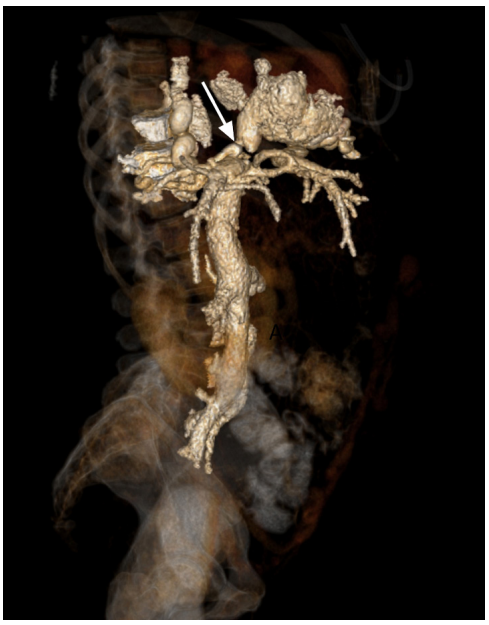
The Netherlands Journal of Medicine

PUBLISHED IN COLLABORATION WITH THE NETHERLANDS ASSOCIATION OF INTERNAL MEDICINE



Abdominal varices, and stenosis of the inferior vena cava: what is your diagnosis?

AUGUST/SEPTEMBER 2019, VOL. 77, NO. 07, ISSN 0300-2977

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The Netherlands Journal of Medicine

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ISSN: 0300-2977

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Contents

EDITORIAL

Therapeutic drug monitoring of flucloxacillin

P.L.A. van Daele

I favour therapeutic drug monitoring. It potentially decreases the risk of toxicity while increasing therapeutic effects of medication. Yet, therapeutic drug monitoring is not necessary for every prescribed drug. It is especially useful in drugs with a small therapeutic window, for example, lithium or antiepileptics, or when there is a strong relation between drug concentration and effect.

In the current issue of the journal, Dijkmans et al. elaborate on therapeutic drug monitoring in patients treated with flucloxacillin who are scheduled to switch from intravenous to oral administration. They state that orally administered flucloxacillin has variable absorption and by performing an oral absorption test (OAT), it is possible to identify patients with inadequate or decreased flucloxacillin absorption. In their paper, they describe two tests, one with and one without interruption of the intravenous administration. Both tests perform equally well, however the test in which the intravenous administration is not interrupted is much easier to conduct. In the study, just over 13% of patients showed, in the authors' opinion, an inadequate increase in serum level.[1]

There have been previous reports on therapeutic drug monitoring of beta-lactam antibiotics but most have focused on critically ill patients in an intensive care unit, demonstrating that in such situations drug monitoring can be useful to optimise antibiotic exposure and maximise effectiveness, thereby potentially improving outcome.[2-5] It is unclear whether this conclusion also holds true for the current study as the patient population is different and apparently less ill, knowing that they can switch route of administration. It would have been informative if we would have known the outcome of those who failed the test. Did they switch therapy? Was there an increase in flucloxacillin dose? Was their outcome worse? And what to do with patients with mild infection, who never need intravenous therapy? Need they be tested?

The authors plea that other institutions adopt their above-mentioned approach of OAT in the management of patients with severe *S. aureus* infections. It would be been more convincing if they had demonstrated that their approach also improves *outcome*.

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REVIEW

The value of FDG-PET CT scans to evaluate bone marrow in haemato-oncological conditions

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ABSTRACT

In the past decade, ^{18}F-fluorodeoxyglucose positron emission tomography combined with computed tomography (^{18}F-FDG PET-CT) scans have been increasingly implemented in the diagnostic process of several haemato-oncological conditions. Accurate assessment of bone marrow activity observed on ^{18}F-FDG PET-CT is crucial for a correct diagnostic conclusion, subsequent treatment decision, and follow-up strategies. By systematically considering the arguments of the level of ^{18}F-FDG uptake, distribution pattern, coinciding changes of the bone structure, and the clinical context, interpretation and validity may improve. This review aims to give a comprehensive overview of the different patterns of ^{18}F-FDG uptake on PET/CT in common benign, clonal, and malignant haematological conditions, accompanied by illustrative cases.

KEYWORDS

Bone marrow, FDG-PET CT scan, haemato-oncological diseases, inflammation

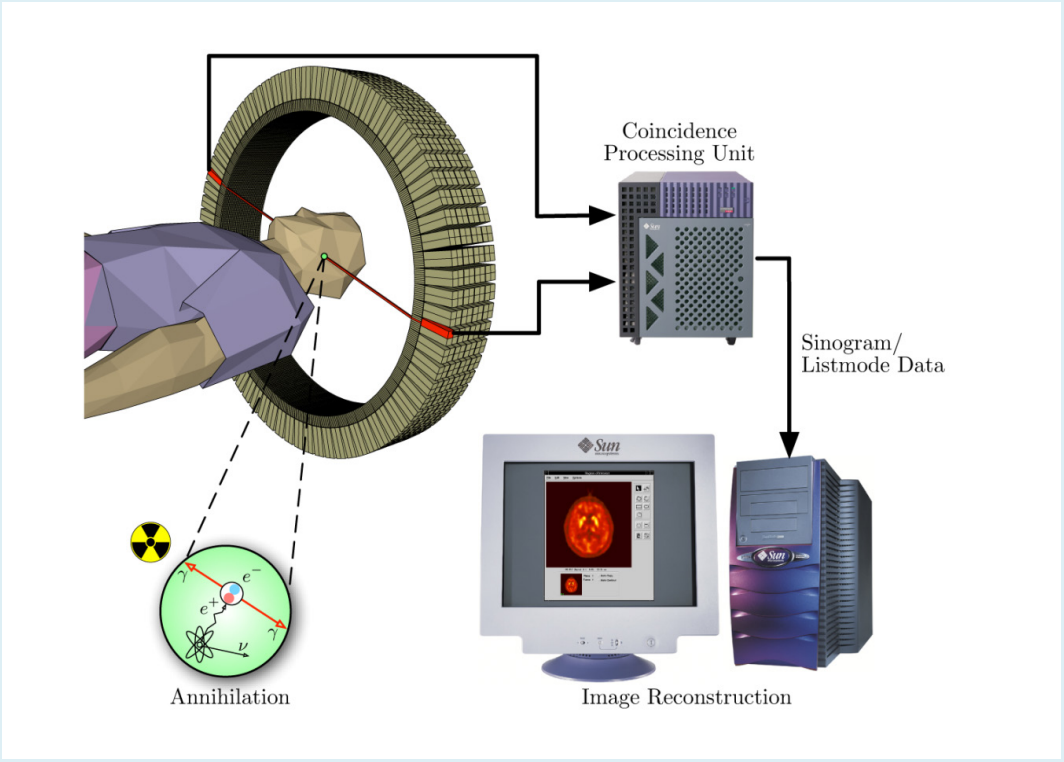
INTRODUCTION

^{18}F-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET) combined with computed tomography (CT) (^{18}F-FDG PET/CT) is an established imaging modality in the diagnosis, prognosis, and evaluation of patients with various haematological conditions, based on uptake of its tracer ^{18}F-FDG in cells with high glycolytic rates.[1,2] However, haematopoietic cells in the bone marrow compartment have constitutively considerable rates of glycolysis, which also results in ^{18}F-FDG uptake on PET/CT scans. Given this physiological background signal, it is of importance to recognize specific patterns of FDG uptake in the bone marrow in order to accurately interpret ^{18}F-FDG PET/CT scan findings.
This review aims to give a comprehensive overview of the different patterns of ^{18}F-FDG uptake in PET/CT scans in common haematological conditions, accompanied by illustrative cases.

BASIC PRINCIPLES OF FDG-PET/CT

The most commonly used tracer is a glucose analogue, fluorodeoxyglucose (FDG), labelled with the radionuclide ^{18}F, with a half-life of 109 minutes. ^{18}F-FDG is taken up by metabolically active cells and subsequently phosphorylated. Because of this, it is unable to enter the citric acid cycle and thus accumulates intracellularly. Most cells rely on glucose as their primary energy source under steady state conditions, and cells with high energy demand increased rates of glycolysis, in both the presence and absence of available oxygen. Based on this principle and due to often significant ^{18}F-FDG uptake in tumour cells, ^{18}F-FDG-PET/CT is a sensitive technique to detect and stage oncological diseases and assess treatment responses.[3-6] However, other factors influencing the metabolic rate of the haematopoietic system, for example inflammation or increased haematopoiesis, should be discriminated. The basic principles of PET and CT are further explained by figure 1.

Figure 1. *Basic principles of FDG-PET/CT.[1] Positron emission tomography (PET) is a quantitative technique that visualises coincident registrations of two opposite 511 keV photons on a 'line of response' resulting from annihilations of positrons emitted from an unstable radionuclide and free electrons available in the tissue. Tomographic reconstruction of enumerable lines of response results in a 3-dimensional image of the distribution of radioactivity in a particular volume. The amount of tracer uptake in a volume of interest (VOI) is commonly reported as standardised uptake value (SUV), which is a semiquantitative measure adjusted for the injected dose of radioactivity (MBq) and body weight (g).[2, 3]*
Computed tomography (CT) scanning is based on the measured attenuation of X-rays with uniform and specific energies emitted by an external source beam. Both the emitter and the detector, placed on the opposite site, rotate around the patient during scanning. Given the known attenuation coefficient of tissues in the body, 3-dimensional anatomical images can be reconstructed.[4] In hybrid PET/CT systems, this density-map based on the CT allows correction of the attenuation of photons that have resulted from annihilations, resulting in increased image quality



Physiological FDG uptake in bone marrow

About 4% of total body weight is bone marrow tissue. Depending on age, adult bone marrow produces between 10^{11}-10^{12} new haematopoietic cells per day, thereby maintaining the number of circulating blood cells.[7] In addition, the haematopoietic system is capable of responding quickly by increasing haematopoietic activity on demand, for example, during severe infection or repopulation after myeloablative chemotherapy.

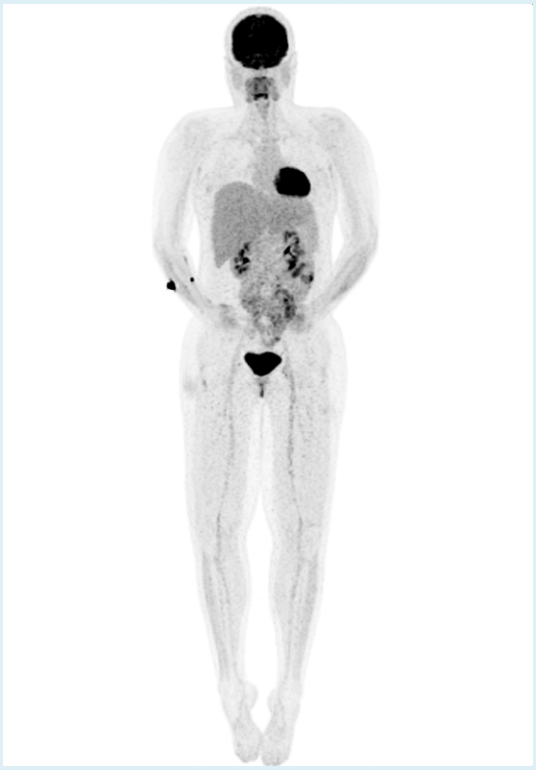
Under physiological circumstances, a high ^{18}F-FDG uptake is visible in the brain, liver, and heart, as well as its renal excretion (figure 2).[8,9] Active haematopoiesis under physiological circumstances is homogeneously distributed over the central bone marrow compartment (e.g., the spine, pelvic bones, and proximal femur and humeral bones), with typical standardised uptake values (SUVmax) in bone marrow being less than liver uptake.[10,11] In children, the distribution of ^{18}F-FDG is also homogeneous, but can be more extensive due to higher activity of the bone marrow.[8,12]

By considering four arguments, the differential diagnosis of abnormal ^{18}F-FDG uptake patterns can be narrowed down to a reasonable working diagnosis:

Level of ^{18}F-FDG uptake (SUVmax): normal (SUVmax ≤ 3); moderately increased above liver FDG values (SUVmax ≥ 4, ≤ 10); extensively increased (SUVmax ≥ 11).

Distribution of ^{18}F-FDG uptake: central compartment versus peripheral epiphysis, involvement of the spleen, lymph nodes and/or organs, and diffuse versus focal.

Figure 2. *Normal pattern. Physiological distribution of FDG, demonstrating high uptake in the brain parenchyma and myocardium, and moderately increased in the liverparenchyma. Excretion of the tracer FDG via the kidneys, ureter and urine bladder*



Coinciding changes of the bone structure as observed by CT, which result from osteoblasts/osteoclasts activity and bone marrow cellularity.
Clinical context, including medical history and laboratory findings.
In the following paragraphs, different benign and malignant haematological conditions supported by illustrative cases are described using these four arguments. Table 1 provides an overview of ^{18}FDG PET-CT scan findings for each haemato-oncological condition.

