case of major bleeding or presence of von Willebrand syndrome, aspirin is a contraindication; ^hydroxyurea intolerance or resistance, use anagrelide or peg-INF-α.**

ET patients belonging to the low-risk or intermediate-risk category and without any symptoms do not need therapy; however, aspirin is recommended to prevent microvascular disturbances as erythromelalgia, although major bleeding or presence of von Willebrand syndrome are contraindications for the use of aspirin. High-risk ET is an indication for the use of hydroxyurea (HU), which inhibits thrombocyte, erythrocyte and leucocyte production, combined with low-dose aspirin if thrombosis or microvascular symptoms are present, of course in the absence of contraindications (*table 2*).[23,32-35] In the MRC-PT-1 trial researchers compared HU plus aspirin with anagrelide plus aspirin in ET patients at high risk for thrombosis, observing that HU plus low-dose aspirin is superior to anagrelide plus low-dose of aspirin.[36]

The administration of aspirin to PV patients has been widely investigated. In 1986, the PVSG concluded that aspirin was ineffective and dangerous, due to increased gastrointestinal bleeding and intracerebral haemorrhage, based on a randomised trial of 163 PV patients receiving either 900 mg/day aspirin plus dipyridamole or radioactive

phosphorus (^{32}P).[37] However, more studies on the administration of aspirin have been done, resulting in the conclusion of the safe use of a considerably lower dose of aspirin in PV patients. The Gruppo Italiano Studio Policitemia Vera demonstrated the safe use of low-dose aspirin (40 mg/day) in PV patients.[38] The study by Landolfi *et al.*[39] showed a significant reduction in the combined risk of cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, pulmonary embolism or major venous thrombosis with 100 mg/day of aspirin. Therefore, low-dose aspirin plus phlebotomies are recommended in the low-risk and intermediate-risk category.[23]

In 1953, the most effective treatment of PV included phlebotomies combined with radioactive phosphorus (^{32}P) resulting in prolonged survival; however ^{32}P was shown to be leukemogenic.[40] The PVSG study group conducted a randomised trial comparing phlebotomy alone with ^{32}P plus phlebotomy and with chlorambucil plus phlebotomy. Patients treated with phlebotomy alone showed a higher incidence of thrombosis in the first three years of treatment. After three to five years of study, a considerable number of patients treated with ^{32}P or chlorambucil developed acute leukaemia, lymphoma and carcinomas of the gastrointestinal tract and skin, compared with those treated with phlebotomy alone. Therefore, patients treated with phlebotomy alone had a better overall median survival of 13.9 years than patients treated with chlorambucil (8.9 years) or ^{32}P (11.8 years).[33] The PVSG also compared HU with phlebotomy; a slightly higher incidence of acute leukaemia, less myelofibrosis and fewer deaths among the patients treated with HU were apparent.[41]

Interferon-α is able to inhibit *in vitro* proliferation of haematopoietic progenitors and inhibition of the thrombopoietin-induced MPL receptor signalling resulting in megakaryopoiesis repression. The use of IFN-α in PV patients was shown to be effective and non-leukemogenic. However, the use of IFN-α has been limited due to its toxicity, parenteral administration and costs.[42,43] The development of pegylated (peg) forms of IFN resulted in improved tolerance, efficacy and fewer side effects.[44,45] Peg-IFN-α has been demonstrated to have clinical advantages, high rates of molecular response and lower toxicity in phase II trials in PV as well as ET patients.[46,47]

PV patients belonging to the low-risk or intermediate-risk category with high haematocrit level are treated with phlebotomies in order to obtain normal haematocrit levels (<0.45 l/l) plus low-dose aspirin, if no contraindications are present. If PV patients show poor compliance to phlebotomy or if they show progressive myeloproliferation, cytoreductive therapy should be given. The high-risk group should be treated with myelosuppression, with HU as the drug of choice (*table 2*). Anagrelide or peg-INF-α is used in PV and ET patients in case of intolerance or resistance to HU, to control platelet count or in those who develop side effects to HU; however, long-term efficacy and safety features are still unknown.[23,35,48]

The prognosis of PMF patients is worse than that of ET or PV patients (median survival six *vs* 20 years) and the disease course is not significantly modified by drug therapy, therefore treatment of PMF is mainly palliative. However, there is a wide heterogeneity in presentation and evolution among PMF patients. Therefore, the International Prognostic Scoring System (IPSS) uses five risk factors for estimating the survival of PMF patients at the time of diagnosis: age >65 years, constitutional symptoms (weight loss, fever, excessive sweating), haemoglobin level <10g/dl, leucocyte count >25 x 10^9/l and circulating blasts >1%. Based on this system PMF patients can be categorised in the low-risk group (0 risk factors present), intermediate-1 (1 risk factor present), intermediate-2 (2 risk factors present) and high-risk group (≥3 risk factors present).[49] IPSS has been modified to Dynamic IPSS (DIPSS) with the same five risk factors to estimate survival during the disease course, while acquisition of additional risk factors modifies patients outcome.[50] Recently, the DIPSS was upgraded to DIPSS-plus by incorporating three independent prognostic factors, including the need for red cell transfusion, thrombocytopenia <100 x 10^9/l and unfavourable karyotype (including +8, -7/7q-, i(17q), -5/5q-, 12p-, inv(3) or 11q23 rearrangement). Based on the DIPSS-plus PMF patients are categorised in the low (no risk factors), intermediate-1 (1 risk factor), intermediate-2 (2 or 3 risk factors) and high (≥4 risk factors) risk group. Unfavourable karyotype and thrombocytopenia both predict leukaemic transformation in PMF patients. If the patient needs red cell transfusion, the patient belongs to the intermediate-risk group, while the patient displays two risk factors: anaemia and red cell transfusion need.[51]

A wait-and-see approach is justified in PMF patients belonging to the low- or intermediate-1 risk group, while the median survival of these patients exceeds 15 and six years respectively.[51] This relatively long median survival does not justify the risks of an allogeneic stem cell transplantation (alloSCT) or the start of investigational drug therapy. There is also no evidence to support the use of conventional drug therapy in low- or intermediate-1 risk group patients if the patients do not have complaints which can be treated (*table 3*).[13] However, if PMF patients suffer from splenomegaly, the first drug of choice is HU and in the worst case splenectomy is indicated. Indications for splenectomy include symptomatic portal hypertension, drug-refractory splenomegaly with severe symptoms, transfusion-dependent anaemia, marked thrombocytopenia and uncontrollable haemolysis due to severe complications that can occur. Irradiation therapy of the spleen transiently reduces spleen size and reduces

Table 3. *Treatment of PMF according to their risk stratification*

Risk category	PMF
Low	Wait-and-see or conventional drug therapy
Intermediate-1	Wait-and-see or conventional drug therapy
Intermediate-2	Hydroxyurea* or experimental drugs or alloSCT
High	Hydroxyurea* or experimental drugs or alloSCT

*Hydroxyurea intolerance or resistance, use peg-INF-α.

the incidence of pancytopenia. Patients usually experience relief of constitutional symptoms when splenomegaly is treated. In the case of non-hepatosplenic extramedullary haematopoiesis (located mainly in the thoracic vertebral column or in lymph nodes, lung pleura, small bowel, peritoneum, urogenital tract and heart) low-dose irradiation therapy is indicated.[52,53]

In patients belonging to the intermediate-1 risk group who suffer from the risk factor they display, conventional drug therapy should be given; anaemia can be treated with androgens, danazol, corticosteroids, thalidomide or lenalidomide. Thalidomide plus prednisone and lenalidomide plus prednisone show higher response rates with decreased toxicity. Thalidomide and lenalidomide are also effective in PMF patients with unfavourable karyotype. A recent study by Holle *et al.*[54] showed an improvement in haemoglobin and thrombocyte counts and a reduction in spleen size and bone marrow fibrosis in patients with PMF, post-ET and post-PV myelofibrosis treated with thalidomide. However, side effects are toxicity and mainly neurotoxicity. More promising might be lenalidomide, which shows fewer side effects with similar improvement in haematopoiesis.[53] The use of erythropoiesis-stimulating agents in myelofibrosis is not recommended due to the risk of splenomegaly exacerbation.[52,55]

PMF patients in the intermediate-2 and high-risk group have an indication for therapy, as well as regular therapy as investigational drug therapy, due to the low survival rates in these patients (*table 3*). In the presence of thrombocytosis, leukocytosis, splenomegaly or bone pain, there is an indication for hydroxyurea. Anaemia can be treated as indicated for the intermediate-1 risk group and splenectomy is also indicated as stated above.[23,53,56-60]

The only potentially curative treatment in PMF patients is allogeneic stem cell transplantation with an overall three-year survival ranging from 30 to 60%. AlloSCT can induce graft versus host disease (GvHD), which can be divided into acute GvHD and chronic GvHD, with an incidence of about 30 to 43% and 30 to 48%, respectively.[61-63] However, despite the high rate of death and the high risk of chronic morbidity due to GvHD, alloSCT is justified in PMF patients belonging to the intermediate-2 or high-risk group, while the median survival of these patients is three years and one year[64] respectively (*table 3*). The three-year overall survival of PMF patients after alloSCT ranges from 37 to 58%.

Future treatment

New therapeutic strategies include JAK inhibitors and imatinib mesylate. Imatinib mesylate (tyrosine kinase inhibitor) is used in the treatment of chronic myelogenous leukaemia and has been shown to reduce spleen size and to reduce the proliferative activity in PV patients.[65] Several JAK inhibitors have been developed since the discovery of the $JAK2^{V617F}$ mutation in 2005, among them ruxolitinib (INCB018424), SAR302503 (TG101348), CYT387, lestaurtinib (CEP701) and SB1518.

Ruxolitinib is a JAK1 and JAK2 inhibitor which was tested in a phase I/II trial. Patients showed responses after one to two months including reduction of spleen size and improvement of constitutional symptoms including fatigue, weight loss, night sweats and pruritus. A more than 50% decrease in total symptom score after 24 weeks occurred in 46% of the patients compared with 5% for the placebo group. Haematological side effects were anaemia and thrombocytopenia (grade 3 or 4). Non-haematological toxic effects were low grade and infrequent. After 60 days the overall survival of the patients treated with ruxolitinib was higher compared with the placebo group (hazard ratio = 0.67). Allele burden was minimally decreased and ruxolitinib was shown to be effective in patients with the $JAK2^{V617F}$ mutation, but also in patients without the JAK2 mutation.[66,67] Ruxolitinib is now being tested in a phase III trial.

In a recent study by Tefferi *et al.* 51 patients were enrolled in the phase I/II COMFORT trial experiencing a very rapid relief of symptoms related to the presence of myelofibrosis and splenomegaly. However, the occurrence of serious anaemia and thrombocytopenia, loss or lack of response, disease progression, patient/physician choice often associated with lack of response, and death during the study prompted 47 patients to discontinue with ruxolitinib treatment. During treatment discontinuation, acute relapse of symptoms and splenomegaly were experienced by most patients, which sometimes required hospitalisation. This observation stresses the need for careful disclosure of the ruxolitinib withdrawal syndrome to myelofibrosis patients. Further, treatment discontinuation should be done under close supervision in a gradual tapering schedule, although the tapering schedule does not guarantee that the withdrawal symptoms will not occur.[68] However, these side effects and the occurrence of ruxolitinib withdrawal syndrome do not counteract the benefits MPN patients with myelofibrosis experience with ruxolitinib treatment.

SAR302503 is a selective JAK2 inhibitor inducing rapid spleen size reduction and improvement of constitutional

symptoms. Further, the majority of patients with leukocytosis and thrombocytosis at baseline achieved normal blood counts. A significant decrease in the *JAK2*^{V617F} allele burden was observed. Grade 1 self-limiting side effects were nausea, diarrhoea and vomiting. Haematological side effects of grade 3 to 4 were anaemia, thrombocytopenia and less frequently neutropenia.[69] SAR302503 is being tested in a phase II trial at the moment.

CYT387 inhibits the JAK1 and JAK2 gene. First results are promising; improvement in spleen size, anaemia and constitutional symptoms. Side effects were headache and thrombocytopenia.[70] CYT387 is currently under investigation in a phase I/II trial.

Lestaurtinib inhibits JAK2 and JAK3 and improves spleen size, transfusion dependency and cytopenias. No effect was seen on the *JAK2*^{V617F} allele burden. Side effects were diarrhoea, anaemia and thrombocytopenia.[71] Currently, lestaurtinib is under investigation in a phase II trial.

SB1518 is a highly selective JAK2 inhibitor and was well tolerated in a phase I trial with a decrease in spleen size and improvement in clinical symptoms.[72] SB1518 is currently being tested in a phase I/II trial.

Another promising drug might be pomalidomide, a second-generation immunomodulatory drug. Pomalidomide was shown to improve anaemia (in 25% of patients treated with 0.5 mg/day and in 36% of patients treated with 3.0 mg/day) and platelet count in patients with ≤100 x 10^9/l (in 58% patients treated with 0.5 mg/day).[73,74]

Hypomethylating agents have also been investigated. The most promising is decitabine, which was tested in a phase II study in 21 MPN patients with myelofibrosis, showing a reduction of 61% in circulating $CD34^+$ cells. ITF2357, a histone deacetylase inhibitor, was shown to resolve pruritus in most patients, to reduce splenomegaly in 38% of the patients and showed a trend in reducing the *JAK2*^{V617F} allele burden.[75]

Everolimus (RAD001) inhibits the mammalian target of rapamycine (mTor) and was shown to reduce spleen size, to complete resolution of systemic symptoms and to reduce anaemia. Side effects were worsening of anaemia in 30% of the patients and grade two neutropenia or thrombocytopenia, although infrequent.[76]

The JAK inhibitors are the most promising new drug strategies for MPN patients with improvement in quality of life and relatively minimal side effects. However, the long-term safety of these agents and whether they prolong survival should be determined. Therefore JAK inhibitors should only be started as a form of therapy in myelofibrosis patients belonging to the intermediate-2 or high-risk group.

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REVIEW

Walled-off pancreatic necrosis

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ABSTRACT

Acute severe pancreatitits may be complicated by the development of 'walled-off pancreatic necrosis' (WOPN), which is characterised by a mixture of solid components and fluids on imaging studies as a consequence of organised pancreatic tissue necrosis. We present here an overview of the definition, clinical features, and diagnostic and therapeutic management of this clinical condition, which is mostly based on consensus as adequate clinical trials are lacking.

KEYWORDS

Pancreatitis, walled-off, review

INTRODUCTION

The term 'walled-off pancreatic necrosis' (WOPN) was first used in 2005 to define a mixed fluid-solid collection [i.e. a picture that is composed of solid components and fluids], with a similar appearance to pancreatic pseudocyst, which occurs after severe acute pancreatitis.[1-3] Previous designations for the condition are organised pancreatic necrosis, post-necrosis pseudocyst, pancreatic sequestration or necroma.[3-6] In 2006, the term 'walled-off pancreatic necrosis' was officially accepted at the American Gastroenterological Association meeting.[7] However, the new nomenclature had various interpretations and consensus about its radiological characteristics and therapeutic options was lacking. In a PubMed search (June 2011) of "walled-off pancreatic necrosis" we only found 18 entries, but some articles were not totally related to the item, so no more than ten articles about WOPN are currently available.[1-3,6-11] We have performed a comprehensive review of this topic.

DEFINITION OF WOPN

In 1992, the Atlanta Classification added clear terms and definitions for the diagnosis of acute pancreatitis and its complications. This allowed the comparison of the results of different working groups in the medical community and simplified the common management of patients around the world.[12] In recent years, new concepts or terms, such as WOPN, have been postulated and this classification will probably have to be updated.[13]

In 1996, Baron *et al.* first used the term 'organised pancreatic necrosis' to describe a transitional collection between pancreatic necrosis and pancreatic pseudocyst that contained different amounts of fluid and necrotic tissue.[4] This entity was caused by the necrosis and liquefaction of pancreatic and peripancreatic tissue, with or without pancreatic duct communication.[3-5]

A temporary proposed classification of acute pancreatitis postulated the new term 'post-necrotic pancreatic and peri-pancreatic collections'.[14] These collections consisted of different proportions of fluid and solid necrosis and can be identified three to six weeks after the episode of acute pancreatitis. When the collections are fully developed, the presence of a thin wall without epithelium may lead to a misdiagnosis of pancreatic pseudocyst. Once walled-off collections are present, WOPN can be diagnosed.[6,14]

WOPN occurs in 1 to 9% of cases of acute necrotising pancreatitis.[5,6,8] Acute biliary pancreatitis is the most common cause of WOPN (50 to 70%) and other aetiologies are alcohol abuse and idiopathic.[8-10] Only a few cases of WOPN are caused by chronic pancreatitis (4-16%).[1,2,6] No difference in the frequency of WOPN formation between men and women has been clearly demonstrated.[7] The most frequent locations of WOPN are the pancreatic body and tail (80 to 92% of the cases), and extension to the paracolic gutters often occurs.[1,6,8-10] The mean size of published WOPNs is between 11 and 17 cm.[1,2,6,8-10]

CLINICAL FEATURES

WOPN typically occurs later in the course of pancreatitis, several weeks (>3-6 weeks) after the start of the attack.[7] After the first episode of acute pancreatitis, WOPN patients might be asymptomatic (50%) or present with symptoms (50%) such as abdominal pain, malaise, relapsing or recurrent pancreatitis, feeding intolerance or weight loss.[1,6] In severe cases, WOPN can obstruct the gastrointestinal tract, fistulise to adjacent anatomic strictures, and compress or erode into blood vessels or the bile duct. [11] WOPN can be infected or aseptic. [1,6] A third of the patients have infected WOPN, sometimes after percutaneous drainage or endoscopy treatment, which could be the source of infection. There is no clear correlation between the symptoms and WOPN infection. If infection is present, gas can be observed on the computed tomography (CT) but only a positive test after percutaneous puncture and gram staining will confirm the infection. The most commonly isolated bacteria in WOPN are *E. coli*, *K. pneumoniae*, *E. faecalis* and *S. aureus*.[6,7] Splenic vein thrombosis is seen in 40% of cases.[6]

DIAGNOSTIC METHODS

No specific clinical chemistry tests define WOPN.[7] The degree of pancreatic enzyme elevation does not correlate with the degree of necrosis.[7] WOPN can be identified with the use of initial and subsequent CT scans that show progression of the initial early necrosis to WOPN which occupies and expands the initial necrotic areas. On CT, WOPN appears as a mostly heterogeneous collection (mixture of fat, fluid and solids) usually without gas.[3,11] Gas within a WOPN collection does not always mean infection. For the most part it is due to fistulisation to the stomach or more commonly the duodenum, in which case it may be sterile. When WOPN fistulises to the colon it is always infected. CT accuracy in the differential diagnosis between WOPN and pseudocyst is about 79 to 84%.[3] A correct diagnosis is crucial because it influences the management of the pancreatic collection. Magnetic resonance imaging (MRI) and endoscopic ultrasound scans provide a better definition of the solid component inside necrotic collections.[3,13,14]

MANAGEMENT OF WOPN

This new term (WOPN) creates a challenge for identifying the most appropriate management. WOPN rates have probably been underestimated in the past because of an unclear definition, multiple names and incorrect diagnosis. The management of asymptomatic patients is unclear. Discussions centre on the need for, time and duration of management.[2,6] In symptomatic patients, infection evidenced by fever, leukocytosis and/or sepsis syndrome is the most common indication for the treatment of WOPN.[6] Other indications are: progressive increase in size, pain, gastric or duodenal outlet obstruction that interferes with feeding or causes persistent nausea or vomiting, biliary obstruction, portal thrombosis, fistulous connection between WOPN and adjacent strictures or clinical deterioration.[1,10,11] The start of WOPN treatment has ranged from 42 to 72 days (range 20 to 300 days) after the onset of acute pancreatitis.[1,2,6] However, there is no absolute time frame and intervention is based upon the severity of clinical symptoms and degree of organisation. There are several treatment options: percutaneous drainage, endoscopic drainage, laparoscopic drainage, surgical necrosectomy and mixtures of these techniques.[1,2,11] WOPN was historically believed to be less amenable to endoscopic or percutaneous treatment because of non-viable solid components. More recently, there has been a paradigm shift in the management of WOPN toward less invasive approaches.[8] The goal of these techniques is to provide minimal access necrosectomy equivalent to open necrosectomy.[8] The therapeutic options are:

- Percutaneous drainage (PD) and combined endoscopy plus PD

The solid component of WOPN limits the management of patients with percutaneous drainage, so the resolution rate is low.[2,9] Percutaneous therapy alone had a worse success rate and more prolonged length of stay, complications, need for surgery and deaths compared with combined therapy.[11] Percutaneous therapy is only effective if multiple large drains are used with frequent upsizing, removal of solid debris and aggressive irrigation. The main indications for PD are: PD combined with endoscopic procedures and puncture to rule out infection.[2,10]

Gluck *et al*. proposed combined therapy (percutaneous drainage and endoscopy). They first inserted a percutaneous drainage tube. If effective, they waited for the clinical outcome; if not, they immediately performed endoscopic therapy plus ERCP in selected cases.[11]

- Endoscopy

Baron described the endoscopic treatment of WOPN in 1996.[4,8,9] The main advantage of endoscopic therapy is the avoidance of surgical necrosectomy, because this procedure is associated with high morbidity and mortality. In addition, endoscopic necrosectomy is associated with a lower risk of pancreatic-cutaneous fistula compared with percutaneous drainage or surgical procedures.[10,11] A few articles about per-oral transgastric necrosectomy in infected pancreatic necrosis have been published, but we are going to focus on articles dedicated to WOPN.[15-18]

One problem of endoscopic treatment is that it is inconvenient for patients because it takes at least three sessions.[6,8,9,11] The endoscopic procedure is also a major interventional procedure associated with major morbidity in 10 to 26% of cases (most commonly bleeding and perforation), mortality of 2 to 7% and need for laparotomy in 0 to 23%.[1,2,6,8-10] Moreover, endoscopy is not feasible in patients with WOPN located more that 1.5 cm from the gastrointestinal lumen or coagulopathy.[11] The transgastric route is the most frequent access used (73 to 85%), but the duodenal route is also employed.[8-10] Endoscopic ultrasound guidance is often used, but not always.[8,9]

Figure 1. *Abdominal CT: Mixed solid-liquid collection (WOPN) Star: solid component*

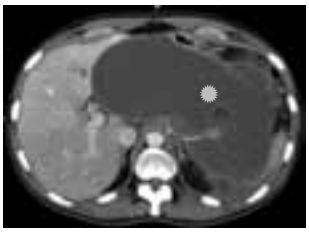

Figure 2. *Abdominal CT: Patient from figure 1: check-up one year after open surgical necrosectomy*

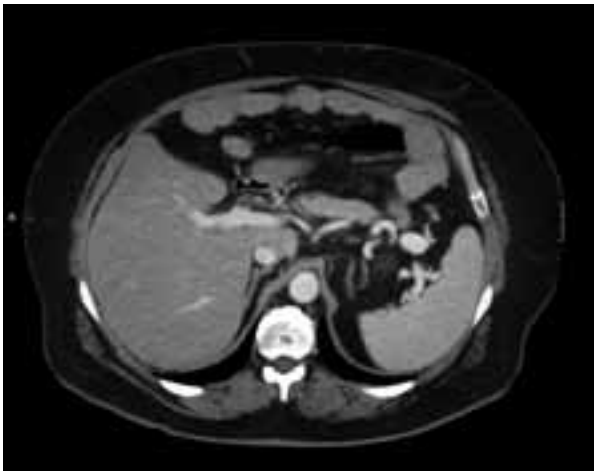

Figure 3. *Abdominal CT: Mixed solid-liquid collection (WOPN)*

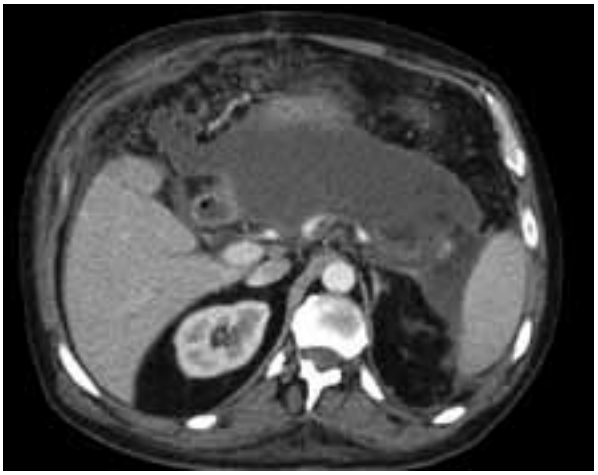

Table 1. *Messages*

WOPN is a new name for an old entity (necroma, organised pancreatic necrosis,..)
WOPN is a transitional collection after pancreatic necrosis that contained various amounts of fluid and necrotic tissue, occurring 6 weeks after an acute pancreatitis attack
CT and MRI are the best diagnostic methods; differential diagnosis with pseudocyst is crucial
Asymptomatic patients would probably not be treated
Transmural endoscopic debridement of WOPN should be the first therapeutic technique performed in symptomatic patients
Surgery should be done only in selected cases (WOPN over 15 cm or affecting both paracolic gutters) after the failure of endoscopic techniques

Simple endoscopic drainage of WOPN has been found to be less effective than transmural endoscopic debridement (NED). [8,10]. NED is successful in approximately 90% *vs* 50% with standard endoscopic drainage.[1,8-10] This outcome is probably due to the fact that standard endoscopy does not allow correct drainage of solid debris. The wider tract fistula and direct cleaning performed in NED improve the results of endoscopy.[8,10]

Papachristou *et al.* described 53 WOPN patients initially managed by endoscopic drainage. Endoscopy alone solved the situation in half of the cases, endoscopy and percutaneous drainage in 25% and surgical management was required in 25%.[1] Two later studies compared only irrigation-based debridement with NED, demonstrating that NED achieves better outcomes (high successful resolution rate and low rates of surgical rescue, percutaneous drainage and recurrent collection).[8,10] In 2011, Gardner *et al.* published a multicentre study of 104 patients with WOPN treated with NED. All the patients were symptomatic. Successful resolution of WOPN was achieved with NED in 95 of 104 patients (91%). Recurrent collection and recurrent pancreatitis were the main causes of failed NED. The mean time to resolution of WOPN was 4.1 months. BMI >32 was a risk factor for failed NED. In conclusion, Gardner *et al.* stated that NED is the most efficacious technique for treating WOPN with an acceptable safety profile.[9]

Varadarajulu *et al.* described a new EUS-based approach to WOPN management consisting of creating multiple transluminal gateways to facilitate effective drainage of the necrotic contents with fewer procedures than conventional endoscopy. The associated success rate was 91.7%.[10]

Fischer *et al.* described six patients treated with a novel endoscopic laparoscopic drainage technique. Only one

patient required surgery. An average of six endoscopic sessions was needed (range 4-11).[2]

- Surgery

The classical indications for surgical therapy of WOPN are infection, complications or failed non-surgical therapies.[11] Surgical minimally invasive necrosectomy is technically feasible and acceptable outcomes are achieved.[18] The laparoscopic approaches to pancreatic necrosectomy can be classified by access route (transperitoneal, retroperitoneal, transgastric) and type of scope (endoscope, laparoscope or nephroscope).[6,19] The main pitfall of the laparoscopic approach is incomplete or unsuccessful drainage.[2] Laparoscopic and hybrid techniques that utilise wide external drainage have high rates of pancreatic fistula formation.[6]

An open approach should be used when endoscopic or laparoscopic treatment fails.[1,2] Operative management of WOPN involves open debridement, lavage of the cavity followed by closed packing and/or drainage.[6] Open debridement for necrotising acute pancreatitis is associated with a high morbidity (55%) and mortality (14%); no data about surgical necrosectomy for WOPN are available but will probably be lower.[11] Several complications have been reported: pancreatocutaneous fistula (up to 53%), enteral fistulae (16%) and abdominal wall hernias.[6,9] Necrosectomy in WOPN patients is not easy but is less technically demanding than necrosectomy performed in necrotising acute pancreatitis.[9]

Three prognostic factors for which WOPN requires a surgical approach have been proposed: preoperative diabetes mellitus, size bigger than 15 cm and WOPN on both sides of the abdomen.[1,8]

Munene *et al.* treated ten patients with open transgastric debridement and internal drainage for symptomatic non-infected WOPN. No mortality was observed, morbidity was 20%, and no pancreatic fistula occurred. Symptoms resolved in 90% of patients.[6] The limitations of this technique are: lack of opposition of the gastric wall to WOPN and extension via paracolic gutters. The main problem of internal WOPN drainage is that it could lead to continuous retroperitoneal contamination. The advantages of this surgical technique compared with the endoscopic approach are similar morbidity, no mortality, reduced length of hospital stay and fewer procedures.[6]

CONCLUSION

WOPN is a new term for an established pancreatic condition. There have been very few studies of WOPN. Indications and management guidelines remain unclear and no randomised clinical trial about WOPN has been conducted. Asymptomatic patients probably would not be treated. Transmural endoscopic debridement of WOPN is efficacious with an acceptable safety profile and probably should be the first therapeutic technique to be performed in symptomatic patients. Surgery should only be performed in selected cases: WOPN over 15 cm or affecting both paracolic gutters after endoscopic techniques have failed.

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REVIEW

BK virus infection in transplant recipients: Clinical manifestations, treatment options and the immune response

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ABSTRACT

Polyomavirus BK (BKV) is ubiquitously present amongst the general population establishing a latent, seemingly asymptomatic infection in immunocompetent individuals. In transplant recipients, however, BKV reactivation is common and can lead to distinctive pathological entities in different patient groups: in renal transplant (RT) recipients, it is associated with nephropathy (BKVN) and ureteral stenosis, and in haematopoietic stem cell transplant (HSCT) recipients with haemorrhagic cystitis (HC). Furthermore, BKV employs several potentially oncogenic mechanisms to promote its replication in cells and has been inconsistently linked to the development of malignancies. BKVN is currently a major cause of allograft failure in RT recipients. HC causes prolonged hospital stay and increased mortality in HSCT recipients. Despite its discovery more than 40 years ago, few advances have been made with regard to therapeutic strategies. Current therapies aim to restore the impaired immune response, e.g. by lowering immunosuppressive agents in RT recipients. However, this is a double-edged sword since it also increases the chance of rejection. Therefore, more specific and effective treatment strategies are urgently needed. Here, we will review the current knowledge on the structure and lifecycle of BKV, characteristics of the BKV-specific immune response, its clinical manifestations and the strengths and limitations of available treatments methods.

KEYWORDS

Haemorrhagic cystitis, nephropathy, polyomavirus BK, viral immunity, ureteral stenosis

INTRODUCTION

In 1971, Gardner and co-workers were the first to isolate polyomavirus BK (BKV) from both urine and ureteral epithelial cells of a Sudanese renal transplant (RT) recipient who presented with renal failure and ureteral stenosis. They named the virus after the initials of this patient.[1] Since then, numerous publications on various aspects of this virus have been published.

BKV seems to be ubiquitously present amongst the general population and up to 100% of tested individuals may be seropositive, with peak seroprevalences reported to occur in children and young adults.[2,3] Up to now, BKV has not definitively been associated with disease in immunocompetent individuals. However, in immunocompromised individuals, the virus frequently reactivates and currently poses a major challenge to transplantation medicine. In this review, several aspects of this virus such as structure and lifecycle, BKV-directed immunity, as well as clinical manifestations and therapeutic strategies are discussed.

STRUCTURE AND LIFECYCLE

BKV is a small, ~45 nm in diameter, non-enveloped DNA virus with a double-stranded circular genome that comprises ~5000 base pairs. BKV shows 70 to 75% sequence homology to other polyomaviruses such as JC virus (JCV), and Simian vacuolating virus 40 (SV40).[4-7] The viral capsid is composed of the structural virion proteins (VP) 1, 2 and 3, and accommodates the viral minichromosome. Pentameres of VP1, arranged in an icosahedral lattice, form the outer capsid.[8] On the inside, the VP1 pentameres have a central groove in which VP2

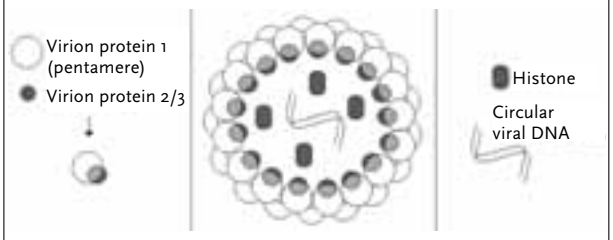
or VP3 is inserted.[9] All three structural proteins contain DNA-binding motifs.[10] A simplified visualisation of the virus is presented in *figure 1*.

The lifecycle of BKV, visualised in *figure 2*, is initiated by the binding of VP1 to certain sialic acid motifs on N-linked glycoproteins and/or to gangliosides GD1b and GT1b on the cell membrane.[11-13] After attachment, BKV traverses the cell membrane by caveolae-mediated endocytosis.[14] Caveolae arise from lipid rafts, plasma membrane regions enriched in cholesterol and the aforementioned gangliosides.[15] Next, BKV is transported towards the endoplasmic reticulum via microtubules.[16,17] Disassembly of the outer capsid is essential for the exposure of VP2 and VP3 which mediate entry into the nucleus via importins.[18] The precise mechanism of capsid disassembly is not known but seems to involve an early acidification step and ultimately leads to cleavage of VP1 molecules and capsid rearrangement.[17] The viral minichromosome consists of circular double-stranded DNA wound around histones.[19]

The BKV genome can roughly be divided into three regions as depicted schematically in *figure 3*: the non-coding control region (NCCR), which contains the origin of replication, a bidirectional promoter-enhancer region and binding sites for host transcription factors;[20,21] the early region, containing genes coding for the tumour antigen (TAg) proteins; and the late region, which contains genes coding for agnoprotein and VP1, 2 and 3.