Benign conditions of increased FDG-update in bone marrow

Haematopoietic activity can be physiologically extensively increased in certain clinical conditions such as systemic inflammatory response to infection, chronic inflammation, or growth factor administration following myelotoxic chemotherapy. The central mechanism is the increased presence of growth factors, which leads to a higher affinity of glucose transporters for deoxyglucose.[8,11,13] Here, the pattern of FDG uptake of the central compartment resembles normal haematopoiesis, for example, diffuse and homogeneous, yet demonstrates an increased intensity (figure 3). Under certain circumstances, the spleen may also be involved, reflecting splenic extramedullary haematopoiesis.[11,14,15]

Systemic inflammation during infection

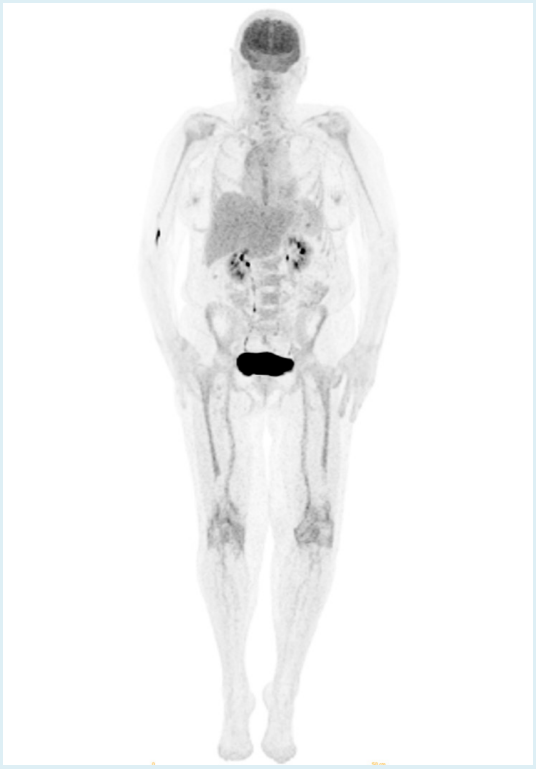
Inflammation defines an immunological process caused by tissue damage and/or infection. Inflammation leads

Table 1. *Overview of FDG PET-CT scan findings in haemato-oncological conditions*

Disease	Typical Level (SUV)	Pattern	CT findings
Inflammation	7	Homogeneous FDG uptake in BM and LNs. Diffuse uptake in spleen	Normal
CSF treatment	7	Diffuse FDG uptake in BM and spleen	Normal
Primary myelofibrosis	2-4	Normalisation of FDG uptake; sometimes, patchy patterns of fibrotic BM	Osteosclerosis
Erdheim Chester (EC)	6-15	Focal BM lesions. Extramedullary haematopoiesis (spleen, liver, central skeleton)	Symmetrical cortical thickening of bone. Osteosclerosis in long bones
Langerhans Cell Histiocytosis (LCH)	7	Asymmetrical, focal BM lesions in flat bones	Osteolysis in flat bones
Haemophagocytic lymphocistiocytosis (HLH)	3	Diffuse FDG uptake in spleen and BM	Hepatosplenomegaly
Leukaemia	11	Diffuse FDG uptake in BM and spleen	Hyperdense BM
Multiple myeloma	4	Diffuse BM pattern; rarely, shift to peripheral sites	Bone fractures (if present), osteolytic bone lesions
Hodgkin lymphoma		Focal osteolytic bone lesions, diffuse osteopaenia	
Non-Hodgkin lymphoma		Focal lesions, mostly of LNs contiguous pattern	

FDG = fluorodeoxyglucose; BM = bone marrow; LN = lymph node; CSF = colony stimulating factor.

Figure 3. *Reactive haematopoiesis. Increased intensity of FDG uptake in the central compartment*



to an increased number of glucose transporters within the affected tissue, while infiltrated granulocytes and macrophages also use glucose as their energy source.[16] Both events result in a homogeneous increased FDG uptake in the bone marrow and lymph nodes with a possibly moderate SUV.[8,17] Inflammation due to infection induces systemic effects resulting in a diffuse FDG uptake in the bone marrow and spleen. The latter must not be misinterpreted as a splenic infection or splenic tumour which appears as focal lesions.[8,9,15] To avoid misinterpretation, it is important to correlate the patient's clinical presentation including inflammatory parameters with the FDG-PET/CT-scan.

Growth factors

Granulocyte-colony stimulating factor (G-CSF) may be used to accelerate granulocytic recovery following myelotoxic chemotherapy or compensate for disease-related impairment of granulopoiesis. A moderate increase in FDG uptake can be observed in the bone marrow within these patients.[9,11]

Elevated, diffuse FDG uptake due to increased haematopoiesis may be misinterpreted for a bone marrow malignancy or disorder. Thus, information regarding medical history and laboratory findings are important to establish a correct diagnosis.[18] Discontinuation of G-CSF treatment leads to a rapid decrease and normalisation of FDG uptake in the bone marrow.[11]

As elevated FDG uptake can be demonstrated as soon as three days after the start of treatment with G-CSF and normally resides after three days post-treatment completion,[11,19] it is advised to perform FDG-PET imaging with a delay of at least five days after discontinuing G-CSF. To summarize, increase of haematopoiesis due to an either endogenous or exogenous inflammatory stimulus induces a specific pattern of FDG accumulation characterised by a homogenous and highly intensive FDG uptake observed at the physiological locations.

Clonal diseases of increased FDG uptake in bone marrow

Erdheim Chester (EC), Langerhans Cell Hystiocytosis (LCH), and Haemophagocytic lymphocistiocytosis (HLH) are rare disorders within the histiocytic cell lineage. EC is a non-Langerhans cell, multisystemic granulomatosis, characterised by accumulated foamy histiocytes present in the long bones and large blood vessels. In some cases, the heart and central nervous system are involved. Recently, several mutations have been identified supporting the clonal nature of the disease and its pro-inflammatory cytokine phenotype. In 50% of all cases, patients present themselves with focal bone pain and are mostly in their adult age. Focal moderately-active lesions in the bone marrow are seen on the FDG-PET/CT scan which may also be visualized by bone scintigraphy (figure 4).[20-23] Typical bone involvement is bilateral with symmetrical cortical thickening.[24] Osteosclerosis is seen in the long bones, except for epiphyses, the axial skeleton, and mandible. In addition to osseous manifestations, histiocytes often accumulate in different tissues leading to involvement of the kidneys, heart, lungs, and brain. Due to the rarity of this disease, it can be easily overlooked or mistaken for a primary bone malignancyFDG is not a cancer-specific agent, and knowledge of the differential diagnosis of benign FDG-avid bone alterations that may resemble malignancy is important for correct patient management, including the avoidance of unnecessary additional invasive tests such as bone biopsy. This review summarizes and illustrates the spectrum of benign bone conditions that may be FDG-avid and mimic malignancy, including osteomyelitis, bone lesions due to benign systemic diseases (Brown tumour, Erdheim-Chester disease, Gaucher disease, gout and other types of arthritis, Langerhans cell histiocytosis, and sarcoidosis. Histological biopsy is needed to confirm diagnosis. PET-CT scans are useful for initial assessment and follow-up of lesions, including pituitary involvement.[14,24-26]

Figure 4. *Erdheim Chester. Symmetrical and moderately increased FDG uptake intramedullary in the distal femur and iproximal tibia*

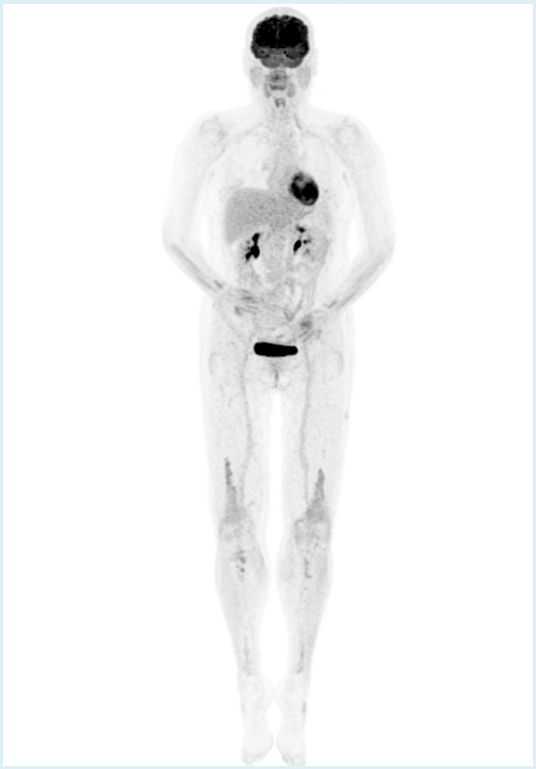
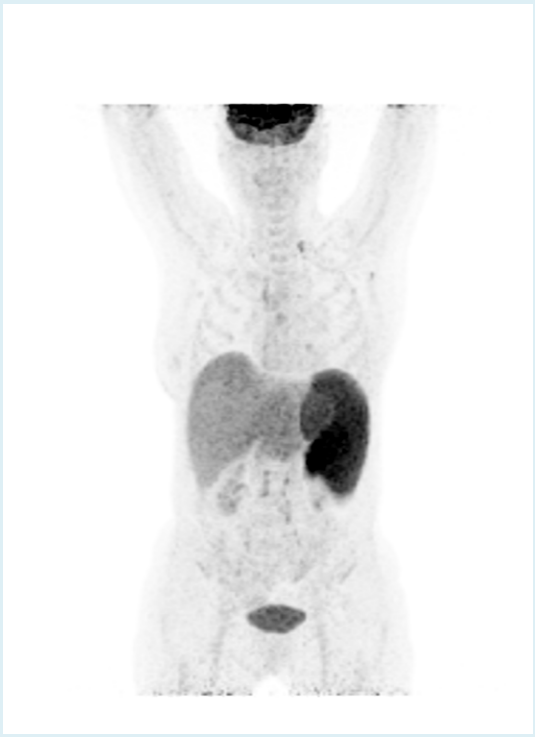


Figure 5. *Haemophagocytic lymphohistiocytosis. Extensive diffuse FDG uptake in the enlarged spleen, which is discordant with the physiological FDG uptake in the bone marrow*



LCH is another rare disease of abnormal clonal proliferation of myeloid dendritic cells, defined as Langerhans cells. This disease is usually diagnosed during childhood. Recently, it was discovered that BRAFV600E mutations characterise the disease in the majority of patients.[27-29] These cells are able to infiltrate tissue, preferably bone tissue such as flat bones like the skull, pelvis, and ribs, resulting in osteolysis. FDG uptake is asymmetric with a moderate SUVmax, depending on the phase and metabolically-active sites of disease.[30] Osteolysis is absent in EC. During early stages, LCH can be mistaken for malignant bone tumours. Similar to EC, histopathologic examination of one of the infiltrated tissues is needed to confirm diagnosis.[14] In general, EC and LCH may be difficult to discriminate in clinical practice due to considerable overlap in clinical presentation, imaging findings, and laboratory findings.

Finally, HLH is a non-malignant syndrome and characterised by overproduction of cytokines resulting in activation of cytotoxic T cells, natural killer (NK) cells, and macrophages, ultimately culminating in uncontrolled hyperinflammation. The excessive immune activation results in the clinical hallmarks of the disease, i.e. fever, hepatosplenomegaly, cytopaenias and hyperferritinaemia. Hereditary or primary HLH often manifests itself during infancy or childhood due to a variety of underlying heterogenous genetic mutations. Acquired or secondary HLH may result from a strong immune activation in response to infection (in particular Epstein Barr Virus), malignancy, or an autoimmune disorder.[31] On images, PET-CT demonstrates a diffuse FDG uptake pattern in the spleen and a normal uptake in the bone marrow (figure 5).[32-35] Other organs may be affected as well.

Malignant conditions of increased FDG uptake in bone marrow

Primary myelofibrosis

Myelofibrosis is a clonal disorder involving a multipotent haematopoietic stem cell. This disease is characterised by excessive, though often ineffective, proliferation associated with pro-inflammatory cytokines, bone marrow fibrosis, and extramedullary haematopoiesis. Constitutional symptoms, cytopaenia due to bone marrow failure or transformation to acute myeloid leukaemia, symptomatic hepatosplenomegaly, and thromboembolic events may complicate its clinical course.