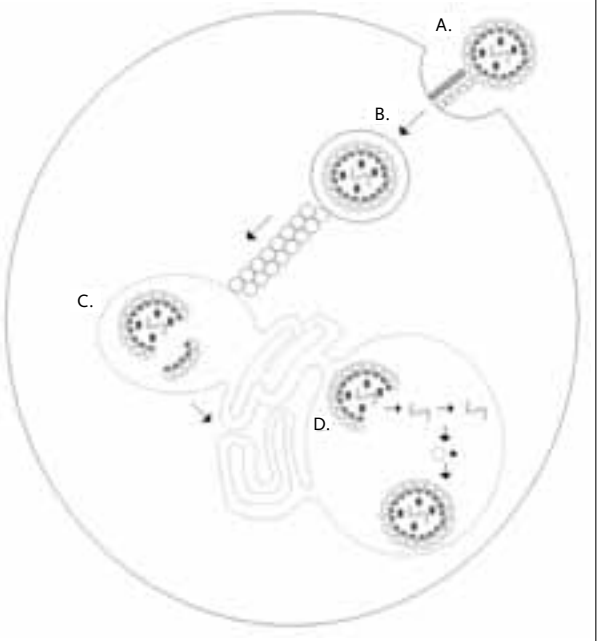
Counter-clockwise transcription of the early region is the first step of replication and leads to the production of the TAg proteins. Three T antigen proteins are produced: large TAg (LTAg), truncated TAg (TruncTAg), and the small T antigen (stAg). Multiple LTAg molecules form a dodecameric complex that binds to the viral origin of DNA replication. Here it acts like a helicase by opening up and unwinding the DNA to initiate clockwise transcription of the late regions.[22,23] LTAg was also demonstrated to bind

Figure 1. *The BKV capsid and minichromosome*



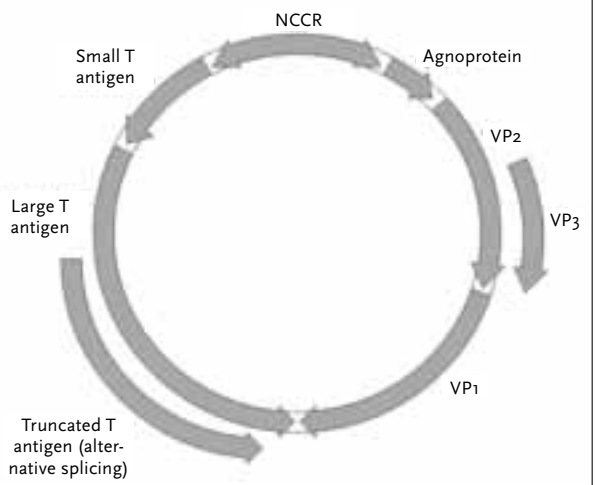
The BKV virion consists of the structural viral proteins (VP), VP1, VP2 and VP3. VP1 complexes into pentameres and forms the outside of the capsid. VP2 and VP3, both originating from the same gene, insert into a groove on the inside of the VP1 pentamere. Together with VP1, they bind the viral minichromosome, which consists of circular DNA wrapped around histones.

Figure 2. *Lytic lifecycle of BKV*



A) BKV attaches to sialic acid motives on N-linked glycoproteins and/or to gangliosides on the cell membrane. B) By caveolae-mediated endocytosis, BKV enters the cell and is transported to the endoplasmic reticulum (ER) via microtubules. C) After internalisation, BKV capsid rearrangement occurs and leads to exposure of VP2 and VP3 D) BKV enters the nucleus via importins where it pirates the cell's replication machinery. Ultimately, BKV progeny accumulates inducing rupture of the cell and release of daughter virions.

Figure 3. *A schematic overview of the BKV genome*



The non-coding control region (NCCR) contains a bidirectional promoter/enhancer site to which host transcription factors can bind. First, the early region is transcribed to produce the tumour antigen (TAg) proteins. Twelve large TAg proteins then complex into a helicase-like structure that attaches to the NCCR and subsequently initiates transcription of the late region which contains genes encoding agnoprotein and the structural viral proteins (VP) 1, 2 and 3.

to heat shock proteins, members of the retinoblastoma protein family, and p53, as such driving the cell into the S phase and preventing cell cycle arrest.[24-26] Knowledge on the function of BKV stAg is limited. In mice, stAg of murine polyomavirus (mPyv) complements LTAg in driving cell cycle progression by several mechanisms, such as activation of the promoter of the proto-oncogene c-myc.[27,28] TruncTAg results from alternative splicing of the LTAg transcript and its functions remain to be elucidated.[29] Transcription of the late region genes leads to the production of the structural VP1, VP2 and VP3 proteins, as well as the non-structural agnoprotein. Little is known on the functions of agnoprotein but it seems to mediate assembly of BKV virions.[30] JCV agnoprotein was found to inhibit double-stranded DNA repair and may as such increase the production rate of more virulent mutant viruses.[31] Intranuclear accumulation of daughter virions ultimately results in rupture of cell membranes, thereby releasing virus progeny into the extracellular space.[32,33]

IMMUNE RESPONSE

The precise route of transmission of BKV is still unclear but may involve salivary, faecal and possibly even transplacental transmission.[34-36] Recently, it was demonstrated that certain defensins, small cationic molecules involved in neutralising a broad spectrum of pathogenic microbes, are able to inhibit BKV infection in an early stage of the virus lifecycle. The human α-defensin 5 (HD5), which is present in the small intestine and in the urogenital tract, reduces the binding of BKV to the cell surface, probably via electrostatic binding to the BKV capsid. Moreover, HD5 aggregates virions, thereby sequestering them away from cells.[37]

Immune and non-immune cells express various receptors that recognise viral nuclear acids and/or viral proteins. Triggering of these sensors induces the production of pro-inflammatory, chemotactic, anti-viral and pro-apoptotic mediators that are crucial for the activation of innate and adaptive immune responses aimed at restricting viral replication.[38] The receptors that recognise proteins and/or nucleic acids of BKV have not been identified thus far, but a recent study showed that BKV infection enhanced expression of the double-stranded RNA sensor, Toll-like receptor 3, the cytokine IL-6 and the chemokine IL-8/CXCL8 in renal collecting duct cells.[39] We observed that expression of TLR3 and the cytosolic dsRNA sensors MDA5 and RIG-I in kidney transplant biopsies was enhanced during BKV infection [Heutinck *et al.* 2012, in press]. These findings raise the question to what extent dsRNA receptors mediate anti-viral immune responses against BKV.

Using microarrays, the global effects of BKV infection on gene expression have been addressed in human proximal tubular epithelial cells and endothelial cells.[40,41] In both studies, BKV infection was found to activate genes involved in cell division, DNA replication and apoptosis. Surprisingly, the virus did not promote transcription of pro-inflammatory cytokines, type I interferons or chemokines. In tubular epithelial cells only two inflammatory genes, PTX3 and MICB, were upregulated after infection with BKV.[40] In BKV-infected endothelial cells, immunological defence genes, amongst which IL-15, tended to be down-regulated or unaffected.[41] One could therefore hypothesise that BKV employs immunosuppressive strategies by inhibiting the expression of genes involved in the anti-viral response. In line with this hypothesis, induction of IL-6 and IL-8 transcription occurred only within the first six hours after BKV infection in collecting duct cells, but was not maintained.[39]

The adaptive immune response and in particular T cells play a crucial role in the clearance of most viral infections. Given the occurrence of BKV-associated disease in transplant recipients and to a lesser degree in HIV-infected patients, T cells are likely to be important. Indeed, BKV-specific T cells are detectable in the peripheral blood of both healthy individuals and transplant recipients.[42-47] T cells are directed against epitopes from all viral proteins, except for agnoprotein which does not seem to be immunogenic.[42,43,48] T cells specific for the structural proteins VP1, VP2 and VP3 appear prior to T cells that recognise TAg proteins. The latter seem to play a leading role in the control of BKV infection and correlated with a drop in viral load.[43] *In vitro* analysis of T-cell reactivity against overlapping peptide pools suggests that CD4+ T cells dominate over CD8+ T cells.[42,49] However, this finding might have been biased by the use of peptide pools that favour presentation in major histocompatibility complex (MHC) class II molecules. BKV-specific T cells can be polyfunctional with regard to production of IFNγ, IL-2, and TNFα.[42,50] Interestingly, polyfunctional T cells directed against LTAg were less frequently detectable in patients with high viral loads and/or BKV nephropathy (BKVN), compared with patients with a rapid viral clearance or no reactivation.[42,51] *In vitro* expanded BKV-specific CD4+ and CD8+ T cells appeared to express cytotoxic mediators and could eliminate peptide-loaded targets.[52-54] Direct *ex vivo* analysis of the cytotoxic potential of BKV-specific T cells is hampered by their low frequency in peripheral blood. Nevertheless, the availability of BKV-peptide-HLA multimers will enable examination of the phenotype of BKV-specific T cells in the future. Notably, several studies revealed a significant cross-reactivity between BKV and JCV multimers.[44-47]

Development of both cellular and humoral immunological memory is important in the immune response against BKV. As mentioned previously, BKV-specific antibodies

are ubiquitously present amongst the population. However, their presence alone does not protect against BKV reactivation, BKVN or HC.[55,56] Nevertheless, negative or low antibody titres prior to transplantation have been proposed as risk factors for BKV viraemia and BKVN.[57-59] Also, the report on BKVN in a patient with hyper-IgM syndrome suggests that immunoglobulin class-switching and affinity maturation may be important in the control of BKV infection.[60] BKV reactivation is associated with significantly increased levels of BKV-specific IgG and IgM. Antibodies were neither quantitatively nor qualitatively related to viral load or to recovery from BKVN,[43,57] indicating the importance of other, and probably cellular immune responses.

BKV persists latently in healthy individuals, thus having developed ways to evade the immune system. As of yet, little is known regarding this subject. One study of particular interest showed that BKV and JCV produce a microRNA (miRNA) that suppresses the expression of ULBP3, a protein recognised by the activating receptor NKG2D present on natural killer cells and CD8+ T cells. Expression of this miRNA reduced the effector function of NK cells *in vitro*.[61] In the case of SV40, this virus encodes another viral miRNA that accumulates during infection and paradoxically reduces the expression of TAg proteins, apparently without affecting viral replication. This downregulation of TAg proteins was proposed to reduce immunogenicity.[62] Lastly, BKV replication leads to the emergence of 'quasispecies'; virions with mutated NCCRs and/or structural proteins of which several may be found in a given individual. Selective pressure might lead to the rise of mutants that are capable of evading immunological surveillance.[63,64]

CLINICAL MANIFESTATIONS AND THERAPEUTIC STRATEGIES

The cellular reservoir of latent BKV infection in immunocompetent individuals seems to comprise numerous cell types, including cervical squamous epithelial cells, peripheral blood leucocytes, salivary gland cells, prostate glandular epithelial cells, and urothelial cells.[34,65-68] BKV was also found in the urine in 7% of healthy individuals, but never in plasma.[69] In immunocompromised patients, BKV has been associated with several clinical manifestations amongst which most prominently BKVN, ureteral stenosis and late-onset HC. Also, the association of human polyomaviruses with malignancies remains a topic of ongoing discussion. Other less apparent associations include encephalitis, retinitis, respiratory tract infections and vasculopathy.[70-73] In the next paragraphs, we will discuss the main clinical manifestations of BKV infection and its possible role in malignancies in more detail. *Table 1* gives an overview of therapeutic strategies available for BKVN and HC.

NEPHROPATHY

BKVN occurs in about 5% of RT recipients, mostly within one year after transplantation.[55,74,75] Patients generally do not present with any symptoms other than a decrease in renal function. BKVN is also observed in native kidneys of HSCT recipients, lung and heart transplant recipients, as well as in immunocompromised HIV-infected patients. Even though BKVN is not specifically monitored in these patients, its prevalence seems to be lower.[76-79]

Table 1. *Therapeutic interventions targeting BKV*

Intervention	Applicability		Proposed mechanism of action	Reported adverse events	Effectiveness	References
	BKVN	HC				
Tapering of immune suppression	Yes	No	Reconstitution of immune responses directed against BKV	Rejection of the allograft kidney	Effective	[74;92;94;104;106;108-110]
Cidofovir	Yes	Yes	Inhibitor of viral replication, mechanism unknown	Severe anterior uveitis, potentially nephrotoxic	Doubtful	[118-121]
Leflunomide	Yes	Yes	Pyrimidine depletion, tyrosine kinase inhibition	Thrombocytopenia, (haemolytic) anaemia and thrombotic microangiopathy	Doubtful	[125-129]
IVIg	Yes	Yes	Antibody-mediated neutralisation	Paradoxical increase in viral load	Doubtful	[122-124]
Fluorochinolones	Yes	Yes	Inhibition of large T antigen helicase activity	None	Doubtful	[130-132]
Statins	Yes	Yes	Prevention of caveolae-mediated endocytosis	None	Very doubtful	[133]

Development of BKVN has been associated to specific immunosuppressive agents such as the calcineurin inhibitor tacrolimus (TAC), the ionosine monophosphate dehydrogenase inhibitor mycophenolate mofetil (MMF), therapy with polyclonal anti-T cell antibodies, and number of corticosteroid pulses given for the treatment of rejection.[55,80-83] Other studies suggest that the cumulative intensity of the immunosuppressive regimen rather than one specific agent increases the risk for BKVN.[84,85] Altogether, it remains unclear whether development of BKVN is attributable to qualitative and/or quantitative differences in immune suppression.

Given the strong association with renal allografts, kidney damage may be involved in the development of BKVN. Indeed, mPyv was found to reactivate and replicate to a significantly higher degree in damaged mouse kidneys.[86] However, transplantation factors leading to graft damage, such as cold ischaemia duration and donor origin (living or non-living), have been inconsistently associated with BKVN in humans.[87,88] The immune system is pivotal in keeping BKV at bay but may also contribute to the pathogenesis of BKVN. In one study, detectable circulating BKV-specific CD8+ T cells were observed in two out of 15 RT recipients with particularly high plasma BKV viral loads. Interestingly, those two patients were the only ones who lost their grafts.[89] Other immunological factors involved in the development of BKVN might be allo-HLA-reactivity and heterologous immunity, the latter concerning T cells that cross-react to both BKV- and allo-antigens. Furthermore, one could reason that allo-HLA molecules presenting BKV peptides are not recognised by host BKV-specific effector-memory T cells, thereby at least temporarily allowing BKV to escape immunological surveillance. In this regard, murine renal allografts were indeed found to be more susceptible to mPyv infection than isografts.[90] Murine Pyv infection also led to an increase in allo-reactive T cells that, however, lacked cross-reactivity to the virus. The authors propose that virus-induced allograft inflammation and a subsequent increase in donor antigen presentation might explain this finding.[90] Nevertheless, CD4+ T cells with cross-reactivity against BKV VP1 and allo-HLA antigens have been observed in humans.[89] The number of HLA mismatches and rejection episodes are also reported inconsistently as risk factors for the development of BKVN in human RT recipients.[55,80-82,87,88,91-99]

Lastly, viral factors have been proposed as the cause of BKVN. NCCR and/or capsid mutants may enhance BKV virulence.[64,100,101] Indeed, in RT recipients with overt viral activity, i.e. viraemia and BKVN, more mutants were detected.[64,102] However, extensive virus replication would logically lead to the emergence of more mutants, thereby confounding associations with clinical disease severity. Of specific interest is the report on more cytopathology in kidneys infected with multiple NCCR mutants.[102]

The renal disease spectrum seems to begin with viruria and ends with extensive irreversible kidney damage and graft failure. It is therefore of paramount importance to intervene in an early phase to prevent graft loss. Screening for active BKV replication may involve the detection of viral DNA by quantitative PCR in urine and in blood. Monitoring of the urine may also comprise the detection of BKV-infected 'decoy cells' or aggregates of BKV virions, the so-called 'haufen'. Solitary point prevalence measurements of urinary BKV viral load, positive in 20 to 57% of RT recipients,[88,103] and/or decoy cells, positive in 13 to 42% of renal RT recipients,[75,104] were found to have low positive predictive value for the development of BKVN.[55,75] However, sustained viruria as well as the presence of haufen were found to have a higher predictive value.[94,104,105] Viraemia only occurs in immunocompromised patients, with 7 to 29% of RT recipients showing BKV viraemia at least once after transplantation.[83,88,94] Moreover, viruria always precedes viraemia.[94] As such, viraemia seems to reflect a state of more elaborate infection. Indeed, high plasma viral loads and sustained viraemia were found to be even better predictors for the development of BKVN than the presence of viruria.[75,94,106]

Ultimately, BKVN is a histopathological diagnosis. Histopathological grades of severity have been defined ranging from stage A: viral cytopathic changes of near-normal renal parenchyma and no or minimal tubular atrophy, interstitial fibrosis or inflammation, to stage C: diffusely scarred renal tissue with extensive tubular atrophy, interstitial fibrosis and inflammation.[107] Since BKV affects the kidney in a random, multifocal manner, false-negative biopsy results may occur, especially in an early stage of disease.

Currently, reducing immunosuppression is the only established mode of therapy and aims to restore the anti-viral immune response. Graft loss due to BKVN is significantly higher in RT recipients with BKVN than in control RT recipients, and may be especially high when tapering of immunosuppression is not applied.[92,108] The combination of regular screening for BKV replication and subsequent pre-emptive adjustment of immunosuppressive therapy seems to be particularly effective.[74,94,104,106,109,110] Given the lack of an evident link between one specific immunosuppressive agent and the development of BKVN, there is no standard strategy for adjusting immunosuppressive therapy. *Ex vivo* and *in vitro* analyses with different immunosuppressive agents revealed that BKV-specific T cells were particularly inhibited by TAC and not so much by MMF or prednisone,[111] indicating TAC as a first target of modification. Upon *in vitro* infection, BKV activates the intracellular PI3K/Akt/mTOR pathway. Subsequent titration with sirolimus reduced LTAg expression in a dose-dependent manner.[112] Another option may, therefore, involve the use of the mTOR-inhibitors sirolimus or

everolimus, which also did not inhibit interferon-γ (IFNγ) production by BKV-specific T cells *in vitro*.[111] However, so far few and conflicting reports on the clinical efficacy of mTOR inhibitors in treating BKVN have been published.[113-116]

Beyond tapering and/or altering immunosuppression, other anti-viral agents have been proposed. Cidofovir, known to be nephrotoxic, showed *in vitro* inhibitory activity against polyomaviruses.[117] Since polyomaviruses do not express the known target of cidofovir, viral DNA polymerase, its mechanism of action is unknown. Several studies proposed to administrate cidofovir simultaneously to reducing immunosuppressive agents,[118,119] or when the latter alone proved ineffective.[120,121] Unfortunately, randomised controlled trials are lacking and several confounders including, most importantly, the concomitant tapering of immunosuppression, complicate the interpretation of the effectiveness of cidofovir. Although long-lasting nephrotoxic effects have not been reported, severe anterior uveitis occurred in up to 7% of patients, sometimes leading to permanent visual impairment.[119] Treatment with intravenous immunoglobulins (IVIg) might help to neutralise BKV particles.[122,123] Surprisingly, IVIg was recently associated with a paradoxical increase in BKV viral load rather than a decrease.[124] The pyrimidine synthesis inhibitor leflunomide may be effective by inhibiting tyrosine kinase activity and by inducing pyrimidine depletion.[112] Apart from a doubtful clinical effect, leflunomide also has a high rate of side effects such as (haemolytic) anaemia, thrombocytopenia and possibly also thrombotic microangiopathy.[125-129] Fluorochinolones have been described to inhibit LTAg helicase activity. Nevertheless, also here the reports on clinical efficacy are contradicting.[130-132] Lastly, one study reported that statins inhibit the formation of caveolae and as such may block virus cell entry.[133]

URETERAL STENOSIS

The original patient B.K. presented with a stenosis of his graft ureter. On further examination, a segment of the ureter appeared to be ischaemic and fibrotic, and large numbers of virions were observed in epithelial cells lining the ureter lumen.[1] Other publications on supposedly BKV-related ureteral stenosis in RT recipients followed, reporting a prevalence of 2 to 6%.[83,134-136]

There has been discussion on the association of BKV with ureteral stenosis. However, its prevalence was found to be significantly higher in RT recipients who developed viraemia than in patients who did not.[83] Apart from a proposed role for BKV in a reversible form of ureteral stenosis in HSCT recipients with haemorrhagic cystitis,[137] to our knowledge BKV as the cause of irreversible ureteral stenosis has not been reported in non-renal transplant patients. Treatment generally consists of (temporary) percutaneous nephrostomy and the concomitant lowering of immunosuppressive agents.

HAEMORRHAGIC CYSTITIS

BKV reactivation is common in HSCT recipients, viruria and viraemia, occurring at least once during follow-up in 47 to 94% and in 23 to 53% of recipients, respectively.[138-140] HC occurs mainly in HSCT recipients and can be caused by conditioning, e.g. with cyclophosphamide and total body irradiation, but also by several viruses amongst which CMV, adenovirus and indeed BKV. The virally induced form of HC usually occurs after engraftment and is therefore referred to as late-onset HC, which occurs in 6 to 29% of HSCT patients, generally within the first two months after transplantation.[140,141]

Patients present with haematuria, painful voiding, bladder cramps, and/or flank pain. Four degrees of disease severity are currently recognised: grade I: microscopic haematuria; grade II: macroscopic haematuria; grade III: haematuria and clots; and grade IV: haematuria with clots, clot retention and renal failure due to obstructive nephropathy. More often, late-onset HC is of a higher severity grade than early-onset HC.[140,142] Bleeding may be so severe that patients require red blood cell and/or platelet transfusion, ultimately even necessitating cystectomy in some severe and refractory cases.[143,144] Not only was HC reported to prolong hospital stay,[145] it also seems to have a significant negative effect on overall patient survival.[146,147]

Also here, the connection of BKV to late-onset HC remains a topic of discussion. Various groups found an association with solely BKV reactivation, defined as detectable virus in urine and/or blood,[146,148-150] whereas others could only relate HC to very high urinary BKV viral loads.[140-142,151] Of specific interest is one study reporting on a correlation between the degrees of viruria and haematuria.[139] Lastly, with 81 to 100% of late-onset HC patients having viruria and 75% viraemia,[138,140,142,146] BKV viral replication seems to occur more frequently in these patients than in HSCT patients in general. Together these studies suggest that BKV reactivation contributes to the pathogenesis of late-onset HC.

The pathogenesis of HC has been proposed to involve two steps: I) Severe immune suppression together with urothelial damage due to conditioning and irradiation, creating an environment favourable for viral replication, as well as leading to an increase in immunological danger signals and antigen presentation. II) Attack of virus-infected host urothelial cells by donor T cells.[152] In support of this theory, BKV-associated HC patients

showed more signs of immune hyperactivity such as graft versus host disease (GVHD) than patients with adenovirus-associated HC.[140,150,152,153] Other inconsistently reported risk factors include donor origin, i.e. cord blood or haploidentical graft, NCCR mutants, treatment with antithymocyte globulins, full conditioning instead of reduced intensity conditioning, and conditioning with busulphan.[140,145,148,149,152,154] Taken together, the pathogenesis of HC is complex but may very well involve immune reconstitution rather than immune suppression.

Due to the risk of GVHD and to the possibility that HC is caused by immune reconstitution, tapering of immunosuppressive therapy is an unattractive treatment option in this clinical context. In many patients, symptoms can be relieved by (intravenous) hydration. Cidofovir has been proposed in the treatment of HC, especially since it may also be given locally by bladder instillation, thereby reducing (cumulative) nephrotoxicity of cidofovir alone or in addition to the several other nephrotoxic agents often used in treating HSCT recipients.[155] Interestingly, both patients treated with cidofovir and patients treated only with supportive care achieved remission.[140] The self-limiting nature of late-onset HC, apparently occurring in a significant number of patients, has been confirmed by other studies.[138,156,157] Remission of symptoms varied from two to seven weeks after haematuria, and did not differ significantly in duration between cidofovir or supportively treated patients.[140] With regard to other virus-targeting strategies, prophylactic treatment with ciprofloxacin led to a significant reduction in the occurrence of HC in one retrospective analysis,[158] but not in another.[159] To our knowledge, no publications have addressed the use of leflunomide or IVIg in the treatment of BKV-associated HC. Taken together, it seems that treatment of BKV-associated HC should mainly be supportive. Supportive treatment strategies, not directly targeting the virus, are beyond the scope of this review and have been reviewed in detail by Harkensee and co-workers.[160]

BKV AND MALIGNANCY

BKV has been associated with several human neoplasms, amongst which bladder and prostate carcinoma, brain tumours, tumours of pancreatic islets, Kaposi sarcoma, Ewing sarcoma and osteogenic sarcoma.[161-168] Of specific interest are the cases of a simultaneous pancreas and kidney transplant recipient with BKVN and a metastasised bladder carcinoma, and a kidney transplant recipient without BKVN who developed a renal cell carcinoma.[169,170] In each of them, the primary tumours as well as the metastases contained BKV DNA and expressed high amounts of LTAg. Nevertheless, numerous other studies did not find BKV to be related to malignancy.[171-179]

In rodent cells, BKV drives malignant transformation *in vitro*, giving rise to full-blown tumours after subsequent inoculation back into the animals.[180,181] Furthermore, the majority of transgenic mice expressing a single copy of the BKV early region developed renal and lymphoid malignancies.[182] Human embryo fibroblast and foreskin cells, however, were not inclined to such transformation,[183,184] possibly owing to the presence of specific human tumour suppressor genes.[185]

The previously mentioned actions of LTAg and of agnoprotein would render an infected cell less capable of arresting the cell cycle for DNA repair and may drive a cell towards a continuously dividing state. Not only does BKV benefit from the ensuing increase in host-derived transcription factors, it can thereby also contribute to malignant transformation. In permissive cells, BKV infection results in either cell lysis, leading to release of viral particles, or latency, which is characterised by low expression of viral genes and immune evasion. Infection of non-permissive cells may lead to an aberrant form of replication with continuous expression of only the early region of the BKV genome. In these cells, TAg proteins accumulate, which may ultimately result in malignant transformation. Interestingly, BKV species with mutated NCCRs were found to possess altered replicative and/or transforming capabilities.[167,186-189]

In conclusion, BKV possesses oncogenic potency and is theoretically able to at the least contribute to malignant transformation of cells. A definitive association with specific human malignancies remains to be proven. For a more in-depth review on this topic, we refer to the publication by Abend and co-workers.[190]

CONCLUSION

BKV is ubiquitously present amongst the general population. When immunological surveillance is hampered, BKV reactivates and causes BKVN and/or ureteral stenosis in RT recipients, and late-onset HC in HSCT recipients. Treatment options targeting viral replication are still limited. The most effective therapy in RT patients is improvement of the host immunological defence by lowering immunosuppressive drugs. In refractory cases of BKV-associated disease, antiviral agents such as cidofovir, leflunomide, IVIg and fluorochinolones may be applied. The effectiveness of these agents is, however, doubtful and some of them can cause severe side effects. BKV has also been implied to be involved in human malignancies, yet its precise role remains to be elucidated. All compartments of the immune system seem to be involved in keeping BKV at bay, virus-specific T cells being of particular importance. In order to develop novel effective treatment strategies and vaccines, more research

towards the characteristics of the BKV-specific immune response is necessary.

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ORIGINAL ARTICLE

Low complication rates in the use of port-a-caths in oncology patients

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ABSTRACT

Background: Port-a-caths (PACs) represent an important component of the care of cancer patients, in particular for administration of chemotherapy. We sought to analyse the longevity and complications of PACs in cancer patients in a large community hospital.

Methods: We retrospectively analysed the indications, duration of use, complications and reasons for removal of PACs in cancer patients treated in our centre from January 2005 to December 2010, and compared these with findings in patients who received a PAC in the same period for reasons not related to cancer.

Results: During the study period 152 cancer patients received a total of 170 PACs; in the same period, 21 patients received a total of 35 PACs for reasons unrelated to cancer. The total analysis comprised 70,919 days of PAC use. Most cancer patients had a solid tumour (97%). PACs were removed because of a complication in 25 cases in cancer patients (14.7%) *vs* 15 cases in non-cancer patients (42.9%, p<0.01). Culture-proven infection was the reason for PAC removal in 16 cases in cancer patients (23.5%) *vs* eight cases in non-cancer patients (42.1%; p=NS). The total number of PAC-associated infections was 20 in cancer patients (0.35 infections per 1000 PAC days) *vs* 19 in non-cancer patients (1.43 infections per 1000 PAC days; p<0.01). No PAC-associated thrombosis was found.

Conclusion: In clinical practice the use of PACs in cancer patients is safe with lower complication rates when compared with PAC use in patients without malignancy.

KEYWORDS

Cancer, chemotherapy, infection, oncology, Port-A-Cath

INTRODUCTION

Venous access is problematic for oncology patients receiving repeated courses of cytotoxic therapy. Totally implantable ports connected to a central venous catheter were first introduced in 1982 and soon replaced subcutaneously tunnelled catheters such as Hickman, Groshong and Broviac lines.[1,2] These totally implantable venous access ports (TIVAPs), among which port-a-caths (PACs), now represent an important component of the regular care of cancer patients by providing a simple way of accessing the venous system for administration of chemotherapy, antibiotics, analgesics, blood products and fluids, and for the collection of blood. Although in general these devices are safe, their use can be associated with significant complications, most notably infection and thrombosis.

Previous studies have examined complication rates of PAC use in cancer patients.[3-11] Such knowledge is significant considering the importance of PACs for the clinical care of cancer patients and for guiding preventive measures. This in particular holds true for the main complications described in the literature: infection and thrombosis. In the current study we retrospectively analysed the indications, duration of use, complications and reasons for removal of PACs in patients with malignancies treated in our centre (a large community hospital in the Netherlands) from January 2005 to December 2010. In addition, we analysed the microbial causes of PAC-associated infections in these patients and their impact on PAC use and removal. In order to obtain insight into complications that may relate to cancer specifically, we compared findings in cancer patients with those in patients who received a PAC in the same period for reasons not related to cancer.

MATERIALS AND METHODS

Patients

We performed a retrospective analysis of 173 adult patients (>18 years of age) who received a total of 205 PACs in the Reinier de Graaf Hospital in Delft, the Netherlands between January 2005 and December 2010. The analysis was approved by the institutional medical ethics committee.

Study design

PAC removals within two days after implantation were excluded since these were considered to be related to the surgical procedure. A single type of PAC was used (Deltec™, Smiths Medical). The PACs were inserted by surgeons from the Department of Vascular Surgery in the operation room under general or local anaesthesia using a standardised surgical technique. The access route was chosen according to the patient's anatomy, preferably the right subclavian or external jugular vein. Prophylactic antibiotics were not routinely administered. The PACs were accessed and cared for by trained nursing staff. Lock with heparin solution was done after every PAC access and every four weeks if the PAC was not in use. Patients did not receive routine anticoagulant therapy. PAC-associated infection was defined as 1) a positive culture of blood obtained from either a peripheral vein or from the port and 2) clinical suspicion of PAC infection as reflected by local symptoms or absence of another infectious source.[12] For the analysis of PAC-associated infections, multiple positive blood cultures with a single pathogen in one clinical episode were counted as one PAC-associated infection with this pathogen.[12] The occurrence of a PAC-associated infection was defined as a complication; other non-infection-related complications were analysed by studying reasons for PAC removal making use of patient hospital records. Diagnostic procedures were done as ordered by the physician; systematic venographies were not performed. Minor complications such as local pain, skin irritation and/or transient inability to draw blood from the PAC were not analysed.

Statistical analysis

Data are expressed as means, medians, interquartile range and ranges as indicated. Differences between cancer patients and non-cancer patients were analysed by the Mann-Whitney U test, Chi square test and log-rank test. A p value below 0.05 was considered to be statistically significant.

RESULTS

Patients

From January 2005 to December 2010, 152 patients with a malignancy received a total of 170 PACs; in the same period, 21 patients received a total of 35 PACs for reasons unrelated to cancer (*table 1*). In both groups, more women than men received a PAC (73.7% amongst cancer patients and 61.9% amongst non-cancer patients). The vast majority of patients with a malignancy suffered from a solid tumour, with breast and colorectal cancer as the

Table 1. *Patient characteristics and indications for port-a-cath placement*

	Total	Cancer patients		Non-cancer patients	
Number of PACs (%)	205	170 (82.9%)		35 (17.1%)	
Number of patients (%)	173	152 (87.9%)		21 (12.1%)	
Female (%)	125 (72,3%)	112 (73.7%)		13 (61.9%)	
Male (%)	48 (27.7%)	40 (26.3%)		8 (38.1%)	
Mean age (range) at time of PAC placement	51.8 (18-80)	51.7 (26-77)		53.5 (18-80)	
Diagnosis (%)		Breast cancer Colorectal cancer Upper GI cancer Ovarian cancer Lymphoma Other	72 (47.4%) 50 (32.9%) 9 (5.9%) 11 (7.3%) 4 (2.6%) 6 (3.9%)	Neuromuscular disease[1] Congestive heart failure CIVD[2]	12 (57.1%) 8 (38.1%) 1 (4.8%)
Indication					
• Chemotherapy		152 (100%)		-	
• Immunotherapy[3]		14 (9.2%)		10 (47.6%)	
• Analgesics		-		2 (9.5%)	
• Dopamine		-		8 (38.1%)	
• Bisphosphonate (APD)		-		1 4.8%)	
Mean (range) number of days in situ					
• Total	70,919	57,642		13,277	
• Per PAC	346 (9-2064)	339 (9-2064)		379 (13-1839)	

[1]Dystrophia (n=4), chronic inflammatory demyelinating polyneuropathy (n=6) and multiple sclerosis (n=2); [2]Common variable immunodeficiency; [3]Refers to monoclonal antibodies: in cancer patients trastuzumb (Herceptin®, antibody directed against epidermal growth factor receptor-2) or bevacizumab (Avastin®, antibody binding to the vascular endothelial growth factor preventing binding to the receptor), in non-cancer patients gammaglobulin (Gammagard®).

predominant diagnoses (47.4% and 32.9%, respectively). In non-cancer patients neuromuscular disease was the most frequent diagnosis (57.1%). The total analysis comprised 70,919 days of PAC use, of which 57,642 days in cancer patients and 13,277 days in non-cancer patients. In cancer patients all PACs were used for administration of chemotherapy. In 14 cases (9.2%) it was also used for immunotherapy. In non-cancer patients ten PACs (47.6%) were inserted for immunotherapy and eight PACs (38.1%) for chronic treatment with dopamine for heart failure (*table 1*).

Longevity of PACs

Table 2 shows the longevity and reasons for removal of the inserted PACs. In cancer patients, 20% of PACs were in use at the end of follow-up, compared with 31.4% in non-cancer patients (p=NS). *Figure 1* is a Kaplan-Meier plot showing that the average survival of the PACs was similar in cancer and non-cancer patients (mean time to removal 927 days *vs* 899 days, p=0.9 by log-rank test). The percentage of PACs removed during the follow-up period was 40% in cancer patients and 51.5% in non-cancer patients (p=NS). The mean number of days a PAC was *in situ* at the time of removal was 309 days and 500 days in cancer and non-cancer patients respectively, p=NS). In cancer patients, most PACs were removed because therapy was completed (63.2 *vs* 15.8% in non-cancer patients, p<0.01). Twenty-five (14.7%) and 15 (42.9%) of PACs were removed for complications (infectious or non-infectious) in cancer and non-cancer patients respectively (p<0.01).