Figure 6. *Primary myelofibrosis. Extramedullary haematopoiesis in periferal bone marrow, enlarged spleen with moderate FDG uptake. Compared to the acute myeloied leukaemia patient (figure 4) there is less visible FDG uptake*

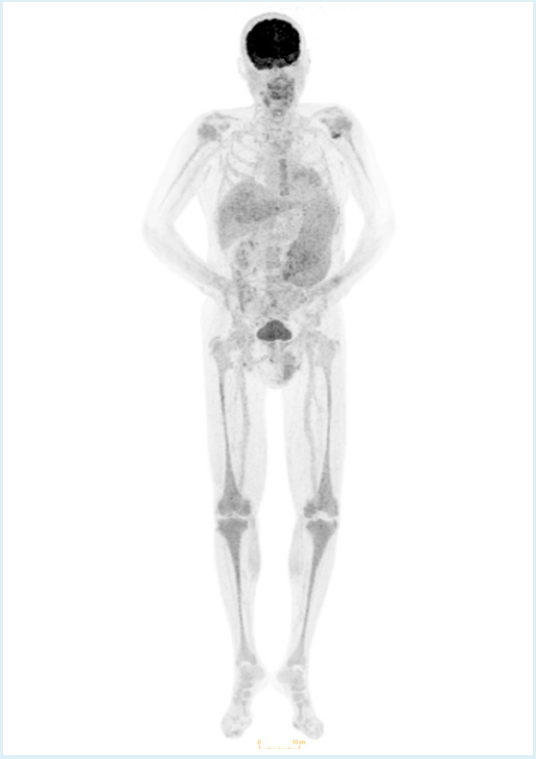
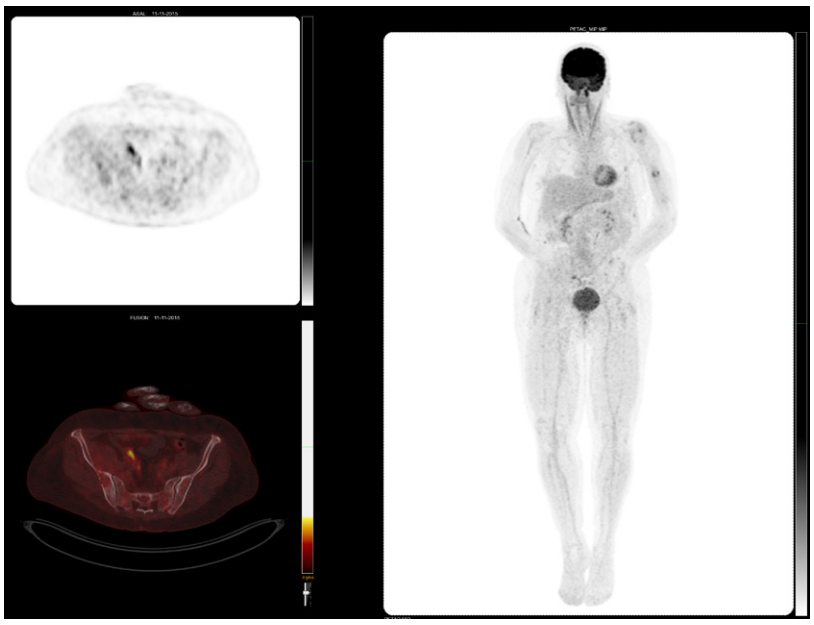


Figure 8. *Acute myeloid leukaemia. Extensively increased FDG uptake throughout the whole skeleton, including smaller bones of the hand and feet. Enlarged spleen with increased FDG uptake*

Figure 7. *Multiple myeloma. Multiple small foci with little or moderately increased FDG uptake diffusely spread over the bones. Most prominent FDG-avid lesion is located mid-diaphysis in the left upper arm*



In over 50% of patients, a typical mutation in the JAK2 gene (V617F mutation) can be identified, which results in an alleviation of the protein's physiological inhibitory status, thereby facilitating the erythropoietin and thrombopoietin receptors to become autonomous with respect to growth factor. Currently, the JAK1 and JAK2 inhibitor ruxolitinib and interferon are non-curative, but are effective therapies, suppressing inflammatory cytokine production and thereby reducing associated clinical symptoms as well as haematopoietic progenitor proliferation. Allogeneic stem cell transplantation remains the only potential curative treatment for now.[36]

With PET-CT, the bone marrow shows a diffuse, homogeneous pattern of FDG uptake (figure 6). Due to extramedullary haematopoiesis, an increased uptake may also be seen in the spleen, liver, and central skeleton. Spleen involvement seems to be positively correlated to bone marrow disease stage and represents the degree of extramedullary haematopoiesis. Intensity in bone marrow uptake decreases over time due to fibrosis and osteosclerosis, resulting in hypocellular and fibrotic parts lacking FDG uptake with narrowing normal haematopoiesis to only distal compartments and an increased FDG uptake in the spleen.[16,37,38]

Multiple myeloma

Multiple myeloma (MM) originates from neoplastic plasma cells, which often accumulate in the bone marrow, cortical bones, and sometimes the extraosseous localisations. This disease often presents with spontaneous bone fractures, hypercalcaemia, renal insufficiency, and anaemia.[39] Bone marrow biopsy will show an excessive amount of plasma cells.[6,40] FDG-PET/CT scans may show focal osteolytic bone lesions and diffuse osteopenia with a moderate FDG uptake (figure 7). Higher and mixed FDG uptake are associated with fast progression and poor prognosis. PET-CT has a central role in both visualising extramedullary plasmacytomas as well as evaluating treatment response in non-secreting myeloma.

Acute leukaemia

Acute leukaemias originate from a clonal proliferation of malignantly transformed myeloid or lymphocytic progenitor cells, ultimately replacing normal haematopoiesis and inducing progressive and severe bone marrow failure. Due to pancytopaenia, patients are susceptible to infection, bleeding, and suffer from anaemic symptoms.

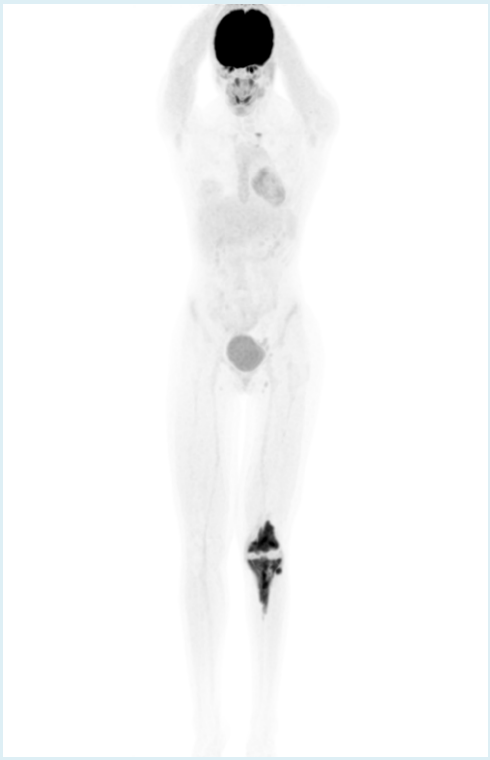
FDG-PET may be used when extramedullary disease is suspected. Due to the diffusely infiltrating hallmark of the disease, FDG-PET/CT demonstrates a typical diffuse pattern in which, depending on the proliferating rate of the disease, an extensive SUVmax can be found (figure 8).[41] Under extensive disease conditions, the residual normal haematopoiesis may have shifted to peripheral sites, e.g., the tibia, resulting in hypercellular bone marrow that is visible as hyperdense bone marrow by CT scan. In addition to bone marrow localisation and depending on the specific leukaemic subtype, leukaemic cells may be present in extramedullary organs, e.g. in the liver, spleen, lymph nodes, and skin.[41,42] Recently, we described a case of recurrent acute myeloid leukaemia with pericardial and abdominal myeloid sarcomas lacking bone marrow involvement.[43]

Mature lymphoma

Both for Hodgkin lymphomas (HL) and non-Hodgkin lymphomas (NHL), the attributable role of FDG-PET/CT has been extensively increased.[44-49] HL and NHL are neoplasms derived from mature lymphoid B or T cells. The stage of differentiation of these malignant immune cells determines the behaviour of the neoplasm.[44]

Staging of lymphoma is based on the Ann Harbor classification and involves the sites involved (nodal, extranodal) and disease distribution. FDG-PET/CT scans are routinely used in staging and assessment of treatment outcomes in Hodgkin and high-grade NHLs such as

Figure 9. *Intra-osseous lymphoma. Extensive focal FDG uptake in the knee joint, crossing the metaphysis, without gross destruction of the cortical bone or extension into surrounding soft tissue*



diffuse large B-cell lymphoma and Burkitt lymphoma, as disease can be typically visualised by high SUV levels.[45] For diseases with lower rates of metabolic activity such as follicular lymphoma, this modality is less suitable due to the difficulty in interpreting normal to only slightly increased SUVmax levels. Consequently, FDG-PET/CT is not advised to be used in intermediate and low-grade NHLs.[49-52]

Both HL and high-grade NHL may show focal abnormalities on the FDG-PET/CT image (figure 9). Yet, due to a (respectively) lymphatic versus haematogenous spreading, HL of stage II and higher demonstrate FDG avidity in contiguously linked lymph nodes. In contrast, NHL is characterised by skipped lesions with FDG avidity in different lymph nodes throughout the body, together with involvement of different organs and bone marrow.[42] Finally, as definite diagnosis of high-grade lymphomas requires histopathologic examination, PET-CT may support this by visualising the most intense and thereby preferred location of biopsy.[41,45]

CONCLUSION

PET-CT has increasingly been implemented in the diagnostic process of haemato-oncological conditions. In this review, we described patterns of ^{18}F-FDG uptake in the bone marrow in benign, inflammatory, and haemato-oncologic conditions, and provided arguments that may aid in the accurate interpretation of ^{18}F-FDG PET-CT in clinical practice.

DISCLOSURES

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

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REVIEW

Monoclonal gammopathy of renal significance (MGRS): histopathologic classification, diagnostic workup, and therapeutic options

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ABSTRACT

Monoclonal gammopathy of renal significance (MGRS) includes all kidney disorders caused by a monoclonal protein (M-protein) secreted by a small plasma cell clone or other B-cell clones in patients who do not meet the diagnostic criteria for multiple myeloma or other B-cell malignancies. The underlying disorder in patients with MGRS is generally consistent with monoclonal gammopathy of undetermined significance (MGUS). MGRS-associated kidney disorders are various and the list is still expanding. The kidney disorders can manifest as glomerular diseases, tubulopathies, and vascular involvement with varying clinical presentations.

Diagnosis is often challenging because of the wide spectrum of MGRS, and it is difficult to establish a pathogenic link between the presence of the M-protein or serum free light chains and kidney diseases; further complicating accurate diagnosis is the high incidence of MGUS and/or kidney disorders, independent of MGRS, in elderly patients. However, MGRS can significantly impair kidney function. Because treatment can stop and also reverse kidney disease, early recognition is of great importance. A combined haematologic and nephrologic approach is crucial to establish the causative role of the M-protein in the pathogenesis of kidney disease. Clone-directed therapy, which may include autologous stem cell transplantation in eligible patients, often results in improved outcomes. In this review, we discuss the histopathologic classification of MGRS lesions, provide a renal and haematologic diagnostic workup, discuss treatment options for MGRS, and introduce a Benelux MGRS Working Group.

KEYWORDS

MGRS, MGUS, paraproteinaemia-associated kidney disease, plasma cell dyscrasia, monoclonal immunoglobulin, free light chain

INTRODUCTION

A circulating monoclonal protein (M-protein) is present in approximately 3% of people aged 50 years and older

and increases to 5% after 70 years of age.[1,2] Patients with this circulating M-protein are diagnosed with monoclonal gammopathy of undetermined significance (MGUS) if the M-protein is < 30 g/l and a bone marrow (BM) examination shows < 10% monoclonal plasma cells with absence of end organ damage or other multiple myeloma defining events.[2,3] Monoclonal gammopathies can be completely asymptomatic in the case of MGUS, but monoclonal gammopathy is sometimes associated with potentially severe organ damage whereby the criteria for overt multiple myeloma (MM) or other malignant lymphoproliferative disorders such as B-cell non-Hodgkin lymphoma, including Waldenström's macroglobulinaemia (WM) or chronic lymphocytic leukaemia (CLL), are not met (table 1).

Therefore in 2012, the term monoclonal gammopathy of renal significance (MGRS) was introduced by the International Kidney and Monoclonal Gammopathy Research Group (IKMG) to describe a spectrum of kidney diseases due to M-protein secreted by a small B-cell, lymphoplasmacytic, or plasma cell clone not meeting any current haematologic criteria for specific therapy.[1] The term MGRS was recently updated by the IKMG and now

Table 1. *Definitions of B-cell and plasma cell proliferative disorders*

Plasma cell disorder	Criteria
Multiple myeloma	Clonal plasma cells in BM ≥ 10% or biopsy-proven bony or extramedullary plasmacytoma, and any one or more of the following myeloma defining events[1]: - Hypercalcaemia: serum calcium > 0.25 mmol/l higher than upper limit of normal or > 2.75 mmol/l - Renal insufficiency: creatinine clearance <40 ml per min or serum creatinine > 177 µmol/l - Anaemia: (Hb < 6.2 mmol/l) - Bone lesions: one or more bone lesions on skeletal radiography, CT, or PET-CT Or any of the following biomarkers of progression[2]: - Clonal BM plasma cells ≥ 60% - 'Involved:uninvolved serum free light chain ratio' ≥ 100 - > 1 focal lesions on MRI studies
Smoldering multiple myeloma	Serum M-protein ≥ 30 g/l or urinary M-protein ≥ 500 mg/24 h and/or clonal BM plasma cells 10–60% Absence of SLiM-CRAB or amyloidosis
Waldenström's macroglobulinaemia	Lymphoplasmacytic infiltrate in BM ≥ 10% Serum monoclonal IgM of any level Symptoms of tumour mass/infiltration (adenopathy, anaemia etc) IgM-mediated symptoms can be present
Smoldering Waldenström's macroglobulinaemia	Lymphoplasmacytic infiltrate in BM ≥ 10% Serum monoclonal IgM of any level Absence of symptomatic tumour mass/infiltration (adenopathy, anaemia etc)
Non-IgM MGUS	Serum M-protein < 30 g/l Clonal plasma cells in BM < 10% Absence of SLiM-CRAB or amyloidosis
IgM MGUS	Serum IgM M-protein < 30 g/l BM involvement with lymphoplasmacytoid cells < 10% Absence of anaemia, constitutional symptoms, hyperviscosity, lymphadenopathy, hepatosplenomegaly, or any organ damage attributed to the lymphoproliferative disorder
Chronic lymphocytic leukaemia	Presence of ≥ $5x10^9$/l clonal B lymphocytes in peripheral blood Phenotype: CD5+, CD19+, CD23+, CD20+/-, sIg +/-
Monoclonal B lymphocytosis	Clonal B lymphocytes < $5x10^9$/l in peripheral blood Presence of CLL phenotype No evidence of lymphoma, infection, or autoimmune conditions