PAC-associated infections

PAC-associated bloodstream infection occurred in 25 of 173 patients (14.4%) (*table 3*). Amongst cancer patients, 18 (11.8%) were diagnosed with PAC-associated infection during the study period, *vs* seven (33.3%) non-cancer patients (p=0.02). The total number of PAC-associated

Table 2. *Numbers and reasons for port-a-cath removal*

PACs	Total (n=205)	In cancer patients (n=170)	In non-cancer patients (n=35)
Number of PACs in situ at closure of data collection (%)	45 (22.0)	34 (20.0)	11 (31.4.)
Number of PACs removed (%)	86 (41.9)	68 (40.0)	18 (51.5)
Number of days in situ[1]			
• Mean	353	312	500
• Median	224	215	247
• Range	6-2064	6-2064	24 -1809
Number of patients with PAC removed	77	64	13
	60	51	9
• Female	17	13	4
• Male			
Reason for removal (% of total removed)	46 (53.5)	43 (63.2)	3 (15.8)
• Treatment completed	24 (27.9)	16 (23.5)	8 (42.1)
• PAC infection[2]	4 (4.7)	2 (3.0)	2 (10.5)
• Occlusion[3]	9 (10.5)	4 (5.9)	5 (26.3)
• Malfunction[4]	3 (3.5)	3 (4.5)	0
• Other[5]			

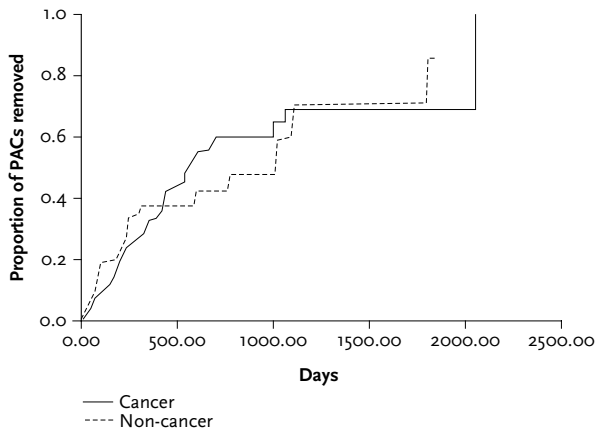
[1]p=NS for difference between patients with cancer and non-cancer patients; [2]PAC infection is defined as positive culture from blood obtained from the port or a peripheral vein and clinical suspicion of PAC as defined by symptoms or ruling out other foci; [3]Defined as inability to infuse fluids into the PAC system, confirmed by administration of radiological contrast fluid into the port; [4]For example nicking of the line, port moved away into deeper (breast) tissue, port turned away; [5]Due to progressive disease in the chest wall covering the port, necessity to insert a Levine shunt, fat necrosis around the PAC.

Table 3. *Port-a-caths in patients with blood stream infections (BSI) and causative organisms*

	All PACs	Cancer	Non-cancer	P- value
Number of PACs inserted	205	170	35	
Number of patients with PAC and BSI	25 (14.4%)	18 (11.8%)	7 (33.3%)	0.02
Number PACs with BSI (% of total)	30 (14.6)	18 (10.6)	12 (34.3)	<0.01
Number of episodes of positive blood cultures[1]	39	21	18	<0.01
Number of different organism in these cultures	43	21	22	
Number of days PAC in situ prior to positive blood culture				0.01
Median	167	100	414	
IQR	55-553	36-234	125-902	
Causative organisms				
Gram-positive	29	14	15	NS
• *Staphylococcus aureus*	10	5	5	
• Coagulase negative staphylococci	16	7	9	
• *Enterococcus*	1	-	1	
• *Streptococcus pneumoniae*	1	1	-	
• Other *streptococci*	1	1	-	
Gram-negative	13	6	7	NS
• *Escherichia coli*	2	1	1	
• *Pseudomonas aeruginosa*	2	-	2	
• *Klebsiella oxytoca*	1	-	1	
• *Klebsiella pneumoniae*	1	1	-	
• *Serratia marcescens*	1	1	-	
• *Rhizobacteria*	1	-	1	
• *Stenotrophomonas maltophilia*	1	-	1	
• *Enterobacter*	2	1	1	
• *Acinetobacter*	1	1	-	
• *Aeromonas hydrophilia*	1	1	-	
Yeasts	1	1	-	-
Candida glabratum	1	1	-	

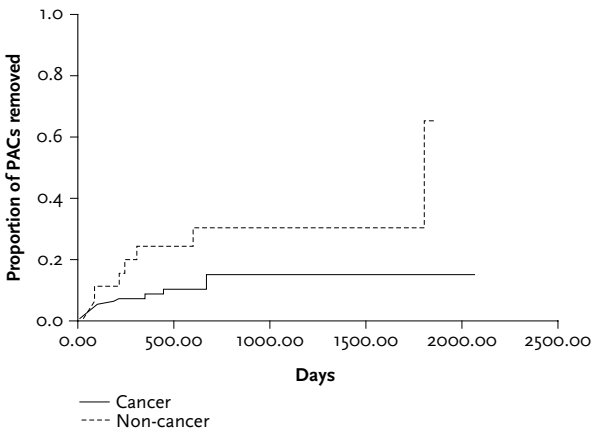
[1]One blood culture per episode (i.e. if four blood cultures were positive for a particular pathogen during the same infection, only one culture was counted).

Figure 1. *Cumulative proportion of port-a-caths (PACs) removed for any reason*



Cases were censored at death or end of follow-up. P=NS by log rank test for difference between PACs in patients with cancer and PACs in other patients

Figure 2. *Proportion of port-a-caths (PACs) removed for infectious complications*



P=0.03 by log-rank test for difference between PACs in patients with cancer and PACs in other patients

infections was 21 in cancer patients (0.36 infections per 1000 PAC days) *vs* 18 in non-cancer patients (1.4 infections per 1000 PAC days; p<0.01 *vs* cancer patients); Of interest, the median time that a PAC was *in situ* before a bloodstream infection occurred was shorter in cancer patients than in non-cancer patients (100 *vs* 414 days respectively, p=0.01). The cumulative proportion of PACs removed for an infectious complication is shown in *figure 2*. Causative organisms did not differ between cancer and non-cancer patients (*table 3*). In both groups, gram-positive pathogens, in particular *Staphylococcus aureus* and coagulase-negative staphylococci, were most prevalent (more than two-thirds of all bloodstream infections).

DISCUSSION

In the last decades, much attention has been given to the achievement of an adequate means of venous access in cancer patients that is suitable for long-term use, in particular for repeated administration of chemotherapy and blood drawing for testing. Totally implantable venous access ports, such as PACs, are preferred to other approaches for many different reasons, including a reduced risk for infection and thrombosis, less visibility and fewer restrictions on daily activity.[13] We here report on our experience with PACs in a large community hospital in the Netherlands during a six-year period (January 2005 to December 2010), comparing indications, duration of use, complications and reasons for removal in 170 cancer patients and 35 patients without malignancy, comprising more than 70,000 days (which is almost 200 patient-years) of PAC use.

The complication rate of PACs in cancer patients in part depends on the type of malignant disease (solid tumour or haematological malignancy) and neutrophil counts in peripheral blood.[13] In the current analysis the vast majority of oncology patients had solid tumours, in particular breast and colorectal cancer (*table 1*), and only three patients had leucocytopenia at the time of PAC-associated infection (data not shown). Hence, our results predominantly apply to patients with solid tumours and normal leucocyte counts. The current study excluded early complications of PAC placements, such as pneumothorax, primary malposition and arterial perforation, since these are related to the surgical procedure. The overall rate of removal of PACs for infectious or non-infectious complications was lower in cancer patients compared with non-cancer patients. Furthermore, the risk that a PAC will be removed for infectious reasons is lower in cancer patients than in non-cancer patients. Although a definitive explanation for this difference is lacking, it may be related to a higher experience amongst oncology nurses in the management of PACs and/or differences in underlying diseases. For example, insufficient hygienic precautions, inadequate flushing of the system after the introduction of fluids or too long an interval between use of the port make the system at risk for irreversible complications. Insufficient dosing of positive pressure leading to narrowing of the lumen of the catheter due to deposits of fibrin or other substances will eventually obstruct the PAC.[6] Different infection rates in cancer and non-cancer patients could have been caused by differences in susceptibility for infection due to the underlying disease. However, although the most important indication for PAC use in non-cancer patients was immunotherapy in the form of infusion of gammaglobulin, this therapy was provided for neuromuscular disease in all

but one patient (who had a common variable immunodeficiency). As such, infection rates in non-cancer patients are not biased due to a large number of patients with primary immunodeficiency.

Although PACs are associated with much fewer infectious complications than other approaches to obtain prolonged access to the venous circulation, infection remains an issue of concern.[7,13] In clinical practice, the diagnosis of PAC-associated infection can be made with or without bacteriological confirmation.[14,15] In the present analysis we only included culture-proven infection: PAC-associated infection was defined as a positive culture of blood obtained from either a peripheral vein or the port and clinical suspicion of PAC infection as reflected by local symptoms or absence of another infectious source.[12] The incidence of PAC-associated infection amongst cancer patients found here (11.8%) is within the same range as that reported in previous studies: positive blood cultures associated with PACs have been reported to occur in 2.4 to 16.0% of patients,[3,4,11] representing a major cause of hospital-acquired bacteraemia and the most frequent reason for catheter removal.[4,16] The vast majority of PAC-associated infections were caused by coagulase-negative staphylococci and *Staphylococcus aureus*, which is in accordance with earlier investigations.[11,13]

There are no standard criteria for catheter removal in PACs.[12,13] In the presence of uncomplicated infection due to coagulase-negative staphylococci, the PAC may be retained if there is no evidence of persisting or relapsing bacteraemia. For PAC-associated infection caused by pathogens other than coagulase-negative staphylococci, some physicians would retain the port, partially depending on the patient's clinical status. In our analysis, most PAC-associated infections resulted in PAC removal in cancer patients (80% of cases), but not in patients without cancer (42%). This difference was not related to a clear difference in causative pathogens. It is conceivable that medical oncologists are reluctant to continue chemotherapy through a PAC that has been infected and that as a consequence PAC-associated infection more often leads to PAC removal in cancer patients.

The reported incidence of venous thrombosis as a PAC-associated complication varies between zero and 10%.[13] In our centre, thrombosis was never the cause of PAC removal during the six-year study period. Notably, since most cases of catheter-related thrombosis are asymptomatic,[13] this does not exclude that thrombosis did occur in our population. Data on prophylactic anticoagulant therapy are not available for the studied population, but this is not a routine policy in our hospital.

Several earlier investigations have examined the complication rate of PACs in a single-centre setting. No device-related deaths were observed and complications as infection and thrombosis were rare for all types of patients.[5,9,11] In a Dutch retrospective analysis encompassing a period of 7.5 years (1992 to 1999) involving 38 PACs, the most prevalent complications were infection (two cases or 5.3%) and thrombosis (three cases or 7.9%).[5] Although the number of PACs studied was relatively low, these data suggest that the incidence of PAC-associated thrombosis may have decreased in more recent years, probably at least in part as a result of better preventive care by the nursing staff.

Our study has several limitations. Firstly, the study has a low sample size relative to the low incidence of PAC-related problems, which in particular is true for thrombosis. Secondly, the study groups were not comparable with respect to baseline and prognostic variables, which may hamper appropriate comparisons.

The use of PACs is widely implemented in the clinical care of patients with cancer. These devices have a high acceptance among patients, nurses and doctors. The current analysis illustrates the low rate of complications associated with the use of PACs in the setting of a large community hospital in the Netherlands.

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PHOTO QUIZ

A patient with haemorrhagic bullae

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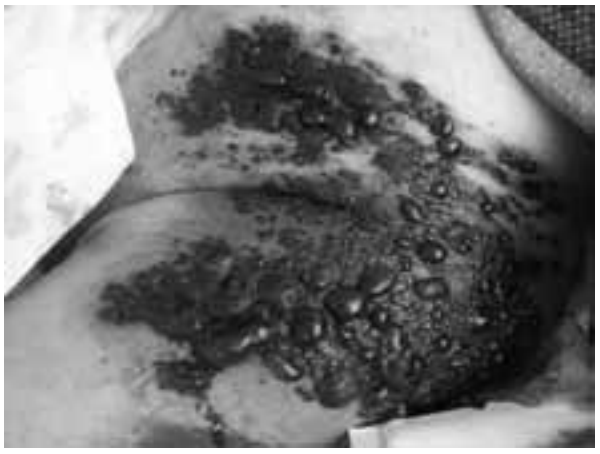
CASE REPORT

A 92-year-old man presented to our outpatient clinic with complaints of fatigue, muscle weakness and upper abdominal discomfort. There was no history of haematemesis, melaena, haematuria or epistaxis. In 2010, he was diagnosed with immune thrombocytopenic purpura (ITP) and was treated with prednisolone 1 mg/kg/day (80 mg/day) with good response. The dose was tapered down slowly without disease relapse. Three weeks prior to presentation, the patient had discontinued the prednisone.

Physical examination revealed purpura and petechiae, especially localised on the extremities. There was no lymphadenopathy or hepatosplenomegaly present. Laboratory findings showed a platelet count of less than 3 x 10^9/l. No other abnormal findings were noted. A bone marrow aspirate smear showed increased production of megakaryocytes without signs of dysplasia in all cell lines. The relapse of ITP was again treated with oral prednisolone 1 mg/kg/day, however, without effect. Also addition of intravenous immunoglobulin did not increase the platelet count. Because of the refractory character of the ITP we decided to give rituximab off-label. Five days after administration of the first dose of rituximab the patient developed haemorrhagic bullous lesions on the left hip (*figure 1*).

Figure 1. *Haemorrhagic bullae in the left groin region*



WHAT IS YOUR DIAGNOSIS?

See page 195 for the answer to the photo quiz.

Netherlands
The Journal of Medicine

CASE REPORT

An unexpected cause of multiple intra-abdominal abscesses in an HIV-positive patient

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ABSTRACT

This case report describes a female HIV-positive patient diagnosed with pelvic actinomycosis using 16S rRNA gene sequence analysis. Actinomycosis is notoriously difficult to diagnose by microbiological culture. 16S rRNA gene sequence analysis allows rapid definitive diagnosis of actinomycosis and is potentially of great value in a clinical setting. This is the first report of pelvic actinomycosis in an HIV-1 infected patient.

KEYWORDS

16s rRNA gene sequence analysis, actinomycosis, HIV-1 infection

INTRODUCTION

Actinomycosis is notoriously difficult to diagnose due to both its variety in clinical presentation and its challenging growth in microbiological culture. As microbiological culture leads to confirmation of the diagnosis in less than 50% of clinically suspected cases, the final diagnosis is often based solely on the distinctive Gram stain of *Actinomyces*.[1-3] Sequence analysis of the 16S ribosomal (r)RNA gene is another available method for the detection of *Actinomyces* species. This molecular technique allows earlier and improved diagnosis of actinomycosis without the difficulties encountered using traditional phenotypic methods.[4,5]

We present the case of a female human immunodeficiency virus 1 (HIV-1) infected patient with pelvic actinomycosis, diagnosed using 16S rRNA gene sequence analysis. We are the first to report pelvic actinomycosis in an HIV-1 infected patient.

CASE REPORT

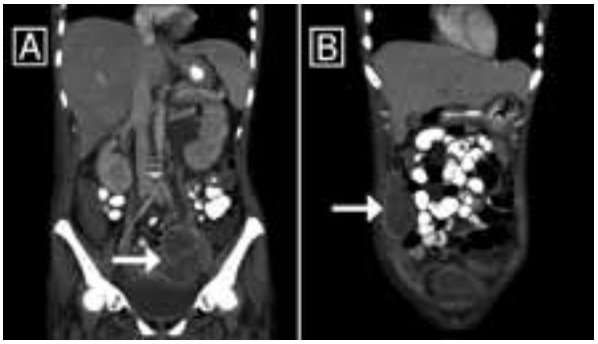
A woman in her early thirties, previously healthy, was admitted to our hospital with a three-month history of fatigue, intermittent fever, poor appetite with marked weight loss and a productive cough with white sputum. The patient's vital functions were normal. Physical examination revealed no abnormalities besides evident cachexia (body mass index 14.0) and mild lower abdominal pain with no palpable mass. Laboratory tests indicated leucocytosis (18.4 x 10^9/l), normochromic normocytic anaemia (Hb 3.5 mmol/l), hypoalbuminaemia (19 g/l), elevated C-reactive protein (139 mg/l) and impaired renal function (estimated creatinine clearance 64.0 ml/min). The chest radiograph showed no abnormalities. Urine analysis indicated leucocyturia. The HIV-antibody test was positive and the CD4 count was 170 x 10^6/l.

Treatment with intravenous (iv) ceftriaxone was initiated for a presumed urinary tract infection and due to the possibility of a *Pneumocystis* pneumonia iv co-trimoxazole was added at a therapeutic dose.

Both blood and urine cultures showed growth of an *E. coli* after which the antibiotics were switched to oral ciprofloxacin. Computed tomography (CT) of the chest showed no intrapulmonary abnormalities after which the co-trimoxazole was stopped. An abdominal ultrasound revealed an abscess-like lesion between the liver and right kidney and hydronephrosis of the left kidney. The CT abdomen revealed a left-sided tubo-ovarian abscess with compression of the left ureter and subsequent hydronephrosis, a large abscess of the abdominal wall and multiple smaller abscesses throughout the pelvis and abdomen (*figure 1*). Percutaneous nephrostomy was performed to relieve the left kidney and the patient's intra-uterine device (IUD) was removed.