[1]Myeloma defining events: organ damage attributed to the underlying plasma cell disorder often abbreviated as 'CRAB' (hypercalcaemia, renal failure, anaemia, and bone disease).
[2]These three biomarkers are abbreviated to 'SLiM' (S ≥ 60% clonal plasma cells in BM; Li = light chains, kappa-to-lambda or lambda-to-kappa ratio ≥ 100; M ≥ 1 focal lesion by MRI).
BM = bone marrow; MGUS = monoclonal gammopathy of undetermined significance; M-protein = monoclonal protein; CLL = chronic lymphocytic leukaemia; Hb = haemoglobin; CT = computed tomography; PET-CT= positron emission tomography–computed tomography; MIR = magnetic resonance imaging; IgM = Immunoglobulin M; CD = cluster of differentiation; sIg = surface immunoglobulin

includes all B-cell or plasma cell proliferative disorders that produce a nephrotoxic M-protein such as smoldering MM, smoldering WM, and monoclonal B-cell lymphocytosis (MBL). Low-grade B-cell lymphomas and low-grade CLL with associated renal disease are also included in the updated definition of MGRS. M-proteins secreted by this clone can be directly nephrotoxic and also indirectly nephrotoxic by complement activation. In addition to kidney involvement, these small B-cell or plasma cell clones can also involve other organs such as skin and peripheral nerves and therefore, the concept of monoclonal gammopathy of clinical significance (MGCS) was recently introduced.[4]

The current treatment guidelines do not recommend anti-tumour therapy in patients with MGUS and smoldering myeloma or asymptomatic WM. These patients are usually monitored as they can remain asymptomatic for years and only in the case of developing or impending symptoms can therapy be introduced.[3,5] In patients with MGRS, this approach may be inadequate, as the M-protein plays a direct role in the pathogenesis of kidney disease despite the absence of high tumour burden. In these cases, the monoclonal gammopathy is of known significance as kidney disease is present with increased risk of progression to end stage renal disease (ESRD). In addition, cases of recurrence after kidney transplantation have been described.[6-15]

In this review, we illustrate the spectrum of MGRS with the histopathologic classification, present a renal and haematologic diagnostic workup that may assist in the diagnosis of MGRS, and discuss treatment options for the underlying haematologic disorder to improve renal and patient outcome. Ideally, patients with MGRS should be evaluated by a multidisciplinary team that includes nephrologists, haematologists, and nephropathologists with expertise in these conditions and therefore, we have established a Benelux MGRS Working Group (Belgium, Netherlands, Luxemburg) that can be consulted in cases of suspected or proven MGRS.

EPIDEMIOLOGY OF MONOCLONAL GAMMOPATHY OF RENAL SIGNIFICANCE

Little is known about the epidemiology of most MGRS disorders. Shaik et al. reported a prevalence of MGRS of 6% in individuals diagnosed with MGUS in the United States[16] and Steiner et al. reported a prevalence of 1.5% of MGRS in 2935 patients diagnosed with MGUS.[17] Regarding specific disease data, amyloid light chain (AL amyloidosis was the cause of ESRD in 1.15% of patients in the Netherlands who started dialysis in 2016.[18] Renal biopsy registries show that a diagnosis of light chain deposition disease (LCDD) is made in 0.3-0.5% of all kidney biopsies, with an identified underlying MGUS in approximately 41% of these cases.[19,20] All other types of MGRS have unknown incidence and/or prevalence as they are only described in the form of case reports and small case series. Although the recognition of MGRS as a new entity has increased in recent years, under-reporting of this disease still exists.

PATHOPHYSIOLOGY AND HISTOPATHOLOGIC CLASSIFICATION

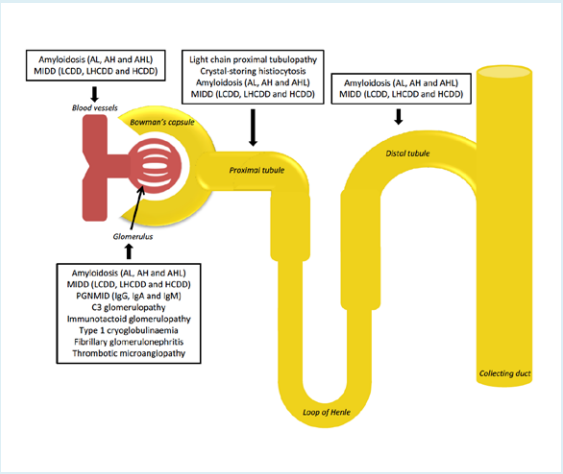
Kidney diseases associated with monoclonal gammopathy are various and the list of recognized disease entities is still expanding. Renal lesions associated with MGRS can be classified by localisation (figure 1) and include glomerular, tubulointerstitial, and vascular patterns of injury, single or in combination.[21-23]

In addition to a direct toxic effect of M-protein deposits, M-proteins can also have autoantibody activity which results in complement activation and subsequent deposition of complement factors in the kidney (indirect mechanism). The type of kidney injury seems to be dependent on the characteristics of the M-protein.[24,25]

Light chain (AL), heavy chain (AH), and heavy and light chain (AHL) amyloidosis

Extracellular deposition of amyloid in glomeruli, tubules, and/or vessels is characteristic for renal amyloidosis. In most cases, the M-protein-related amyloidosis is derived from fragments of monoclonal light chains

Figure 1. *Spectrum of monoclonal gammopathy of renal significance by localisation*



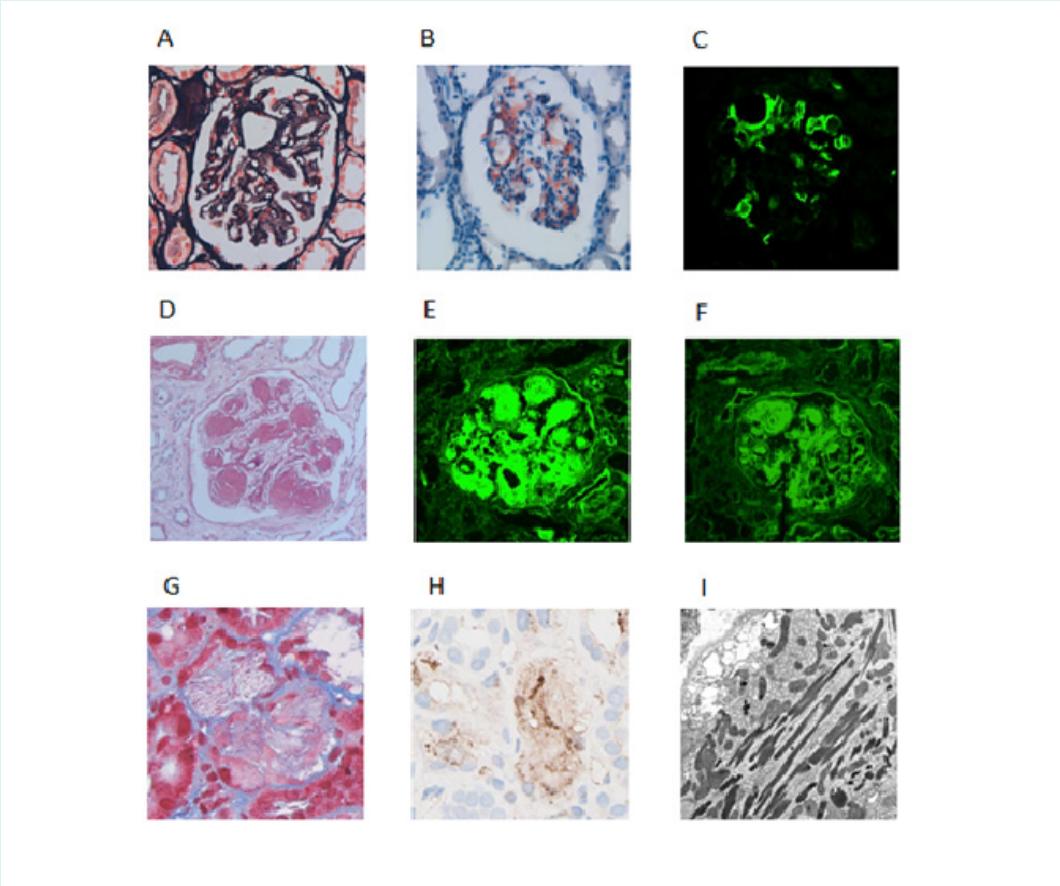
AL= light chain amyloidosis; AH = heavy chain amyloidosis; AHL = heavy and light chain amyloidosis; MIDD = monoclonal immunoglobulin deposition disease; LCDD = light chain deposition disease; LHCDD = light and heavy chain deposition disease; HCDD = heavy chain deposition disease; PGNMID = proliferative glomerulonephritis with monoclonal immunoglobulin deposits

(LCs), which are more often of the λ than κ isotype[26], and rarely from fragments of intact immunoglobulin (Ig) or heavy chains only. With light microscopy (LM) amyloid appears as acellular deposits that stain pale eosinophilic on haematoxylin and eosin, periodic acid–Schiff (PAS)-negative or weak, trichrome-blue or gray, and silver-negative (figure 2A). Amyloid is the only MGRS lesion that is Congo red-positive and identified by apple green birefringence under polarised light (figure 2B). With immunofluorescense (IF) microscopy, monotypic staining of the amyloid deposits can be present (figure 2C). With electron microscopy (EM), amyloid deposits in the kidney appear as nonbranching fibrils that are randomly arranged with a thickness between 7 and 14 nm and can be seen within the mesangium, glomerular or tubular basement membranes, interstitium, and vessels.[21] AL/AH/AHL amyloidosis is a systemic disease and most patients present with two to four organs involved.[27]

Monoclonal immunoglobulin deposition disease (MIDD)

MIDD includes three subtypes depending on the composition of the deposits. Light chain deposition disease (LCDD) is the most common subtype, primarily the κ type. The kidneys are almost always affected and extrarenal involvement is common in the heart, liver, and lungs.[21] Under LM, a nodular glomerulosclerosis pattern is classic for MIDD and nodular mesangial expansion along with thickening of the glomerular and tubular basement

Figure 2. *Selected cases of MGRS identified by renal biopsy*



A. Subtle silverlucency of the mesangium in a case of amyloidosis.
B. Corresponding Congo red staining highlighting mesangial amyloid deposition. Applegreen birefringence was present (not shown).
C. Corresponding lambda light chain immunofluorescence. Kappa was negative, as well as immunofluorescence for the heavy chains.
D. PAS staining of a case of light- and heavy chain deposition disease (LHCDD) showing nodular glomerulosclerosis.
E. Corresponding IgG immunofluorescent staining of LHCDD.
F. Kappa showed similar staining.
G. A case of light chain proximal tubulopathy. Masson trichrome stain showed acute tubular damage with fuchsinophillic crystal formation in the proximal tubules.
H. Kappa staining was positive in the cytoplasm of proximal tubules. Lambda staining was negative (not shown).
I. Electron microscopy showed proximal tubules with abundant needle-shaped crystals.
MGRS = monoclonal gammopathy of renal significance; PAS = periodic acid–Schiff; LHCDD = light- and heavy chain deposition disease; IgG = immunoglobulin G

membranes (GBM and TBM) is also present (figure 2D). A variable degree of tubular atrophy, interstitial fibrosis, and inflammation can also be present as nonspecific findings.

With IF, LCDD presents with monotypical linear amorphous LC deposits in the mesangium, and along the glomerular and tubular basement membranes. In light and heavy chain deposition disease (LHCDD) and heavy chain deposition disease (HCDD), linear deposition of monotypic γ, α, or μ HC along the GBM and TBM are seen (figure 2E, F). The granular deposits appear nonfibrillar and electron-dense and are located in the glomerular subendothelium and mesangium, and on the outer aspect of the tubular basement membrane, as observed by EM.[21,28,29]

Proliferative glomerulonephritis with monoclonal immunoglobulin deposits (PGNMID)

PGNMID is the result of glomerular deposition of monoclonal IgG or rarely, IgA or IgM. LM shows predominantly endocapillary proliferation and/or membranoproliferative glomerulonephritis (MPGN), with or without membranous features. As seen by IF, the IgG deposits are confined to glomeruli and consist of a single light chain isotype and a single heavy chain subtype, most commonly IgG3κ. Positive staining for C3 and C1q indicates activation of complement system. Granular, non-organized deposits, typically in a subendothelial and mesangial distribution are characteristic findings with EM.[21,30-32]

Monoclonal gammopathy-associated C3 glomerulopathy (C3GP)

C3GP results in kidney dysfunction via an indirect mechanism. In most cases, the mechanism by which the presence of an M-protein, mostly IgG, predisposes to C3GP is not clear. A possible mechanism has been suggested whereby the M-protein acts as an autoantibody to C3 convertase or as an autoantibody to other complement-regulating proteins which results in dysregulation of the alternative complement pathway.[15,33]

C3GP is characterized by glomerular deposition of the complement component C3 fragment at least two orders of intensity stronger than any combination of IgG, IgM, IgA, and C1q. Mesangial proliferative, membranoproliferative, or endocapillary proliferative glomerulonephritis are the usual patterns on LM. Electron-dense deposits located in the mesangium and subendothelium can be seen with EM.[34]

Immunotactoid glomerulopathy (ITG)

ITG is often monoclonal in nature, in contrast to fibrillary glomerulonephritis (FGN).[9] ITG is characterized by either an MPGN or diffuse proliferative pattern as observed with LM. Organized and parallel-oriented microtubular M-protein deposits with either κ or λ light chain restrictions are seen. These microtubular glomerular deposits are mostly composed of IgG, are Congo red-negative, contain distinct hollow centers, and stain for complement (C3) by IF. They are focally arranged in parallel arrays and are usually greater than 30-90 nm in diameter as seen by EM.[21,35]

Cryoglobulinaemia-associated glomerulonephritis (CGG)

Cryoglobulinaemia (CG) is composed of three types of which type I and type II are associated with monoclonal proteins. Type 1 CG is characterized by precipitation of M-protein when exposed to a temperature lower than 37°C and dissolve once the serum is heated. It can cause CGG or vasculitis. With LM, the characteristic finding of CG is a MPGN with endocapillary proliferation. Numerous intracapillary infiltrating leukocytes and large PAS-positive intraluminal immune deposits (protein hyaline thrombi) can be seen with LM. Intraluminal Ig and deposition of C3, C4, C1q are seen with IF and substructures like microtubules, fibrils, and finger print deposits can be observed with EM.[21,31,36] In type II CG, the monoclonal Ig is bound to the constant domain of polyclonal Ig heavy chains forming a complex. The most common cause of type II CG is hepatitis C infection. Approximately 10-30% of cases are not related to hepatitis C infection but to B-cell lymphoproliferative disorders.[37]

Paraprotein-associated fibrillary glomerulonephritis (FGN)

Although FGN is often polyclonal in nature, it can also be associated with organized glomerular M-protein deposits which have a fibrillary amyloid-like appearance as observed with EM. An MPGN pattern is commonly seen with LM. Unlike amyloid, lesions are Congo red-negative.[38] IF staining for IgG (mostly IgG4, sometimes IgG1) and C3 is common in these fibrils, which have a diameter ranging from 16 to 24 nm, as observed with EM.[35]

Monoclonal gammopathy-associated thrombotic microangiopathy (TMA)

TMA associated with monoclonal gammopathy is a relatively new entity with few cases described in the literature. TMA with no other cause than gammopathy was first described in three cases that were part of a large cohort of patients with WM and a number of single case reports.[39] The causal relation between the gammopathy and TMA was further supported by an unexpected high occurrence of monoclonal gammopathy in patients with TMA reported by Ravindran et al.,[40] with an incidence of 21% in patients aged 50 and older. How monoclonal gammopathy induces TMA is not fully understood and remains to be elucidated. Kidney injury

in TMA is characterised by thrombus in the glomerular capillaries, swelling of the endothelium, mesangiolysis with microaneurysms, and double-contour formation of the glomerular capillary walls. Acute tubular injury with differing degrees of tubulointerstitial scarring and thrombus in the arteries and arterioles can also be seen.[40]

Light chain proximal tubulopathy (LCPT)

LCPT is characterised by proximal tubular dysfunction secondary to cytoplasmatic inclusions of LCs as crystals within the endolysosomal compartment of proximal tubular cells. The accumulated LCs are almost always of the κ class and are rod- or rhomboid-shaped hypereosinophilic and PAS-negative under LM (figure 2G, H). For the visualization of the intracytoplasmic crystalline inclusions of κ LC in proximal tubular cells by IF, pronase digestion might be necessary.[41] The electron-dense intracytoplasmic inclusions can be seen with EM (figure 2I). Some inclusions appear to be membrane-bound within the endosomes or lie free in the cytosol.[42] Recently, it was shown that LCPT without crystal formation is actually more common than LCPT with crystal formation and is usually associated with accumulation of λ LCs. LCPT with crystal formation seems to be more frequent in younger patients and is associated with Fanconi's syndrome (FS) and a worse kidney function compared with noncrystalline LCPT, as reported in one study.[42] However, little is known about the clinical significance of crystalline vs noncrystalline deposits because of their rarity.[43] It could be argued whether noncrystalline LCPT is pathologic, given the physiologic reabsorption of LCs occurring in the proximal tubule.

Crystal-storing histiocytosis (CSH)

In CSH, the M-protein crystals accumulate within the lysosomes of histiocytes accumulated in the interstitium. LM shows that the crystal-containing histiocytes are accompanied by interstitial fibrosis and tubular atrophy and IF demonstrates that the accumulated crystals are almost always LCs of the κ class. Diagnosis of CSH can be challenging since the crystalline inclusions cannot always be identifyed by IF and pronase-digested tissue or the immunoperoxidase method may be required.[41,44] CSH can occur simultaneously with LCPT. If this is the case, organized cytoplasmic inclusions of needle- or oval-shaped crystals in proximal tubular cells are seen with EM.[21,45]

DIAGNOSIS OF MGRS

When should MGRS be considered?

Clinical manifestations depend on the affected segment of the nephron. Patients present with a progressive decline in kidney function, microscopic haematuria, proteinuria (ranging from subnephrotic to overt nephrotic syndrome), and/or proximal tubular dysfunction.[21] The proximal tubular dysfunction can result in FS which is characterised by normoglycaemic glycosuria, hyperphosphaturia resulting in hypophosphataemia, proteinuria, aminoaciduria, hyperuricosuria, and urinary wasting of bicarbonate. Incomplete FS has also been reported.[31]

Therefore, MGRS should be considered in every patient with renal manifestations combined with an M-protein, especially when classical cardiovascular risk factors or diseases, such as diabetes mellitus, hypertension, and atherosclerosis are absent or well controlled. MGRS should also be considerd in patients presenting with C3GP and TMA, which are known to have a strong association with M-protein and kidney disease, for example, in elderly patients. Although MGRS is commonly seen in patients of 50 years and older[14,46], it has also been reported in younger patients.[9-11,14,47]

Extrarenal manifestations are common in AL amyloidosis and MIDD, with the heart and liver often affected, which could serve as a clinical clue. In type 1 CG, the skin is the most affected organ.[48] However, these patients are difficult to recognize and diagnosis often requires extensive consultation between nephrologist and haematologist.

In the diagnosis of MGRS there are two important objectives: 1) one should try to identify the circulating M-protein and 2) one should try to establish a causal relationship between the M-protein and the observed renal abnormality. Therefore, kidney biopsy is essential and should be performed in all cases of suspected MGRS. Laboratory evaluation in patients suspected of having MGRS is summarised in table 2.

Monoclonal protein testing

Typically, MGRS exhibits low levels of circulating M-protein reflecting the small size of the underlying B-cell or plasma cell clone.[21] With detection limits of 500-2000 mg/l, serum protein electrophoresis (SPE) has insufficient sensitivity to detect low levels of M-protein, particularly free light chains (FLCs) because of their lower half-life compared to intact Ig (table 3).[49-51] Kyle et al. found that an M-protein cannot be detected by routine SPE in > 50% of the patients with AL amyloidosis.[52] Serum immunofixation electrophoresis (IFE) is approximately 10-fold more sensitive than SPE, yet urine IFE and/or serum free light chain assay (sFLC assay) are usually necessary to detect an M-protein.[49]

An sFLC assay utilizes antibodies directed against epitopes that are exposed in "free" LCs but hidden in intact Ig molecules. This assay provides a quantitative measurement of both κ and λ FLCs with a sensitivity of < 5 mg/l, and calculation of a κ/λ ratio can detect unbalanced LC synthesis as a surrogate marker for a monoclonal gammopathy.[49,50] Studies evaluating the diagnostic performance of this test have documented detection of

Table 2. *Laboratory evaluation of suspected MGRS*

Serum measurements
Creatinine
Potassium
Bicarbonate
Calcium[a]
Phosphate[b]
Glucose[b]
Uric acid[b]
M-protein diagnostics including free light chains
2. Urinanalysis
Creatinine
Total protein
Albumine
Phosphate[b]
Glucose[b]
Uric acid[b]
Microscopic evaluation urine sediment
3. 24-hour urine collection
Creatinine
Sodium
Total protein
Albumin
Bence Jones protein
Total volume

Note: The abovementioned parameters need to be performed to assess kidney function, acid-base status, and possible metabolic complications in the context of chronic kidney disease.
[a]Presence of hypercalcaemia should prompt further investigation of possible multiple myeloma.
[b]To detect proximal tubular dysfunction/Fanconi syndrome.
MGRS = monoclonal gammopathy of renal significance.

abnormal κ/λ ratios in 100% of patients with LC MM[49,52], in 76-98% of patients with AL amyloidosis[52-55], and in 92-100% of patients with LCDD.[56-58]

For more than 10 years, sFLC quantification has been performed by an immunonephelometric assay based on polyclonal antibodies (Freelite®, The Binding Site, Birmingham, UK). In recent years, novel assays based on monoclonal antibodies have entered clinical practice.[51,59,60] The Freelite FLC assay and one of the novel assays (N Latex FLC assay®, Siemens Healthcare Diagnostic Products GmbH, Marburg, Germany) seem to have similar diagnostic performance, though current data indicate that they are not interchangeable, especially in monitoring response to therapy.[59,61,62]

Reference intervals for the Freelite serum FLC were defined by Katzmann et al. (normal range κ: 3.3-19.4 mg/l; λ: 5.7-26.3 mg/l; κ/λ ratio: 0.26-1.65).[56] Since FLCs are filtered by the glomerulus and metabolised in the proximal tubules, caution is required when interpreting this assay in the context of renal impairment. As glomerular filtration rate (GFR) significantly declines, the removal of FLCs by reticuloendothelial pinocytosis becomes more important resulting in a prolonged FLC half-life and polyclonal increase in both κ and λ FLCs.[63] In patients with normal GFR, the increased physiological production of polyclonal κ LCs (molecular weight (MW) 22.5 kDa) is masked by the more rapid clearance of this monomeric LC compared to larger dimeric λ LCs (MW 45-50 kDa).[64] As the differential ability to clear κ and λ LCs by the kidney is lost, the κ/λ ratio can slightly increase and, for this reason, adaptation of the normal range to 0.37-3.17 increases the reliability of the Freelite FLC assay in patients with renal impairment.[65] In the BeNeLux region, most clinical laboratories make use of the Freelite FLC assay, which has an adjusted FLC ratio reference range of 0.37-3.17. In terms of M-protein diagnostics, a patient with MGRS and renal impairment would reach complete response (CR) in case of negative IFE in serum and urine. Provided that CR criteria are met and clonal cells in bone marrow are absent, the patient reaches serum CR when the adjusted FLC ratio is normal.

On the contrary, when using the N Latex FLC assay, there is no need for a separate renal reference range since the κ/λ ratio in patients with kidney disease does not differ from the normal values in healthy controls.[66] Adaptation of the κ/λ ratio in the first assay and the unadapted κ/λ ratio in the second assay, illustrate the heterogeneity that exists in FLC measurement. Therefore, follow-up of FLC measurements should be performed using the same assay and the same platform.[62]

International guidelines recommend using an sFLC assay along with SPE and IF as an initial screening panel for monoclonal gammopathies, because of the incremental sensitivity and potential limitations of urinary assessment in this setting.[67,68] Since FLCs only appear in the urine when the proximal tubular reabsorption capacity is overwhelmed, low-level serum FLCs will not be detected by urine tests.[49] Furthermore, urine analysis can be unreliable due to errors in carrying out a correct 24-hour urine collection, or due to inaccurate interpretation of EP results in concentrated urine samples or nephrotic-range proteinuria.[49] On the other hand, several studies have proven the necessity to perform both serum and urine IF for optimal sensitivity in AL amyloidosis and LCDD.[54] In addition, since 24-hour urine collection is necessary to calculate the GFR more precisely and measure the amount of proteinuria, urine IF and EP can be performed on this sample.

Table 3. *Overview of methods for monoclonal FLC detection*

	Serum protein EP	Urine protein EP	Serum IF	Urine IF	sFLC assay
Quantitative or qualitative	Semi-quantitative	Semi-quantitative	Qualitative	Qualitative	Quantitative: independent measurement of κ and λ FLC + calculation of a κ/λ ratio
FLC detection limit (sensitivity)	500-2000 mg/l	20-50 mg/l	150-500 mg/l	20-50 mg/l	κ: 1.5 mg/l λ: 3 mg/l
Advantages	Inexpensive; Easy to perform.	Inexpensive; Easy to perform.	10x more sensitive than serum PE.	-	Valuable as prognostic factor; Valuable for monitoring response to therapy.
Disadvantages	Low sensitivity for detection of low levels M-proteins, FLCs in particular.	FLCs in urine only when tubular reabsorptive capacity is overwhelmed; 24-hour urine collection required; Identification of monoclonal FLCs is a subjective interpretation of EP results; Difficult interpretation of EP results in concentrated urine or proteinuria.	-	FLCs in urine only when tubular reabsorptive capacity is overwhelmed 24-hour urine collection required.	More expensive; FLC assays are not accurate and measurements results are not equivalent between different methods; Assay reactivity of monoclonal and polyclonal κ and λ FLC in specific disease groups needs improvement.

EP = electrophoresis; IF = immunofixation; FLC = free light chain; sFLC = serum free light chain

Kidney biopsy

A kidney biopsy is crucial for the diagnosis of MGRS to determine whether the M-protein is an innocent bystander or the cause of the renal disease. Detailed IF, immunohistochemistry (IHC), and EM studies to identify deposit composition and ultrastructural organisation pattern should be applied. In this respect, it is important to emphasize that EM should be part of the standard work up, since FGN, ITG, type 1 CG and crystalline LCPT can only be diagnosed with EM. An exception can be made for patients with suspected AL amyloidosis, in whom other tissue specimens (e.g., abdominal fat) can be sufficient for diagnosis if clinical criteria for renal involvement are met.[69]

In selected cases, more sophisticated techniques such as immunogold EM or proteomics via laser microdissection and by mass spectrometry (LMD/MS) are required to characterise the component proteins of dense deposits.[70,71] LMD/MS is not only considered the gold standard for accurate typing of amyloidosis, it has also proven to be extremely useful for correct diagnosis and understanding of other MGRS.[72,73] The use of these advanced techniques may require sending the biopsy sample to a specialised centre and is therefore usually reserved for equivocal results or difficult cases.