The two largest abscesses were drained and their contents cultured. The Gram-stain revealed branched Gram-positive

Figure 1. *Computed tomography of the abdomen shows (A) a left-sided tubo-ovarian abscess (white arrow) measuring 5x5x7 cm with compression of the left ureter and subsequent hydronephrosis and (B) a right-sided abscess of the abdominal wall (white arrow) measuring 3x6x9 cm*



rods, suspicious of *Actinomyces*. Anaerobic culture showed growth of colonies compatible with *Actinomyces* spp. Definitive identification was achieved using 16S rRNA gene sequence analysis after which treatment with iv penicillin G (2 million U, four times a day) was initiated.
The patient's condition improved markedly. She was discharged after 15 days with an intravenous penicillin pump (12 million U/24 hours) for six weeks, followed by oral amoxicillin (500 mg, four times a day). On re-evaluation the CD4 count had increased to 380 x 10^6/l and initiation of antiretroviral therapy was postponed.

DISCUSSION

In this case, the patient presented with surprisingly mild and unspecific symptoms given the extent of her pelvic actinomycosis. This illustrates the variable clinical picture that actinomycosis can present and the delay in diagnosis this can cause.[6,7] Rapid reliable diagnostic testing such as 16S rRNA gene sequence analysis is an important tool in overcoming these difficulties.

Actinomycosis

Actinomycosis is a chronic granulomatous infection caused by anaerobic Gram-positive bacteria from the *Actinomyces* genus. These commensal inhabitants of the oral cavity and gastrointestinal tract cause infection after preceding mucosal disruption, resulting in the formation of multiple connecting abscesses.[2,8] *A. israelii* is the most common pathogen in humans.[2,8]
Due to the disease's variety in clinical presentation and invasive spread which is often mistaken for malignancy, the diagnosis is frequently missed making the overall incidence difficult to determine.[3,6] Estimates range from 1/40,000 to 1/119,000 cases per year.[8] Known risk factors include surgery, trauma and IUD use.
Actinomycosis usually involves the cervicofacial (50%), abdominal (20%) and thoracic (15%) regions.[3,8] Clinical symptoms depend on the site of infection and are frequently unspecific including (mild) pain, fatigue and intermittent fever.
Anaerobic culture is the preferred diagnostic test; however, this can be time-consuming and yields results in only 50% of clinically suspected cases.[1,3] The distinctive Gram stain is often the only indication of the diagnosis.[8] Typically, affected tissues produce pus with sulphur granules of 1 to 2 mm containing branched, Gram-positive filaments. Although these sulphur granules are commonly considered pathognomonic for the disease, they are only present in 50% of cases.[2,3] *Actinomycosis* therefore still poses a great diagnostic challenge when using traditional phenotypic methods.
Molecular genetic methods, such as 16S rRNA gene sequence analysis, bypass many of the problems encountered using traditional methods.[9] The 16S rRNA gene is the part of the genetic code most commonly used for the taxonomy of bacteria.[4] It contains both highly conserved genomic sequences and variable sequences which allow species differentiation. Sequence analysis of the 16S rRNA gene is an effective method for precise identification of bacteria which are otherwise rarely isolated.[4] 16S rRNA gene sequence analysis allows rapid definitive diagnosis of actinomycosis and identification of specific *Actinomyces* strains and can be of great value in the clinical setting.[5,10,11]
Actinomycosis requires long-term antibiotic therapy. The treatment of choice is high-dose iv penicillin G (10-20 million U/day) for four to six weeks followed by six to 12 months of oral penicillin or amoxicillin.[8]

Actinomycosis and HIV

Despite the impaired cellular and humoral immunity associated with HIV infection there is no increase in the incidence of actinomycosis in the HIV-positive population.[12] The sporadic examples available in the literature of HIV-positive patients with actinomycosis do indicate an increased prevalence of the atypical acute, invasive, ulcerative form of the disease.[12] Despite this observation, HIV infection does not appear to predispose an individual to actinomycosis.[12,13]

CONCLUSION

Actinomycosis remains notoriously difficult to diagnose due to its variety in clinical presentation and challenging growth in microbiological culture. Molecular genetic methods such as 16S rRNA gene sequence analysis allow

more rapid and accurate diagnosis of actinomycosis than traditional phenotypic methods and are potentially of great value in the clinical setting. Actinomycosis is rare in the HIV-positive population. There are no other reported cases of pelvic actinomycosis in an HIV-1 infected patient.

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PHOTO QUIZ

An unexpected cause of chest pain

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CASE REPORT

A 66-year-old man with a history of COPD and nicotine abuse visited our emergency department because of severe chest pain radiating to the left shoulder blade. The pain was continuous and started suddenly, approximately four hours before presentation. One week earlier, the patient underwent a gastroscopy and abdominal ultrasound because of upper abdominal pain, both without any abnormalities. At physical examination, blood pressure was 190/110 mmHg in both arms and the pulse was 77 beats/min. The patient had a respiratory rate of 20 breaths/min, normal oxygen saturation and temperature, and auscultation of heart, lungs, abdominal, and femoral arteries was normal. The pain could not be provoked by palpation. Chest X-ray and routine laboratory tests (including cardiac enzymes) were normal, except for a slight elevation of the inflammatory parameters (leucocyte count 12.4 x 10^9/l, and C-reactive protein 19 mg/l). ECG showed slight left ventricular hypertrophy. A contrast-enhanced chest-computed tomography (CT), performed to rule out pulmonary embolisms, showed an abnormal aortic wall (see figure page 196).

WHAT IS YOUR DIAGNOSIS?

See page 196 for the answer to this photo quiz.

Netherlands
The Journal of Medicine

PHOTO QUIZ

An unusual cause of ascites

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CASE REPORT

A 72-year-old women with an extensive medical history, including cervical carcinoma, rheumatoid arthritis, hypertension and COPD, was seen at the internal medicine outpatient clinic because of ascites. The day of the scheduled ascites puncture, she presented herself to the Emergency Room with nausea, vomiting and diarrhoea.
Ascitic fluid was obtained in which malignant cells were found. Histologically these were consistent with ovarian carcinoma. However, a tumour of the gastrointestinal system could not be excluded. We performed a computed tomography (CT) scan of the abdomen (*figure 1*) and thorax, which showed no signs of a gynaecological tumour or primary tumour of the gastrointestinal system. A vast amount of ascites and an omental cake were reported by the radiologist. We consulted a gynaecologist, who could not find a gynaecological tumour using transvaginal ultrasound.
We then tried to obtain histological material from the omental cake. Unfortunately the ultrasound-guided puncture failed because the lesion could not be recognised. In consultation with the gynaecologist it was decided to perform a laparoscopic exploration of the abdominal cavity. During the laparoscopy, no tumours of uterus or adnexes were observed. However, multiple miliary omental lesions and an appendiceal mass were reported. Biopsies were taken (*figure 2* shows the histology of the appendiceal mass).

Figure 1. *Abdominal CT-slides showing an abdominal cake and ascites*

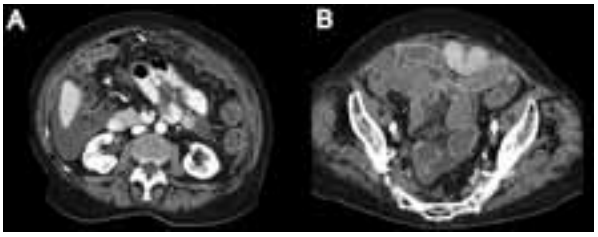
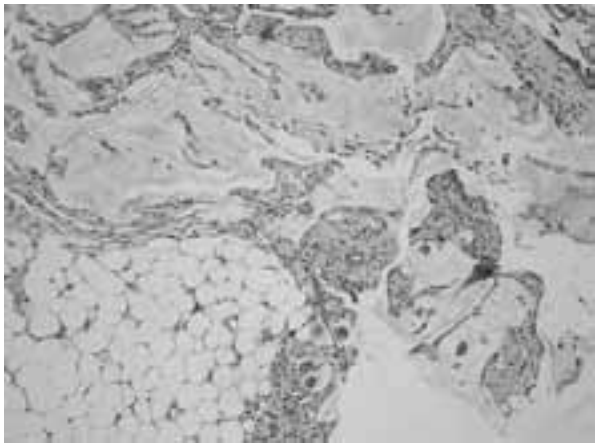


Figure 2. *Histologic material of the appendiceal mass*



WHAT IS YOUR DIAGNOSIS?

See page 197 for the answer to this photo quiz.

Netherlands
The Journal of Medicine

PHOTO QUIZ

A man with painless scrotal swelling

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CASE REPORT

A 65-year-old man with a medical history of obstructive sleep apnoea syndrome presented with complaints of diarrhoea, abdominal pain, nausea, vomiting and fever for one week. Also, he showed signs of biliary obstruction. Further investigation revealed the presence of *Campylobacter jejuni* gastroenteritis and choledocholithiasis with minimal jaundice. A day later the patient developed cholangitis for which an endoscopic retrograde cholangiography (ERCP) with precut papillotomy was performed. Midazolam was used for procedural sedation. However, the procedure was complicated by agitation despite higher doses of midazolam. Several attempts to cannulate the common bile duct were undertaken, but nevertheless unsuccessful. The next day, the patient experienced considerable painless scrotal swelling (*figure 1*). An abdominal X-ray was also performed (*figure 2*).

WHAT IS YOUR DIAGNOSIS?

See page 198 for the answer to this photo quiz.

Figure 1. *Extensive scrotal swelling*

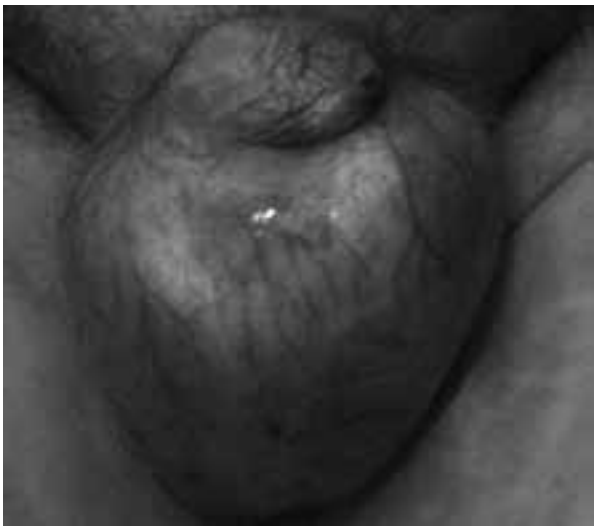
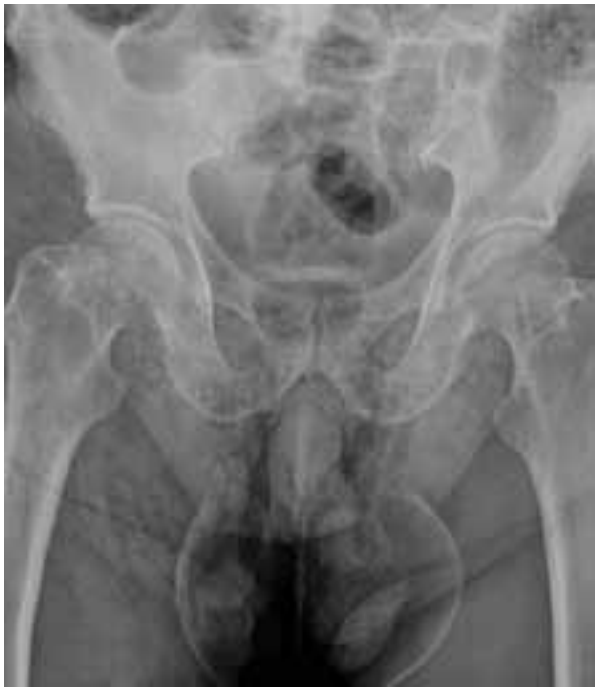


Figure 2. *Abdominal X-ray showing air within the retroperitoneum, as well as subcutaneous and scrotal emphysema*



ANSWER TO PHOTO QUIZ (PAGE 189)

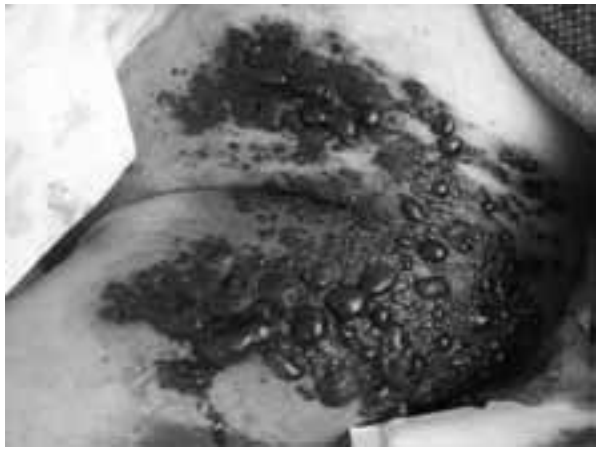
A PATIENT WITH HAEMORRHAGIC BULLAE

DIAGNOSIS

The affected dermatomal pattern on one side of the body, in conjunction with the immunosuppressive treatment, made us think of an opportunistic infection with varicella zoster virus (VZV). The haemorrhagic aspect was suggested to be caused by the underlying thrombocytopenia, which initially persisted despite therapy with rituximab. A polymerase chain reaction on the bullous fluid was positive for VZV DNA, indicating an active VZV infection.

ITP treatment with corticosteroids results in a complete response in 20% of adult patients. Most of the patients, however, will require a second-line treatment. For the clinical management of ITP we refer to a recent paper in this journal.[1] Rituximab, a chimeric anti-CD20 monoclonal antibody, depletes CD20+ B-cells which results in low or even undetectable levels for two to six months, returning to pre-treatment levels within a year. Rituximab also induces complement-mediated cytotoxicity and dysfunction of CD4 T cells leading to abnormal cytotoxic T-cell-mediated responses.[2] The above predisposes the patient to opportunistic infections. Data from 356 patients receiving rituximab monotherapy showed a 30% incidence rate of infectious events; 19% of patients had a bacterial infection, 10% viral infections, 1% fungal infections and 6% infections of unknown aetiology.[3] One randomised controlled trial showed opportunistic viral infections which included three dermatomal herpes zoster infections and four localised herpes simplex infections. Isolated cases of other viral infections have been associated with rituximab, such as cytomegalovirus, West Nile virus, JC virus, BK virus and parvovirus.[4]

Figure 1. *Haemorrhagic bullae in the left groin region*



Our patient was treated with valaciclovir 1000 mg twice a day. He responded very well to drug therapy and there was a major reduction of the bullae and herpes zoster rash. Additionally, after the third rituximab infusion, a significant increase in the platelet count to 70 x 10^9 cells/l was seen.

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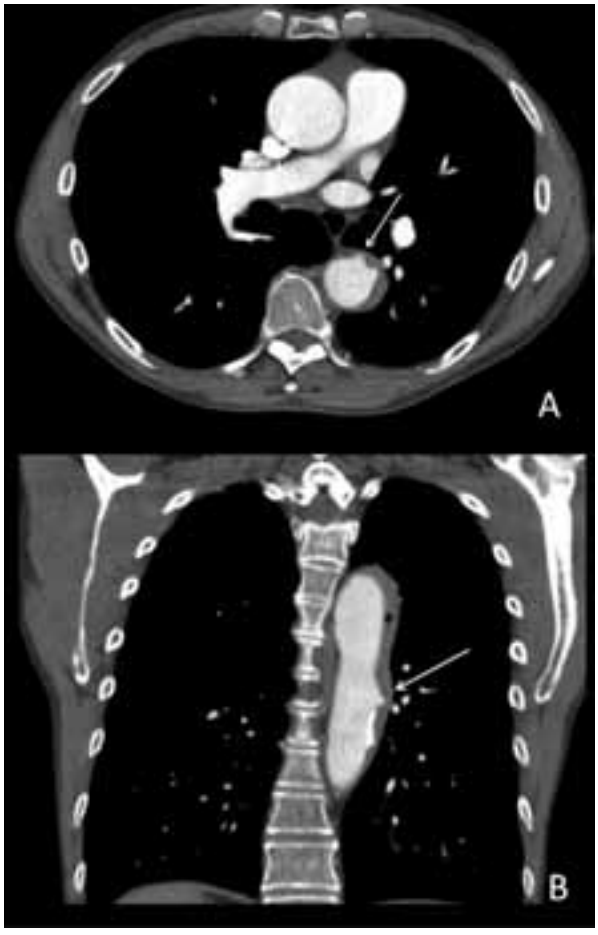
ANSWER TO PHOTO QUIZ (PAGE 192)

AN UNEXPECTED CAUSE OF CHEST PAIN

DIAGNOSIS

The chest CT shows a deep atheromatous ulcer in the descending aorta. A small intramural haematoma was also observed throughout the complete thoracic aorta (thickened aortic wall with slightly increased Hounsefield units). No evidence was found for aortic dissection or pulmonary embolism. Thus, the diagnosis was penetrating aortic ulcer (PAU), which is an ulceration of an atherosclerotic lesion leading to a disruption of the internal elastic lamina of the aortic wall and, subsequently, the development of an intramural haematoma.[1] Diagnosing PAU may be challenging as chest pain has a wide range of differential diagnoses. Sudden onset of severe chest pain in the elderly hypertensive patient is the classical presentation, although symptoms vary greatly.[2] PAU is often overlooked due to its low incidence and the fact that an aortic dissection is considered unlikely if the blood pressure is equal in both arms and vascular murmurs are absent. Unfortunately, other acute aortic syndromes besides aortic dissection, such as PAU or intramural haematoma, are often forgotten. The diagnosis is made by imaging studies, mostly by contrast-enhanced chest-CT.[3] Since PAU has a high risk for progression into a fatal rupture of the aorta, emergent treatment is required. Both invasive (surgical or endovascular) and conservative treatments are considered appropriate.[4] Our patient was treated conservatively (as a 'hypertensive emergency') with labetalol intravenously. His blood pressure decreased quickly and the pain dissolved. Six months later, the blood pressure was normal (using four antihypertensive drugs) and the patient no longer complained of pain. Control CT scans after 24 hours, seven days and six months showed no further progression of the aortic ulcer.