Bone marrow

In order to identify the underlying B-cell or plasma cell clone, which is essential to determine treatment strategy, a detailed haematologic evaluation should be performed in all cases.[21,37] Overt MM or WM should be excluded, and BM aspirate and biopsy generally show only a small increase in plasma cells or B-cells (by definition < 10%) without morphologic abnormalities.[21,37,74] A Congo red stain performed on the BM biopsy could demonstrate amyloidosis which is present in approximately 60% of AL amyloidosis cases.[75] IHC and/or flow cytometry is mandatory to establish clonality and to make the connection between the circulating M-protein and the presence of this M-protein in the kidney.[21,37]

TREATMENT OF MGRS

The aim of the treatment in MGRS is to preserve or improve organ function by targeting the B-cell or plasma cell clone that is responsible for production of M-protein and organ damage. Achieving complete haematologic response will lead to better organ response and thereby prevent progression of organ damage.[37] Current evidence strongly supports the strategy of clone-directed therapy.

Except for amyloidosis, very few data regarding treatment options exists for the other MGRS (sub)types and there are currently no evidence-based recommendations. Based on literature concerning MM and AL amyloidosis, it can be assumed that when treating MGRS, the best results are achieved by targeting the underlying MGUS clone and aiming for a deep and prolonged haematologic response. Treatment depends on the isotype of the underlying clone in the BM (IgG, IgA, or LCs only versus IgM clone), the renal metabolism and potential renal toxicity of the therapy, and presence of neuropathy in the patient.[37]

Plasma cell dyscrasia with IgG, IgA, or LCs only

In case of an IgG, IgA, or LC-only-producing plasma cell clone (non-IgM MGUS), therapy directed at eradication of the plama cell clone with anti-myeloma agents should be considered to preserve kidney function. The most important drug in the treatment of MGRS associated with a plasma cell clone is the proteasome inhibitor bortezomib. Bortezomib has a nonrenal metabolism and is usually given in combination with dexamethasone. Other proteasome inhibitors are currently available, but bortezomib has the most robust data in the treatment of MGRS. In MM patients with renal impairment, bortezomib-containing regimens have demonstrated rapid reduction of tumour load and improved kidney function. In addition, in a study with 27 dialysis patients with MM, bortezomib was used as induction therapy and showed a higher overall response rate compared to induction therapy with conventional chemotherapy, and reduced the transplant-related mortality.[76,77] Bortezomib has been demonstrated to be highly effective drug in AL amyloidosis and seems to be the most effective agent in MIDD as high rates of CR/very good partial response (VGPR) have been described after treatment with bortezomib-based regimens and melphalan-conditioned autologous stem cell transplantation (ASCT).[12,77-79] Furthermore, integration of bortezomib both before and after ASCT overcomes the negative impact of renal failure in AL amyloidosis.[80-81]

For an optimal treatment strategy, it is important to determine whether patients are eligible for ASCT since this therapy can deepen and prolong the duration of remission. Similar to MM, ASCT is preceded by high-dose therapy with melphalan. The melphalan dose is normally reduced in cases of GFR of < 40 ml/min. Induction therapy prior to ASCT can be omitted in cases of a small clone, but proves to be beneficial for patients with a poor performance status due to MGRS. The treatment-related mortality of ASCT is < 1%.

Determination of eligibility for ASCT differs across countries and institutions. However, the decision to undergo ASCT should be made on a case-by-case basis by reviewing the risk-benefit assessment, and the needs and wishes of the patient. In general, patients younger than 70 years of age, with sufficient cardiac function (ejection fraction > 45%), sufficient pulmonary function, a systolic blood pressure of > 90 mmHg, World Health Organisation performance score < 2, and New York Heart Association scores I-II, are eligible for ASCT. Interestingly, kidney disease is not a contraindication to ASCT, although it is crucial that the renal function is stable. Kidney disease has no adverse effects on the quality of stem cell collection or their engraftment and ASCT is possible in dialysis patients.[82-88] However, the procedure is associated with increased risk of transplant-related mortality for patients with kidney disease compared to those with normal kidney function.[86] Patients who do not meet the abovementioned criteria and who also have an active infection are not eligible for ASCT. Overall survival is strongly dependent on the haematologic response according to most AL amyloidosis data.[86,87] Since MGRS is more similar to MGUS with regard to few plasma cells in the bone marrow, it would be fitting to treat patients who are ineligible for ASCT, with six to eight courses of bortezomib in combination with dexamethasone and cyclophosphamide, or bortezomib plus melphalan-prednisone. Treatment with immunomodulatory drugs (IMiD) such as thalidomide and lenalidomide can be used as an alternative, and in a relapse setting, pomalidomide can be used. Unlike lenalidomide, thalidomide and pomalidomide are not excreted by the kidneys and dose modifications are therefore not needed.[89,90] However, lenalidomide has been shown to be more effective than thalidomide and is thus the preferred alternative to bortezomib.

B-cell clone with IgM M-protein

Since IgM MGUS is rare, there is little evidence supporting the choice of treatment in MGRS-related to IgM M-protein. When the underlying BM clone is an IgM M-protein-producing and CD20-expressing B-cell or lymphoplasmacytic clone, rituximab-based therapy is the first choice of treatment. Rituximab can be combined with dexamethasone and cyclophosphamide or bendamustine in MGRS associated with IgM-MGUS. Rituximab can be safely administered without dose modification in patients with decreased kidney function.[91] Approximately 60% of cyclophosphamide is eliminated through the kidneys. Studies describe an increase of cyclophosphamide exposure in patients with kidney disease, however, dose adjustments in these patients remain variable in the literature.[90,92] Bendamustine does not appear to alter the pharmacokinetics in moderate renal impairment but limited data suggest toxicity increases in patients with GFR < 40 ml/min.[93] The benefit of ASCT in these patients has not been demonstrated but since a deep haematologic response is most likely beneficial, this option should also be considered.

The optimal treatment strategy of an IgM-related MGRS disorder should be constructed with the consultation of experts on MGRS, for example, the multidisciplinary Benelux MGRS Working Group, with experience in the use of these drugs.

Supportive care

In all patients, hypertension and/or proteinuria should be treated, preferably with renin-angiotensin system (RAS) inhibitors combined with salt restriction. Diuretics can have an extra antihypertensive and/or antiproteinuric effect. In case of AL amyloidosis, treatment with RAS blockade should be prescribed with caution as these patients have a tendency towards hypotension. In FS, prevention of osteamalacia with bicarbonate, phosphate, and vitamin D supplementation should be considered.[22]

Response to therapy

In MGRS, assessment of the haematologic response to treatment is crucial because the renal response is dependent on the haematologic response. For example, renal response rate in AL amyloidosis was significantly higher in patients with > 90% suppression of the nephrotoxic M-protein.[87] In MIDD, achieving haematologic complete response showed similar benefits for the kidney.[79] In AL amyloidosis, measurement of the sFLC is an essential tool for the assessment of a haematologic response. The response criteria used in AL amyloidosis are CR, VGPR, partial response (PR), and no response (NR)[94], and it seems logical to use these same response criteria in the other MGRS disorders. The use of sFLC assay for response has been suggested for all MGRS involving LCs only.[22] In cases of an undetectable or difficult to measure M-protein, the haematologic response can be assessed with repeated bone marrow examinations using sensitive multicolor flow cytometry. Sometimes, the GFR and proteinuria can be the only parameters used to assess disease activity.[94] It is important to note that the renal response is usually delayed. In one study, a minimum duration of 12 months of haematologic response was needed until onset of renal response was seen in patients with AL amyloidosis.[95]

CONCLUSION

In summary, MGRS consists of a variety of kidney diseases that are caused by the production of an M-protein resulting in kidney deposits and/or autoimmune activity. Histopathologic identification and classification are crucial for determining the optimal treatment for MGRS. For the diagnosis of MGRS, it is important to identify the circulating M-protein and to establish a causal relationship between the M-protein and the renal damage. A kidney biopsy with detailed examination by LM, IHC, IF, and EM is needed in the diagnosis and aids in characterising the MGRS lesion.

Clone-directed therapy is currently the most effective treatment strategy. Choice of therapy will depend on type of clone, eligibility for ASCT, side effects, and scarce literature support. Preferably, patients should be managed by a multidisciplinary team consisting of nephrologists, haematologists, and nephropathologists, with expertise in MGRS for optimal care. In cases of suspected or proven MGRS, the Benelux MGRS Working Group can be consulted during diagnosis and treatment of possible MGRS patients.

DISCLOSURES

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

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ORIGINAL ARTICLE

The simplified oral flucloxacillin absorption test: an accurate method to identify patients with inadequate oral flucloxacillin absorption

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ABSTRACT

Background: The preferred treatment for severe methicillin-sensitive *Staphylococcus aureus* infections is flucloxacillin, a small-spectrum antibiotic administered intravenously (IV) and orally. However, clinicians switch to the less preferred broad-spectrum antibiotics because of the variable absorption after oral administration of flucloxacillin. A classical oral absorption test (OAT) requires overnight fasting and interruption of IV therapy, and is laborious. In the current study, we investigated whether a simplified OAT can be utilized in a clinical setting to guide antibiotic treatment in patients with severe *S. aureus* infections. For this, OAT IV therapy is continued and oral dosing is performed after a one-hour fast and implemented after a small study.

Methods: In 196 patients receiving IV flucloxacillin by continuous infusion, a classical OAT (test A) or simplified version of the OAT (test B) was performed. In both tests, 1 g oral flucloxacillin was given and serum samples were taken prior to intake and at one and two hours after administration. Flucloxacillin concentrations were determined by high-performance liquid chromatography. Adequate absorption was defined as an increase of flucloxacillin concentration of at least 10 mg/l after one or two hours compared to baseline.

Results: In a sample of 196 patients (85 F/111 M), test A was performed in 28 patients, and test B in 168 patients. Age, gender, and baseline values of creatinine and albumin were similar in both groups. The maximal increase of flucloxacillin absorption was highly variable between patients. In 26 (13%) of the 196 patients, the flucloxacillin increase did not reach the value of 10 mg/l. The median (interquartile range, IQR) maximal increase of flucloxacillin absorption was 22.0 (15-31.25) mg/l for test A and 21.5 (13-32.25) mg/l for test B. There was no significant difference in maximal increase of flucloxacillin absorption between test A and B ($p = 0.74$), nor between males and females ($p = 0.95$). Age, creatinine, and albumin were not correlated with flucloxacillin levels.

Conclusions: The simplified version of the OAT is useful to identify patients with adequate oral flucloxacillin absorption, and to ensure the effective continuation of an oral small-spectrum treatment.

KEYWORDS

Flucloxacillin, serum concentration, absorption, *Staphylococcus aureus*

INTRODUCTION

Despite advances in antibacterial therapy and stewardship, the effective treatment of severe *Staphylococcus aureus* infections remains an important clinical challenge. Globally, the incidence of severe staphylococcal infections remains high,[1-2] which is partly due to the increasing use of indwelling catheters, vascular and orthopaedic prostheses, and prosthetic heart valves.[3] Severe *S. aureus* infections are associated with a high mortality rate and with associated complications (including infective endocarditis) being more prevalent compared to other bacterial infections.[4] It is clear that the effective management and treatment of severe *S. aureus* infections is essential. The treatment of choice for severe *S. aureus* infections

depends on concomitant variables including pathogen antibiotic susceptibility, patient factors (including underlying co-morbidities and concurrent medication), and physician preference. In countries with low endemic methicillin-resistant S. *aureus* (MRSA) rates, such as the Netherlands,[5,6] intravenous (IV) flucloxacillin is the preferred choice of treatment because of its bactericidal activity and narrow-spectrum of activity. Continuous IV infusion of flucloxacillin is followed by a course of oral flucloxacillin, which allows for earlier discharge of the patient from the clinic and has a reduced risk of catheter-associated complications. Flucloxacillin is rapidly absorbed with maximal serum concentrations observed at approximately one hour after intake. It has a high degree of protein binding (approximately 90%) and an elimination half-life of one hour.[7] Of note, previous studies have demonstrated a high degree of variability of flucloxacillin absorption following oral administration,[7-9] and the mechanisms underlying the observed variability remain unclear. It is of clinical importance to ensure that oral flucloxacillin is adequately absorbed in patients and that therapeutic serum levels are maintained, in favour of effective treatment of the underlying bacterial infection. Knowledge of the full pharmacokinetic profile of flucloxacillin in serum and the minimum inhibitory concentration (MIC) of the isolate are indicators of adequate oral dosing. The breakpoint MIC for flucloxacillin-susceptible *S. aureus*, defined as the highest MIC value indicating susceptibility, is commonly defined as < 0.5 mg/l of free drug. Given its high binding capacity, this will translate into a total serum drug concentration of 5 mg/l. Therefore, we routinely accept serum flucloxacillin concentrations of at least 10 mg/l as therapeutic levels, as these are associated with protein-free drug concentrations of > 1 mg/l, which is well above the MIC.[10,11] This level of exposure was therefore commonly accepted and associated with the efficacious treatment of susceptible *S. aureus* strains by beta-lactam antibiotics.[11] The oral absorption test (OAT) is used to ensure an efficacious switch from intravenous to oral therapy and has been routinely performed at our institution (Leiden University Medical Centre, the Netherlands). Results have shown that in approximately 10% of patients, the absorption of oral flucloxacillin was insufficient to reach therapeutic levels (i.e., a maximal serum concentration increase of < 10 mg/l).[9] The OAT format was laborious and error-sensitive, as it required the cessation of the continuous IV flucloxacillin administration eight hours prior to oral intake of the test dose. Recently, we demonstrated that a simplified version of the OAT performed similarly to the classical OAT, was easy to administer and could be implemented in hospital pharmacies at low equipment and staff costs.[9] However, our key concern was the small patient sample (43 patients) and retrospective design of that study. In the current study, we aimed to confirm our previous findings in a larger patient population, to investigate whether a simplified OAT can be utilized in a clinical setting to guide antibiotic treatment in patients with severe *S. aureus* infections, and to screen for factors associated with the previously observed inter-individual variability in oral flucloxacillin absorption.

PATIENTS AND METHODS

This study complied with institutional guidelines and Dutch law, as the evaluation concerned daily routine practice that adheres to the law on the medical treatment agreement (WGBO; Wet op de Geneeskundige Behandelings Overeenkomst). Hence, separate medical ethical approval was not needed.

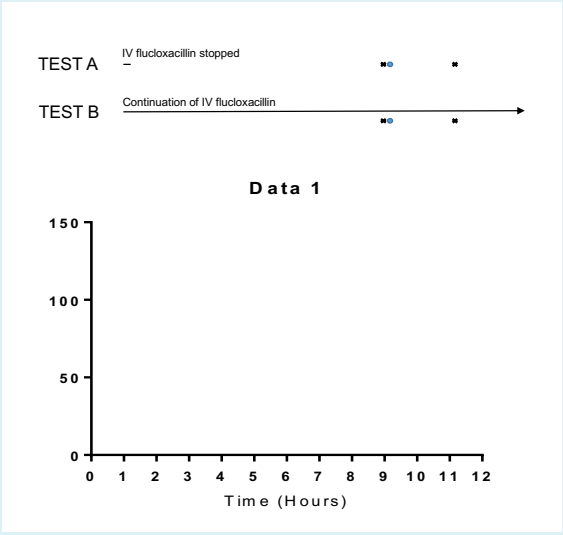
Patients

The evaluation period included adult patients admitted between 2011 and 2017 to Leiden University Medical Centre, Leiden, the Netherlands. Data were retrospectively collected from 196 hospitalised patients receiving continuous IV flucloxacillin and scheduled for oral flucloxacillin treatment. No potentially eligible patients were excluded from the evaluation.

Flucloxacillin Oral Absorption Tests

We evaluated two separate test protocols to assess the oral absorption of flucloxacillin (figure 1). The simplified version of OAT (test B) was already implemented. The classical OAT (test A) was occasionally performed due to ingrained habits. Test A commenced with an overnight fast and interruption of continuous IV infusion of flucloxacillin for eight hours. Thereafter, an oral test dose of flucloxacillin (1 g) was given. Blood samples for serum flucloxacillin concentrations were taken at baseline, and at one hour after oral dosing, according to the expected C_{max} of flucloxacillin. Because of the expected inter-patient variability in C_{max} levels, it was decided to add a measurement at two hours. This also allowed for a better estimation of flucloxacillin absorption in case of diminished gastrointestinal motility. Test B required the continuation of IV infusion of flucloxacillin. An oral test dose of flucloxacillin (1 g) was given after a one-hour fast. Measurement of serum flucloxacillin concentrations were performed at similar time-points as in test A (figure 1).
Adequate absorption was defined as an increase of serum flucloxacillin concentration of at least 10 mg/l from baseline at either sampling time. Because of the high level of protein binding and renal excretion of flucloxacillin,

Figure 1. *Diagram to show test A and test B OAT protocols*



• = OAT; x = blood sample taken for serum flucloxacillin concentration

albumin and serum creatinine were also assessed in patients prior to oral flucloxacillin dosing.

Flucloxacillin assay

Flucloxacillin serum concentrations were determined using a validated high-performance liquid chromatography (HPLC) method with ultraviolet detection (all equipment from Dionex Corporation, Sunnyvale, CA, USA). We added 10 µl of a 1 mg/l of cloxacillin solution (Sigma) and 0.5 ml of acetonitrile (Promochem) to 0.5 ml of a thawed patient serum sample. The samples were then vortexed and subsequently centrifuged for five minutes at 25,000 g. Thereafter, 0.8 ml of the supernatant was transferred to a 10 ml polypropylene test tube, and 3.5 ml of chloroform (Merck) was added. The samples were vortexed and centrifuged for three minutes at 5,500 g. We mixed 0.1 ml of the aqueous upper layer with 0.1 ml of acetate buffer (0.1 mole/l), and 20 ml of this solution was assayed by HPLC.

The chromatographic system consisted of an octadecylsilica Hypersil stationary phase (3 mm particle size, length 12.5 cm, id 4.6 mm), and a mixture of 1 mole/l acetate buffer solution (pH 6), water, and acetonitrile (40 + 710 + 250, vol/vol) as mobile phase. Flow rate was 1.0 ml/min, and detection took place at a wavelength of 210 nm. A flucloxacillin reference solution in serum was pre-treated using similar methodology as in the patient samples. This solution was used to determine the flucloxacillin/cloxacillin signal ratio in patient samples. From this ratio, serum concentrations of flucloxacillin were calculated. The lower limit of quantification was 3 mg/l, and the assay showed linearity for flucloxacillin concentrations up to at least 100 mg/l. For a quality control sample of a predefined concentration (40 mg/l) we found a mean concentration of 44.4 mg/l (111%) after 15 tests over a one-month period, with a coefficient of variation of 4.0%.

Data analysis

Demographics of the study population were summarised. Baseline flucloxacillin levels, age, serum creatinine concentration and serum albumin concentration in relation with the maximal increase of flucloxacillin concentrations were visually explored. The change in maximal increase of flucloxacillin levels between test A and test B, and between males and females was tested using an unpaired Student's t-test. A p of 0.05 was considered statistically significant.

RESULTS

Of the 196 patients (85 females and 111 males) who were treated with IV flucloxacillin, the individualised dose of continuous infusion ranged from 6-12 g/d. Baseline characteristics of patients were comparable between OATs A and B (table 1). Two measurements at two hours after dosing were removed from the analysis due to unrealistic outliers (> 200 mg/l), probably because samples were taken erroneously from the flucloxacillin catheter. There was a difference in maximal increase of flucloxacillin absorption from baseline (figure 2). The median (IQR) maximal increase was 22.0 (15-31.25) mg/l for test A and 21.5 (13-32.25) mg/l for test B (figure 2). There was no significant difference in maximal increase of serum flucloxacillin levels between tests A and B (p = 0.744). No relationship could be identified between any of the covariates and the maximal increase of serum flucloxacillin concentrations (figure 3A-D). The inter-subject variation in flucloxacillin seemed to increase with increasing age. No statistical tests were performed due to insufficient data. In 26 (13.27%) of the 196 patients, the maximal increase of flucloxacillin concentration did not reach the predefined target of 10 mg/l. This was found in 10.7% patients using test A and 13.7% patients using test B.

There was no significant difference in maximal increase of serum flucloxacillin concentration between male and female subjects for test A (p = 0.80) and test B (p = 0.95). Additionally, there was no relationship between both serum creatinine and serum albumin levels and the observed maximal increase of serum flucloxacillin concentration (figure 2). Serum creatinine (n = 143) and albumin (n = 48) samples were not available for all included subjects (figure 3, C and D).

Most of the maximal flucloxacillin concentrations were reached at two hours after dosing (54.6%). In tests A and

Table 1. *Population characteristics, median (interquartile range) [range]*

	Test A		Test B	
Number of patients	28		168	
Gender	10 F	18 M	75 F	93 M
Age (yrs)	68.5 (64-75) [51-94]	54 (46-67) [24-77]	61 (49-70) [20-94]	66 (49-72) [21-91]
Serum Creatinine concentration (µmol/l)	61 (54-67) [33-79]	59 (51-77) [48-90]	80.5 (61-134) [44-1012]	90 (67-139) [29-807]
Serum Albumin concentration (g/l)	39 (33-40) [27-41]	42 (39-43) [27-44]	32 (28-38) [19-44]	27.5 (26-35) [20-55]

M = male; F = female

B, 45.4% of the apparent maximum concentrations were achieved one hour post dose and 54.6%, two hours post dose.

DISCUSSION

In the current study, we confirmed the finding of our previous study, wherein we demonstrated that a simplified version of the OAT was easy to perform and could be implemented in hospital pharmacies at low equipment or staff costs, and performed similarly to the classical OAT.[9] In the current study, we observed that there was no significant difference in maximal increase in serum flucloxacillin levels from baseline between tests A and B, the classical and simplified version of the OAT. As flucloxacillin has a wide therapeutic window, it was deemed that the short-term exposure to higher levels of flucloxacillin in the simplified OAT was safe. As such, the simplified OAT is not only a safe, viable alternative, but also has practical advantages compared to the classical OAT; for example, nursing staff is no longer required to stop and start IV pumps, which saves approximately 20 minutes of nursing staff time per test.

A large inter-subject variability in oral flucloxacillin absorption was observed in our patient sample. It should be noted that outliers were seen in the simplified OAT group, which adds an extra level of variability in absorption. We believe that this is mainly caused by the larger sample size in the simplified OAT group. Median and IQR values for both tests were comparable. Our study highlighted that a significant proportion of all patients (13%) demonstrate insufficient drug absorption of flucloxacillin. This finding is in agreement with the results of our previous study,[9] and of previously published pharmacokinetic data.[7,8,10] Our results further showed that there was no correlation between serum creatinine and serum albumin levels and the observed maximal increase of serum flucloxacillin concentration. In tests A and B, 45.4% of the apparent maximum concentrations were achieved one hour post dose and 54.6%, two hours post dose. This suggests that the C_{max} likely occurred sometime in between the sampling times. Future research may focus on optimising the sampling times to identify sufficient absorption to reduce the required samples from two to one. However, as covariates and high variability may impact the absorption process,[12] two samples are recommended to perform the absorption

Figure 2. *Box and whisker plots for the maximal increase in flucloxacillin at either one or two hours after an oral flucloxacillin dose of 1 g using tests A and B (interruption or continuation of IV flucloxacillin). The horizontal bold line across the box shows the median; the lower and upper lines indicate the 25th to 75th percentile of the data and the whiskers depict 1.5 x the IQR. Data outside this range is indicated with black circles. The dashed line indicates the cut-off value at 10 mg/l*

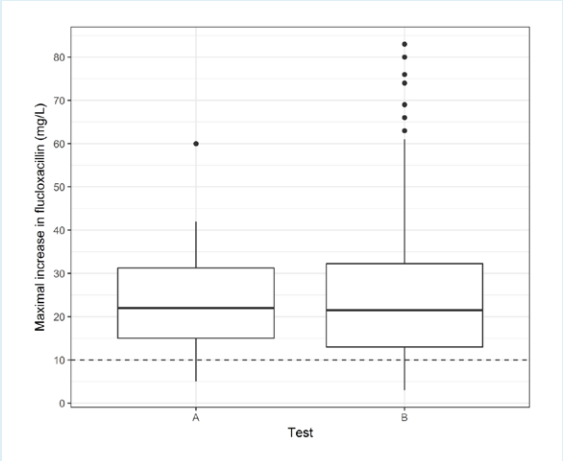
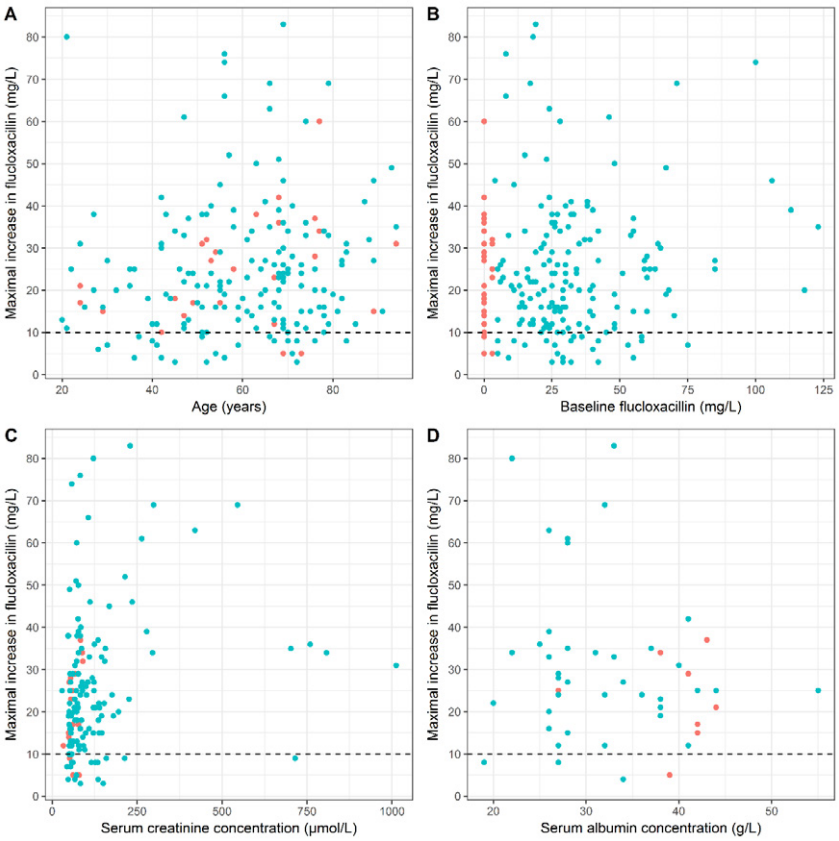


Figure 3. *Maximal increase in flucloxacillin after oral absorption test A (red circles) or B (blue circles) versus age (A), baseline flucloxacillin (B), serum creatinine (C) (n = 143), and albumin (n = 48) (D). Dashed line indicates cut-off for inadequate oral absorption of 10 mg/l*



test at this stage. Since C_{max} mainly depends on the extent and rate of drug absorption, minor or no effects could be expected from renal function or serum albumin. Knowing that flucloxacillin is predominantly excreted by the kidneys and is associated with a high degree of protein binding (approximately 90%), the observed lack of correlation suggests that both renal function and variation in serum albumin are not responsible for the observed inter-individual variability in oral flucloxacillin absorption in our patient sample.