Figure 1. *Contrast-enhanced CT of the thorax in the transverse imaging plane (A) and reconstructed coronal imaging plane (B) shows a thickened aortic wall with slightly elevated Hounsfield units, suggestive of intramural haematoma (black asterisk). The contrast-filled lumen expands within the aortic wall, which is visible in multiple reconstructed imaging planes with vascular calcifications on the luminal side of the aorta (therefore not compatible with thrombus), suggestive of the presence of a penetrating aortic ulcer (arrow). No dissection flap of the aorta was observed*



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ANSWER TO PHOTO QUIZ (PAGE 193)

AN UNUSUAL CAUSE OF ASCITES

DIAGNOSIS

Histology shows a mucoid adenocarcinoma with signet cell differentiation. In combination with the clinical findings we made the diagnosis of metastasised mucoid adenocarcinoma of the appendix. Interestingly, the CT scan showed an omental cake (*figure 1A*), but nothing of interest in the appendiceal area (*figure 1B*). This could be due to the fact that the carcinoma has a signet ring differentiation.

Multiple histological types of appendiceal carcinomas are known, of which signet ring differentiation is the smallest group.[1,2] In signet cells the cell is filled with mucus, leading to an outward position of the nucleus. This type of cancer is exceedingly rare. Dutch numbers are lacking, but extrapolating from US data,[3] a case of appendiceal adenocarcinoma with signet ring differentiation is only seen once every five years in the Netherlands.

The condition is usually an unexpected finding during surgery for another indication. However, 80% of cases present with abdominal pain or acute appendicitis.[2] Other symptoms are a bloated feeling or an abdominal mass. If a physician suspects an appendiceal carcinoma based on the patient's symptoms, regular imaging techniques can be used.

The treatment of choice is a right hemicolectomy.[4] If the condition of the patient does not allow an operation, or when (inoperable) metastases are present, the physician should consider chemotherapy. The prognosis of primary carcinoma of the appendix is dependent on the stage of the disease.[3] Carcinoma with signet ring differentiation tends to have a poorer prognosis because of very early dissemination to the peritoneum,[3] which is probably the reason why it did not show up on the CT scan. Physicians should always keep this type of tumour in mind when malignant cells are found in ascitic fluid without obvious lesions on imaging techniques.

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ANSWER TO PHOTO QUIZ (PAGE 194)

A MAN WITH PAINLESS SCROTAL SWELLING

DIAGNOSIS

Because the first ERCP was unsuccessful, the procedure was repeated a day later, but this time in the operation room with adequate anaesthesia. Surprisingly, cholangiography revealed a fausse route with a duodenal perforation. The abdominal X-ray showed air within the retroperitoneum and subcutaneous, as well as inguinal and scrotal emphysema (*figure 2*). Abdominal computed tomography scan confirmed the diagnosis of scrotal emphysema (pneumoscrotum) related to retroperitoneal perforation due to a fausse route during ERCP. The retroperitoneal perforation as well as the pneumoscrotum resolved with conservative measures, including nasogastric decompression, antibiotics and intravenous fluids.

ERCP is a commonly used and well-tolerated procedure with low overall complication risk (1 to 5%) and mortality rate (0.2 to 0.5%).[1] Possible complications are, among others, pancreatitis, bleeding, infection, cardiopulmonary events and perforation. In our case the patient remained restless despite sedation, potentially increasing the complication risk. So, adequate sedation is essential during procedures such as ERCP. The risk of perforation is minimal (<0.05%) occurring in 0.2 to 0.6% of ERCP cases and originating from several anatomic sites such as the retroperitoneum.[1] Retroperitoneal perforations can cause subcutaneous emphysema, pneumomediastinum, pneumothorax and to lesser extent pneumoscrotum.[2] Pneumoscrotum develops in cases if air dissects down from the retroperitoneum through the anatomic connections between the retroperitoneum, fascial planes of the abdominopelvic cavity, inguinal canal and finally into the scrotal sac.[2] In case of retroperitoneal perforation conservative management with broad-spectrum antibiotics, serial re-evaluations, decompression of the biliary tract, stomach and duodenum, is successful in most patients and approved for the initial treatment.[1,3,4] Surgery should be considered with the co-existence of peritoneal signs (guarding, rebound tenderness), significant duodenal perforation, sepsis or failed conservative treatment.[1,3,4] In our case, conservative management was successful and the patient recovered without life-threatening complications. However, because of their variable course of disease, these perforations are treacherous and can cause morbidity and mortality as well.[5]

When a patient develops a pneumoscrotum after ERCP or another endoscopic intervention, a procedure-related perforation should be considered.

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Netherlands
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SPECIAL ARTICLE

Dutch guidelines for diagnosis and therapy of proliferative lupus nephritis

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ABSTRACT

Proliferative lupus nephritis is a strong predictor of morbidity and mortality in patients with systemic lupus erythematosus. Despite improvements in the management of lupus nephritis, a significant number of the patients do not respond to immunosuppressive therapy and progress to end-stage renal failure. In order to optimise the diagnostic strategy and treatment of patients with proliferative lupus nephritis, guidelines are needed.

In this review, the Dutch Working Party on Systemic Lupus Erythematosus provides recommendations regarding four important areas in patients with proliferative lupus nephritis: I) indications for a first renal biopsy, II) definitions of treatment response, III) selection of treatment options, and IV) indications for a repeat biopsy.

KEYWORDS

Azathioprine, cyclophosphamide, mycophenolate mofetil, proliferative lupus nephritis

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disorder characterised by the production of auto-antibodies most prominent against nuclear antigens. Antibodies against nucleosomes and double-stranded DNA have a central role in the pathogenesis of the disease.[1] The systemic character of SLE is illustrated by the fact that all kinds of tissues and/or organs may be involved in this disease.[2]

In Europe, the incidence of SLE is estimated at 3.3 to 5.0 per 100,000 persons and the prevalence at 25.4 to 91.0 per 100,000 persons.[3] Most patients are women of childbearing potential. Lupus nephritis (LN) occurs in up to 50 to 75% of SLE patients during the course of the disease.[4,5] The incidence of kidney involvement differs with ethnicity: a higher incidence of LN has been reported among Black, Hispanic and Asian patients compared with Caucasian patients.[3,6] Although the clinical presentation may vary among patients, proliferative LN is a major cause of morbidity and mortality.[7,8] Progression into end-stage renal disease (ESRD) despite aggressive immunosuppressive therapy does occur.[9-11]

To date, no guidelines on how to manage patients with proliferative LN (ISN/RPS class III and IV) are available in the Netherlands although European guidelines have been published[12], and international (KDIGO) and US (American College of Rheumatology) guidelines are currently being developed. The Dutch Working Party on SLE has addressed this issue and developed recommendations based on opinions from expert panel meetings with nephrologists, rheumatologists and clinical immunologists, and a critical review of the present literature. A systemic search of the PubMed database was performed (1975 to January 2012), and all English language publications were considered. The following search terms were used: SLE, (refractory) LN, azathioprine, cyclophosphamide,

prednisone, mycophenolate mofetil (MMF), rituximab, hydroxychloroquine, renal biopsy, repeat biopsy, antiphospholipid syndrome nephropathy, induction treatment, maintenance treatment, and response.

The strength of evidence was graded using the following classification: Level A evidence represents data derived from multiple randomised controlled trials (RCT) or a meta-analysis; Level B from a single RCT or a non-randomised study; Level C from expert opinion.

In this article, we present recommendations regarding four important areas in the care of patients with proliferative LN: I) indications for a first renal biopsy, II) definitions of treatment response, III) selection of treatment options, and IV) indications for a repeat biopsy.

Table 1. *Abbreviated International Society of Nephrology/ Renal Pathology Society (ISN/RPS) classification of lupus nephritis 2003*[13]

Class I	Minimal mesangial lupus nephritis
Class II	Mesangial proliferative lupus nephritis
Class III	Focal proliferative lupus nephritis (involving <50% of all glomeruli)
Class IV	Diffuse proliferative lupus nephritis[a,b] (involving ≥50% of all glomeruli) *Segmental lesions:* IV-S (involving <50% of the glomerular tuft) *Global lesions:* IV-G (involving ≥50% of the glomerular tuft)
Class V	Membranous lupus nephritis[c]
Class VI	Advanced sclerosing lupus nephritis without active lesions

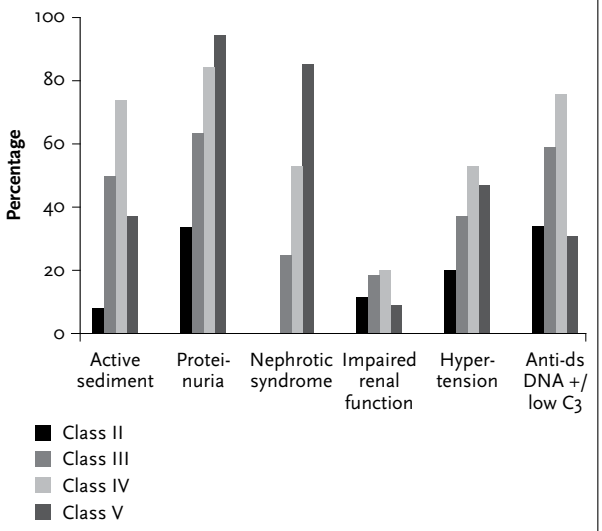
[a]Indicates the presence of active (A), active and chronic (A/C) and chronic (C) lesions; [b]Indicates the proportion of glomeruli with fibrinoid necrosis and cellular crescents; [c]Class V may occur in combination with class III or IV, in which case both will be diagnosed.

INDICATIONS FOR A FIRST RENAL BIOPSY IN PATIENTS WITH SLE

The occurrence of LN should be considered in any SLE patient with a recent onset of impaired kidney function, proteinuria and/or microscopic haematuria (≥5 red cells per high-power field). However, as these clinical features do not permit a reliable prediction of the class of LN (*figure 1*), the diagnosis must be confirmed by kidney biopsy, since this can have clinical consequences on treatment decisions.[2] Six classes of LN are distinguished in the current classification of the International Society of Nephrology and the Renal Pathology Society (ISN/RPS) (*table 1*).[13] These histological findings provide the basis for treatment recommendations. Based on panel discussions, the Dutch Working Party formulated guidelines (as stated in *figure 2)* on when to perform a first renal biopsy in patients with SLE.

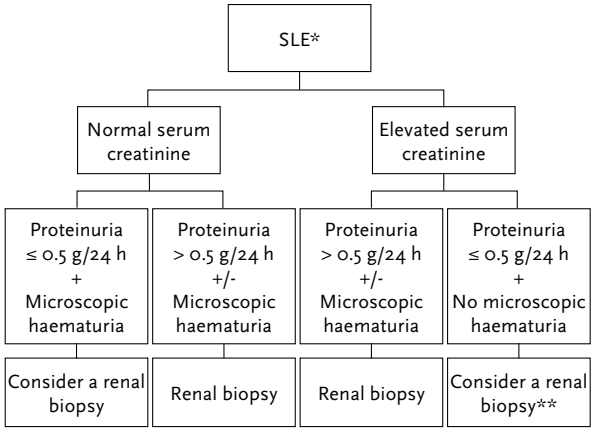
Although clinically silent proliferative LN occurs in a substantial proportion of patients, it is generally accepted to decide not to perform a renal biopsy in SLE patients who have a normal renal function, no haematuria and <0.5 g/24 hours of proteinuria (Level C).[14] In such patients renal parameters should be monitored carefully. In SLE patients presenting with >0.5 g/24 hours of proteinuria, after exclusion of other causes a renal biopsy is indicated, independent of the presence of microscopic haematuria

Figure 1. *Incidence of clinical symptoms in various forms of lupus nephritis*[19]



Lupus nephritis, based on the 1995 classification published under the auspices of the World Health Organization.[80]

Figure 2. *Indications to perform a first renal biopsy in patients with systemic lupus erythematosus*



***Systemic lupus erythematosus: at least 4 ACR criteria positive; **Consider a renal biopsy when either i) a persistent elevation of serum creatinine >30%, ii) other causes of renal impairment are excluded, iii) positive anti-phospholipid antibodies, iv) extra-renal involvement/presence of anti-dsDNA antibodies/ hypocomplementaemia.**

and/or an increase in serum creatinine (Level C). These patients may have focal or diffuse proliferative glomerulonephritis, or membranous lupus.

In SLE patients with microscopic haematuria in the absence of an increase in serum creatinine or proteinuria it is not clear whether a renal biopsy should be performed. Although prompt diagnosis after the onset of LN and subsequent initiation of appropriate therapy are associated with improved outcomes, persistent isolated microscopic haematuria has not been associated with a negative outcome so far and warrants close monitoring of other renal parameters (Level C).[15,16]

An increase in serum creatinine may implicate a proliferative LN. However, is it possible that these patients present without microscopic haematuria or proteinuria? Since clinical features do not permit a reliable prediction of the class of LN, the Dutch Working Party came to an opinion-based agreement that in this setting a biopsy should be considered when the observed increase in serum creatinine is persistent over several weeks and is >30%, together with the presence of either I) extra-renal lupus manifestations and/or serological activity and/or II) the presence of anti-phospholipid antibodies.[17-20] Moreover, in the absence of an obvious extra-renal explanation for deteriorating renal function a kidney biopsy may be warranted to exclude renal pathology other than LN, including a tubulo-interstitial nephritis, vascular disease (e.g. thrombotic microangiopathy or vasculitis), diabetes or drug-induced nephrotoxicity (Level C).

DEFINITIONS OF TREATMENT RESPONSE IN LN

Standard definitions of treatment response have been assessed in proliferative LN.[21-23] However, no single initial renal parameter has been validated as a marker for determining response.[12,23] Nonetheless, changes in renal function have been associated with renal outcome in several studies. In the National Institutes of Health (NIH) trials comparing prednisone, azathioprine and cyclophosphamide, doubling of serum creatinine was associated with the development of renal insufficiency.[24,25] Moreover, in the Euro-Lupus Nephritis Trial a decrease of an initially elevated serum creatinine and/or decrease in proteinuria to <1 g/24 hours at six months were powerful predictors for improved long-term renal outcome.[26] A recent trial conducted by the Collaborative Study Group demonstrated that even patients with a partial response (a ≤25% increase in baseline creatinine and ≥50% reduction in baseline proteinuria to ≤1.5 g/24 hours [but >0.33 g/24 hours] within five years of entering the study) had a significantly better renal survival than patients who did not retain a response, but not as good as in patients with complete response (serum creatinine of ≤1.4 mg/dl [98 mmol/l] and proteinuria ≤0.33 g/24 hours within five years of entering the study).[27] Moreover, the choice of time-point used to address response differs in clinical studies. In the above-mentioned study, the time for attaining a complete response was significantly longer than that required to attain a partial response (median: 10.5 *vs* 5.8 months). These results are consistent with the results of other reports.[28,29] On the basis of these observations, it is comprehensible that studies with only six months of follow-up report a relatively low percentage of complete response rates.

Based on the available literature, the Dutch Working Party assigned the following definitions of response as a guide to the success of therapy (Level C):

A *complete response* includes no disease activity, i.e. proteinuria <0.5 g/24 hours, and/or a serum creatinine within 125% of the baseline value at 6 to 12 months after the start of induction therapy.

A *partial response* is defined as an improvement not sufficient for the definition of a complete response, i.e. a reduction of proteinuria of >50% (and at least <3 g/24 hours), and a serum creatinine within 125% of the baseline value at six to 12 months after the start of the induction therapy.

A *failure* of the initial induction therapy has been defined as a doubling of serum creatinine compared with the baseline value at three months after the start of induction therapy.

A *flare* is an increase in disease activity that requires intensification of the therapy and is defined as an increase of ≥25% in the lowest serum creatinine level measured during the period of induction therapy and/or the development of either a nephrotic syndrome (proteinuria >3.5 g/24 hours and serum albumin <30 g/l), while the lowest protein excretion so far has been ≤2.0 g/24 hours repeatedly, or proteinuria >1.5 g/24 hours in a previous non-proteinuric patient.

Refractory LN includes persistent or worsening renal disease activity as manifested by progressive deterioration of renal function and/or proteinuria despite optimal immunosuppressive therapy and supportive treatment, and involving at least one of the following conditions: I) failure of the initial induction treatment at three months, for which a switch to another induction therapy regime has already been carried out; II) intolerance for cyclophosphamide and mycophenolate mofetil (MMF); III) exceeding a cumulative dose of 15 gram of cyclophosphamide, IV) a second relapse within two years after start of the initial induction therapy, and V) a relative contraindication for high-dose oral or intravenous (iv) prednisone, such as avascular osteonecrosis, previous psychosis on corticosteroids, osteoporosis and/or severe obesity (BMI ≥35 kg/m^2).

TREATMENT OF LN

Induction treatment

Cyclophosphamide-containing regiments have long been considered the gold standard in inducing renal remission and preventing renal flare in patients with proliferative LN.[25,30,31] However, treatment-related toxicity raised a number of concerns.[32,33] Furthermore, while cyclophosphamide induces renal remission in a significant proportion of patients with proliferative LN, the rate of relapse is considerable.[34] In order to reduce the toxicity but not the efficacy, alternative treatment regimens have been evaluated in recent years.

In the Euro-Lupus Nephritis Trial, 90 (mainly Caucasian) patients were randomised to high-dose iv cyclophosphamide (500-750 mg/m^2 six pulses monthly, followed by two pulses tri-monthly) or low-dose iv cyclophosphamide (500 mg fixed dose, six pulses every two weeks) in combination with methylprednisolone (three days, 750 mg) followed by oral prednisone (0.5 to 1.0 mg/kg).[9,35] Following the cyclophosphamide pulses, oral azathioprine (2 mg/kg) was introduced in both treatment arms. After ten years of follow-up, no significant differences were found between the low-dose and high-dose arms with regard to survival, ESRD or doubling of serum creatinine. These data show that the 'Euro-Lupus regimen' achieves good clinical results in the long-term in an European (mainly Caucasian) population with moderately severe disease, and seems to be a good alternative for the high-dose NIH cyclophosphamide regimen, while a considerably lower cumulative dose of cyclophosphamide is given. However, it should be noticed that in the low-dose arm additional cyclophosphamide was necessary during follow-up, increasing the cumulative dose from 3.0 to 5.5 gram.

The first Dutch Lupus Nephritis Study was initiated to analyse the effect of induction therapy with either pulse iv cyclophosphamide or azathioprine combined with methylprednisolone in patients with proliferative LN.[36] In this study, cyclophosphamide was superior to azathioprine in terms of preventing renal relapse and progression of chronic lesions in repeat biopsies at 24 months. The long-term follow-up data of this study confirmed the superiority of cyclophosphamide in the prevention of renal relapses, but sustained doubling of serum creatinine, ESRD, mortality, and renal function did not differ between the two treatment groups after a median follow-up of 9.6 years.[37] These results indicate that azathioprine can not be considered to be the standard induction therapy in patients with proliferative LN and should be reserved for those patients with a strong wish to conceive and with a high risk of premature ovarian failure, who are willing to accept the higher risk of exacerbations.

The benefits of MMF for LN were first reported in uncontrolled studies of patients refractory to cyclophosphamide.[38,39] Subsequently, relatively small randomised controlled trials have been performed.[40-43] The Ginzler study, a non-inferiority RCT, demonstrated that MMF (initial dose 1 g/day, increased to 3 g/day) was significantly better in inducing complete remission (CR) at 24 weeks than the NIH-cyclophosphamide regimen (CR 22.5% *vs* 5.8% respectively).[44] In this study, 56% of the patients were Black.

In view of the small size of the MMF trials, several meta-analyses of RCTs comparing induction therapy with MMF or cyclophosphamide have been performed. The results of these analyses show that MMF appears to be superior to cyclophosphamide in terms of both response and safety (*table 2*).[45-49] However, the results of these meta-analyses should be interpreted with caution, because of the inclusion of relatively small trials, the heterogeneity for race/ethnicity, class of LN, definitions of clinical response, duration of follow-up, and MMF and cyclophosphamide dosing regimens.

Recently, the results of the Aspreva Lupus Management Study (ALMS) were reported.[50] In this superiority RCT, 370 patients with either class III, IV or V LN were randomised

Table 2. *Induction treatment: mycophenolate mofetil versus cyclophosphamide (RR or relative benefit; 95% CI)*

	Mak et al.*	Naveneethan et al.*	Walsh et al.*	Zhu et al.*	Kamanamool et al.**
PR	-	1.07 (0.72-1.60)	-	1.06 (0.71-1.59)	-
CR	-	1.36 (0.82-2.24)	-	1.81 (0.70-4.68)	1.60 (0.87-2.93)
PR/CR	1.05 (0.95-1.17)	1.15 (0.86-1.54)	-	1.20 (0.85-1.69)	1.20 (0.97-1.48)
Treatment failure	-	-	0.70 (0.54-0.90)#	-	-
ESRD	0.45 (0.18-1.12)	0.66 (0.25-1.70)	-	0.58 (0.20-1.65)	-
Death	0.71 (0.37-1.35)	0.35 (0.14-0.86)#	-	0.46 (0.17-1.30)	-
ESRD/ Death	-	-	0.44 (0.23-0.87)#	-	-
Relapse	-	-	-	-	-

***RR <1 in favour of mycophenolate mofetil; **RR >1 in favour of mycophenolate mofetil; #p<0.05 in favour of mycophenolate mofetil; RR=relative risk; CI=confidence interval; PR=partial remission; CR=complete remission; ESRD=end-stage renal disease.**

to MMF (target 3 g/day) or iv cyclophosphamide (target 0.5 to 1.0 g/m^2, six pulses monthly). Although most patients in both treatment groups experienced clinical improvement, MMF was not superior in inducing complete response at 24 weeks (MMF 56.2% and cyclophosphamide 53.0%). In addition, significant differences were not observed with regard to the rates of serious adverse events (MMF 28.0% and cyclophosphamide 23.0%) or infections (MMF 69.0% and cyclophosphamide 62.0%).

In this study, a heterogeneous population in terms of race and ethnicity was included. A subgroup analysis suggested a significantly worse response for cyclophosphamide in non-Asian, non-Caucasian mainly Black patients (MMF 60.4% *vs* cyclophosphamide 38.5%).[51] These findings seem consistent with the results of the Ginzler study where a greater proportion of Black patients were included than in the ALMS study (61.0% *vs* 25.9%).[44] So far, although MMF seems to be superior to cyclophosphamide in the high-risk Black patients, the efficacy of MMF in patients with other ethnicities seems to be comparable with cyclophosphamide.

Taking these studies together, although long-term data are not available, MMF seems to be a reasonable treatment alternative to high-dose iv cyclophosphamide in LN.

As only 60% of the patients with proliferative LN obtain a partial or complete response at 6 to 12 months in the studies discussed so far, new immunosuppressive therapies have been instituted. Given the substantial evidence for the role of B cells in the pathogenesis of SLE and the recent development of monoclonal antibodies to B-lymphocyte-specific targets, B-cell depletion seems to be an attractive approach in LN treatment. Several small, open-label uncontrolled studies suggested that rituximab may be effective in proliferative LN as initial induction therapy.[52-54] However, in contrast to these studies, two randomised, controlled trials did not show any additional significant effect of anti-CD20 as add-on therapy in patients with LN treated with MMF and corticosteroids.[55,56] Therefore, the use of rituximab as a first-line adjunctive agent in induction therapy is not justified (Level A).

Based on the results of the available literature, the Dutch Working Party proposes induction treatment in patients with proliferative LN with either the low-dose cyclophosphamide Euro-lupus regimen or MMF together with (methyl)prednisolone (Level A), as outlined in these protocols (*tables 3 and 4*).

In patients who do not meet the response criteria for partial/complete remission after 12 months of induction treatment or if induction treatment fails at three months, switch of the immunosuppressive agent from either cyclophosphamide to MMF, or from MMF to cyclophosphamide, accompanied by iv methylprednisolone (750 mg) for three days is recommended (Level C).

Table 3. *Induction treatment: mycophenolate mofetil*[50]

Mycophenolate mofetil
Week 1: 1000 mg/day
Week 2: 2000 mg/day
Week 3: 3000 mg/day
Corticosteroids
Prednisone 1 mg/kg/day, maximum 60 mg/day
After 4 weeks prednisone tapered every 4 weeks by 10 mg to 20 mg, followed by prednisone tapered every 4 weeks with 5 mg to 10 mg

Table 4. *Induction treatment: cyclophosphamide*[35]

Cyclophosphamide
A fixed dose of 500 mg iv, 6 times every two weeks
Corticosteroids
Methylprednisone pulse 750 mg iv at day 0, 1 and 2, followed by prednisone 0.5-1.0 mg/kg/day
After 4 weeks prednisone tapered every 2 weeks with 2.5 mg to 5-7.5 mg at 30 months

MAINTENANCE TREATMENT

Immunosuppressive treatment

MMF has been compared with azathioprine or tri-monthly iv cyclophosphamide as maintenance therapy in a small randomised controlled trial in non-Caucasian patients, following induction therapy with cyclophosphamide and corticosteroids. This trial showed that both MMF and azathioprine were significantly better in terms of patient survival, incidence of clinical events (death or chronic kidney failure) and prevention of relapses, if compared with cyclophosphamide.[57] However, differences between MMF and azathioprine could not be assessed due to the small number of patients included in these arms. Furthermore, it should be noted that the death rate in the cyclophosphamide arm was higher than that observed in other (NIH) studies.

Recently, two randomised, controlled trials with different study designs have been conducted to assess the optimal maintenance treatment in proliferative LN. In the MAINTAIN Nephritis Trial, MMF (2 g/day) was compared with azathioprine (2 mg/kg/day) as maintenance treatment after induction treatment with low-dose iv cyclophosphamide (Euro-Lupus regimen).[58] MMF and azathioprine were equally effective in preventing renal flares. In this study, patients were randomised at the start of the induction treatment.

Recently, data from the ALMS Maintenance Trial were published.[59] In contrast to the MAINTAIN Nephritis Trial, only patients achieving partial or complete remission during a six-month induction phase were re-randomised to corticosteroids plus MMF (2 g/day) or azathioprine (2 mg/kg/day) for up to 36 months. In this study, MMF was

superior to azathioprine in delaying the time to treatment failure, which was defined as either renal flare, necessity of rescue therapy, doubling of serum creatinine, ESRD or death (16.4% *vs* 32.4%). The completion rate at 36 months was higher in the MMF group compared with the azathioprine group (62.9% *vs* 48.6%). Superiority of MMF was consistent regardless of type of induction treatment, race or region. The discrepancy in the results between the MAINTAIN and the maintenance phase of the ALMS trial can have several explanations, such as the number of and the difference in ethnicity of the patients included in both studies, a different trial design and differences in study endpoints. Moreover, the randomisation procedure in the ALMS Maintenance Trial selected those patients with a good clinical response. As indicated before, a considerable proportion of patients do not show such a favourable response at six months.

Based on the above-mentioned studies, MMF is superior to azathioprine in maintaining a renal response and in preventing a renal flare in patients who had a response to induction therapy (Level A).

Duration of therapy

It is difficult to precisely define the criteria that allow the identification of patients in whom the dose of immunosuppression can be reduced safely. If the disease is clinically and serologically quiescent the immunosuppression could be tapered slowly. Based on the study by Grootscholten *et al*. duration of therapy of at least five years seems warranted.[60] In this context, the ten-year follow-up data of the Euro-Lupus Nephritis Trial showed that 53% of the patients were still on maintenance immunosuppressive therapy.[9] The Dutch Working Party proposes the following reduction schedule as a guidance in clinical practice (Level C): taper the dose of prednisone to 10 mg every other day at four years after the start of the induction therapy, followed by a 50% dose reduction of azathioprine/MMF six months later and continue this treatment regimen for at least two more years.[37] After this period (6.5 years), the decision to stop immunosuppressive treatment will be left to the discretion of the treating physician and the patient. This advice differs from the tapering schedule as proposed in the ALMS and MAINTAIN trial. In the ALMS trial the dose of corticosteroids was maximally 10 mg until 36 months with no data after 36 months. In the MAINTAIN trial prednisone was dosed at 7.5 mg at six months, 5 mg at 12 months, with further tapering after 24 months.[58,59] There are no data available from controlled studies allowing clearer advice.

Supportive treatment

The importance of concomitant immune modulation with hydroxychloroquine has been highlighted by several recently published studies demonstrating lower rates of renal flare, ESRD and mortality in those patients taking hydroxychloroquine.[61-64] Therefore, unless there are contraindications, the consensus opinion is that all patients should receive hydroxychloroquine (200 to 400 mg) from the start of the induction therapy onwards (Level B). To detect retinal toxicity a baseline examination within the first year of use and an annual screening after five years of use should be performed by an ophthalmologist. For patients with maculopathy or additional risk factors for retinal toxicity (cumulative dose of hydroxychloroquine >1000 g, elderly, kidney and/or liver dysfunction) annual screening should be performed from the initiation of the therapy.[65]

In patients with LN, the indication for supportive treatment depends on the stage of chronic kidney disease and the presence of proteinuria. In general, the strategy aims at reduction of cardiovascular risk factors and should comprise lifestyle modifications (smoking cessation, weight reduction if BMI >25 kg/m^2, increased physical activity and dietary changes, especially salt restriction) together with adequate control of blood pressure (target of <130/80 mmHg, Level A for proteinuria >1 g/24 hours) with angiotensin inhibitors (ACEi) or angiotensin receptor blockers (ARBs) (Level A for proteinuria >1 g/24 hours), and treatment of hyperlipidaemia (Level C). As for stage 3 to 5 chronic kidney disease (creatinine clearance <60 ml/min), treatment options are summarised in *table* 5.[66]

To reduce the risk of corticosteroid-induced osteoporosis each patient should receive calcium and vitamin D supplementation. In addition, a bisphosphonate should be started in each patient receiving >15 mg/day prednisone, or in postmenopausal women and males >70 years of age using prednisone in a dosage of 7.5 to 15 mg/day (see CBO Consensus Osteoporosis 2011).[67] However, in patients with renal failure (creatinine clearance <60 ml/min) and in patients with a pregnancy wish, bisphosphonates should not be given.[67] In addition to the supportive treatment options mentioned above,

Table 5. *Supportive treatment in chronic kidney disease stage 3-5*[66]

Blood pressure
Dietary sodium reduction (5.0 g sodium chloride)
Achieve blood pressure <130/80 mmHg, using an ACEi or ARB as first-line treatment in the presence of >1 g/day of proteinuria
Proteinuria
Achieve proteinuria <1 g/day, using an ACEi or ARB as first-line treatment
Low protein diet (0.8 g/kg body weight per day)
Lipids
Achieve LDL cholesterol <2.6 mmol/l, using statins as first-line treatment

ACEi = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; LDL = low-density lipoprotein.

low-dose acetylsalicylic acid seems warranted in patients with positive anti-phospholipid antibodies for primary prevention of thrombosis and pregnancy loss (Level C).[12] Moreover, coumarines should be considered in patients with a nephrotic syndrome and a serum albumin <20 g/l (Level C).[68]

Treatment of refractory LN

The evidence for any kind of immunosuppressive therapy in refractory LN is weak. Small observational studies provided evidence that rituximab seems to be an effective treatment for patients with active LN that is refractory to standard immunosuppressive therapy.[52,53,69-73] However, the use of the different dosing schedules in these observational studies make an interpretation difficult.[54] Adjunctive treatment with tacrolimus resulted in a significant clinical response in patients resistant to MMF.[74-76] However, although these newly introduced immunosuppressive regimens have proven their efficacy in some cases of refractory LN, the application of high-dose cyclophosphamide (NIH regimen) could still be a possibility. These (adjunctive) regimens are described in *table 6 (*Level C).

INDICATIONS FOR REPEAT BIOPSY IN PATIENTS TREATED FOR CLASS III/IV LN

The benefit of a repeat biopsy during the disease course of proliferative LN is questionable since there is no consensus in the literature. A recent retrospective study showed that in the presence of proliferative lesions in the original biopsy, a repeat biopsy during a clinical flare is not necessary as these patients rarely switch to a pure non-proliferative LN.[77] Moreover, histopathological variables in a protocolised biopsy at two years after induction therapy did not predict renal outcome at 77 months or at 115 months in patients with proliferative LN randomised to iv cyclophosphamide or azathioprine/methylprednisolone.[37,78] In contrast to these findings, Hill *et al*. reported that certain histological findings in repeat biopsies at six months had a better predictive power for subsequent doubling of serum creatinine than the same markers in the initial biopsy.[79]

Given the conflicting results from the literature, the opinion of the Dutch Working Party is that a repeat biopsy is only justified in those patients where it is anticipated that the findings have therapeutic consequences (Level C). First, the persistence of proteinuria after reaching a partial response, despite optimal supportive treatment including salt restriction and treatment with ACEi or ARBs to differentiate between active disease, chronic lesions or transition to focal segmental glomerulosclerosis. Second, failure to respond (either complete or partial response) at 12 months after the start of the initial induction treatment to differentiate between active and chronic lesions.

Table 6. *Treatment of refractory lupus nephritis*

Rituximab*
1000 mg intravenous at day 1 and 15 as add-on therapy
Tacrolimus*[75]
0.1 mg/kg/day, through level 4-10 µg/l as add-on therapy
Cyclophosphamide*[25]
750 mg/m^2 intravenous, increased with 250 mg per dose to a maximum of 1500 mg 6 times monthly, then every 3 months for an additional 2 years
***Prednisone 1 mg/kg/day, maximum 60 mg/day.**

CONCLUSION

In this report guidelines are proposed for the management of proliferative LN, with regard to the following topics: indications for a first renal biopsy, definitions of treatment response, selection of treatment options, and indications for repeat biopsy. This consensus approach provides agreed expert opinion for clinicians and will hopefully support the optimisation of treatment in patients with proliferative LN. Moreover, following this guideline throughout the Netherlands could be a basis for future central registration and follow-up on a national level.

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