The results of both simplified and classical OAT show that 45.4% of the maximum flucloxacillin concentrations were achieved one hour after administration and 54.6%, two hours after administration. This confirms the expected inter-patient variability in C_{max} levels, and justifies the use of two sampling time points in our study.

It should be noted that a substantial proportion of patients (14%) in the simplified and classical OAT groups did not absorb well. This could be explained by genetic variation in drug transporter enzymes or enzymes involved in the first-pass metabolism of flucloxacillin; however, the mechanism underlying the observed inter-individual variability in oral flucloxacillin absorption remains undetermined. Based on our personal experience and expertise, we believe that patients with an adequate level of oral absorption will generally demonstrate consistent levels of absorption in repeated tests. On the other hand, patients who are identified as poor absorbers, generally display variable levels of absorption during retesting. The reason for this variability remains unclear. Factors such as gastric emptying and intestinal motility could play a role; for example, late absorption of rifampin was associated with delayed gastric emptying such as diabetes mellitus.[13] Polymorphisms in the gut could also result in a reduced absorption of flucloxacillin and drug transporter polymorphisms like P-glycoprotein, or first-pass enzymes could further complicate the pharmacokinetic profile of variable flucloxacillin absorption. Another explanation

could be a pharmacogenomic mechanism, since a substantial proportion of the patient sample in our study showed levels of inadequate absorption. Currently, there is no evidence for the involvement of genetic variability leading to a differentiated expression or function of metabolic enzymes such as cytochrome P450 (CYP) or drug transporters, while un-identified polymorphisms in CYP gene expression and/or enzyme activity could indeed play a role in the increased hepatic breakdown of flucloxacillin. Similarly, genetic variation in P-glycoprotein polymorphisms or hepatic enzymes may influence the absorption and first-pass effect of flucloxacillin, resulting in inter-individual differences in flucloxacillin absorption and, hence, peak levels. We do know that a high dose of IV flucloxacillin for a minimum of two weeks prior to oral dosing might be responsible for the induction of increases in gene expression of drug transporters and/or CYP enzymes, as previous studies have reported the induction of hepatic CYP 3A4 and P-glycoprotein by flucloxacillin.[14-16] Therefore, there is a need for novel studies exploring how pharmacogenomics affect the pharmacokinetic profile of flucloxacillin.

Limitations of our study were the retrospective design and the absence of clinical assessments.

In summary, we have designed and confirmed that a simplified OAT can be utilized in a clinical setting to guide antibiotic treatment in patients with severe *S. aureus* infections. The HPLC/ultraviolet flucloxacillin assay can be easily performed by most labs, and can be implemented in hospital pharmacies with limited equipment and staff costs. We have demonstrated that this adapted OAT can be safe, less expensive, less time consuming, and less error-sensitive, compared to the classical OAT, and can adequately identify patients with insufficient oral flucloxacillin absorption without interruption of IV therapy; this is advantageous since serum levels will not drop below therapeutic levels. As the mechanism(s) underlying the observed inter-individual variability in oral flucloxacillin absorption remain elusive, it is vital to develop a quantitative test to clinically assess the efficacy of oral absorption of flucloxacillin to ensure patient safety and the efficacious treatment of underlying bacterial infections. For optimal management of patients with severe *S. aureus* infections, we strongly encourage fellow clinicians to adopt and implement our simplified OAT in clinical practice.

DISCLOSURES

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

ACKNOWLEDGEMENTS

We thank Tessa Nelemans (Master's student, Biomedical Sciences, Leiden University Medical Centre, the Netherlands) for support in data analysis.

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CASE REPORT

Spontaneous haemorrhage of hepatic adenoma in a patient addicted to anabolic steroids

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SUMMARY

This case report describes a patient with a nearly fatal spontaneous haemorrhage of a hepatic adenoma that occurred in association with anabolic androgenic steroid (AAS) use. The patient was addicted to AAS and had been using exceptionally high dosages as well as growth hormone. After cessation of AAS use, testosterone replacement therapy was started to prevent post-AAS-hypogonadism and consequent relapse.

What was known on this topic?
Hepatic adenomas are classically associated with the use of oral contraceptives in women.

What does this add?
Hepatic adenomas may occur in men using high dosages of AAS and lead to spontaneous life-threatening haemorrhage.

KEYWORDS

Anabolic androgenic steroids, performance and image enhancing drugs, hepatic adenoma, illicit drug use

INTRODUCTION

The use of anabolic androgenic steroids (AAS) by amateur strength athletes is widespread, with an estimated prevalence rate of 6%.[1] AAS are often used in cycles and are comprised of an injectable (intramuscular) testosterone ester and one or two other AAS types. Between cycles, no AAS are used, allowing the pituitary-gonadal axis to recover. However, about 5% use AAS continuously. In addition to AAS, other performance and image enhancing drugs (PIEDs) are commonly used, such as clenbuterol or growth hormone.[2]
Side effects are reported by virtually all users of AAS and include acne, gynecomastia, agitation, decreased libido, erectile dysfunction, and depressed mood.[2] Illegally obtained medications such as isotretinoin, tamoxifen, and human chorionic gonadotrophin are used to combat side effects. Little is known about the long term negative health effects of AAS use, but these likely include cardiovascular toxicity[3] and hypogonadism.[4] We present a case of a

Figure 1. *Axial image of a computed tomography scan of the abdomen. A large subcapsular haematoma is visible in the right liver lobe originating from a round, well-demarcated, heterogenous lesion with nodular attenuation, most probably a hepatocellular adenoma. Contrast extravasation inside the haematoma (yellow arrow) is a sign of active bleeding. Note the large size of the iliopsoas and erector spinae muscles*

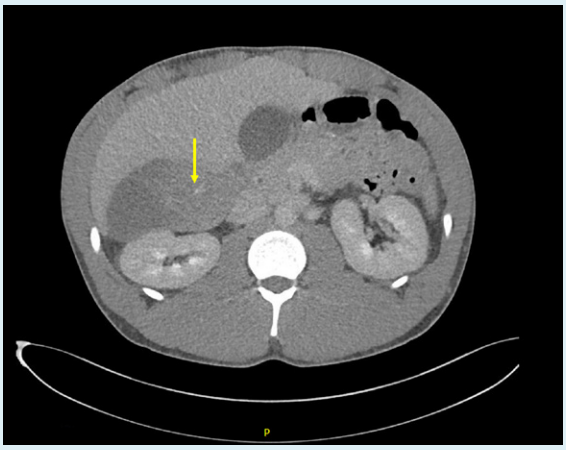
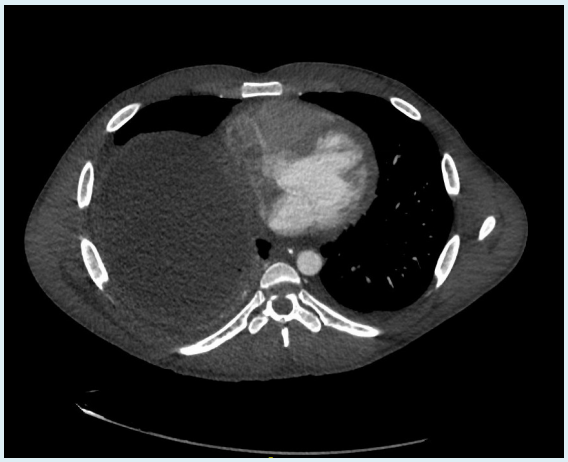


Figure 2. *Axial image of a computed tomography scan of the chest. The liver haematoma compresses the right atrium of the heart obstructing venous return and causing peripheral oedema*



strength athlete who used extreme dosages of AAS and suffered a spontaneous haemorrhage of a hepatic adenoma.

CASE STUDY

A 27-year-old muscular male with an otherwise unremarkable medical history presented at the emergency room with sudden severe abdominal pain in the right upper quadrant. Computed tomography of the abdomen revealed a 15 cm large subcapsular liver haematoma in a lesion typical of adenoma. Two smaller adenomas were present in the liver. There was evidence of active bleeding (see figure 1). Fluid resuscitation and blood transfusion were performed in the intensive care unit. A branch of the right hepatic artery was coiled to control the persistent bleeding and prevent multi-organ failure, as well as the need for emergency laparotomy. Continuous venovenous haemofiltration was necessary for several days because haemorrhagic shock had led to acute tubular necrosis.

The patient admitted he had been using AAS for the past five years. Before this, he had struggled with cocaine, cannabis, and alcohol addiction as well as depression. He started using a limited amount of AAS in cycles separated by several months. This was followed by shortened intervals, an increase in AAS types and dosages, and for the last three years he had been alternating cycles with a high maintenance dose ('blast and cruise'). His cycle prior to the haemorrhage comprised more than 10 different AAS types. During the peak of this cycle he injected 60 ml of AAS per week, which is about 15,000 milligrams of testosterone equivalents. In addition to AAS, he was concurrently using growth hormone (3.5 IU daily), clenbuterol, levothyroxine, tamoxifen, and anastrozole, as well as protein, vitamin, and mineral supplementations. The patient explained his escalated AAS use was due to a high level of ambition and a deep-seated distortion of his self-image.

After the patient was transferred to the gastroenterology ward, he needed opiates for heavy abdominal pains due to pressure of the haematoma on the liver capsule. The haematoma became infected with *Staphylococcus aureus* after haematogenic spread from an intravenous catheter. He received treatment with clindamycin after flucloxacillin had caused Stevens-Johnson syndrome. Antibiotic treatment was complicated by relapsing *Clostridium difficile*-associated diarrhoea for which he received vancomycin. The patient developed severe peripheral oedema caused by intermittent bleeding inside the haematoma that compressed the right atrium of the heart (see figure 2). The oedema resolved after a percutaneous drain was inserted into the haematoma to release eight litres of stale blood.

Almost two months after admission, the patient was discharged. During hospital admission, the patient did not use AAS and his testosterone concentration gradually declined from 195 nmol/l to 12 nmol/l. Gonadotropin levels were undetectable at all times. Replacement therapy with an injectable blend of testosterone esters was started on an outpatient basis when the testosterone concentrations became insufficient, with a limited issue of vials per prescription. The patient was followed-up alternately by an endocrinologist and addiction specialist at short intervals to survey hormone therapy, address the AAS addiction and muscle dysmorphia, and prevent relapse of AAS use. With magnetic resonance imaging, the liver adenoma was not visible anymore. One year after the hospital admission, the patient is in good health and has not used AAS or other illicit drugs again.

DISCUSSION

The occurrence of hepatic adenomas is very rare among men not using AAS, with an estimated incidence of less than 1 per million.[5] Spontaneous haemorrhage is a known complication of hepatic adenoma. The association between androgens and hepatic adenomas was noticed previously in patients treated for hereditary angio-oedema[6] and Fanconi anaemia[7] and has been reported in users of AAS as well.[8] The exact incidence of hepatic adenomas among users of AAS is unknown, but the majority is probably unnoticed. AAS presumably induce hepatic adenomas through the action of oestrogens derived from aromatization of AAS.[9] There appears to be a dose-dependent relationship between sex hormones and liver tumour occurrence, which may

explain the size and spontaneous haemorrhage of the adenoma in our patient.

The presented case illustrates the possible dramatic course of AAS addiction. Although the patient started with separate AAS cycles, he eventually used AAS continuously. The applied dosages in the months before the liver haemorrhage were astronomical with 15,000 mg of testosterone equivalents per week, which by far is the most reported by an AAS user based on our experience in the AAS clinic – an outpatient clinic for past users of AAS in the Netherlands. In an earlier survey, the average dose of AAS equivalents used during a cycle was approximately 1000 mg per week, which is already a tenfold physiological amount.[10]

As many as 30% of AAS users at some point develop a certain degree of AAS addiction. Addiction to other recreational drugs and muscle dysmorphia were risk factors in our patient that predisposed him to AAS addiction.[11] Treating a patient for AAS addiction should be a joint effort between an endocrinologist and addiction specialist. In this patient, due to prolonged suppression of the pituitary-gonadal axis, recovery of normal endogenous testosterone was unlikely in the short term and there was a high likelihood of a permanent post-AAS-hypogonadism.[2] Symptoms of testosterone deficiency would increase the urge to use AAS again. Therefore, we started testosterone replacement therapy as soon as hypogonadism occurred, which took several months due to the long half-life of certain testosterone esters, such as decanoate. Health parameters such as liver enzymes, haematocrit, and cholesterol were monitored. An addiction specialist with an interest in AAS addiction applied cognitive behavioural therapy. Although this treatment was successful in our patient in the first year, the risk of relapse remains high.

DISCLOSURES

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

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CASE REPORT

Limb salvage in antiphospholipid syndrome with repetitive arterial occlusions

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SUMMARY

Outcomes of vascular surgery for patients with primary antiphospholipid syndrome (APS) presenting with acute limb ischaemia (ALI) are poor, with a high rate of postoperative arterial thrombosis and limb amputation. A primary antiphospholipid syndrome 42-year-old male patient presented with acute limb ischaemia. Timely endovascular thrombectomy successfully prevented irreversible tissue damage but failed to maintain this due to recurrent thrombosis. Intensive plasma exchange following repeated endovascular therapy (EVT) ameliorated this thrombotic event. Two weeks post-discharge, thrombotic arterial reocclusion led to readmission and repeated management. Following successful reperfusion, intensive immunosuppressive therapy and anticoagulant agents ensured that the patient was free from recurrent events during the next eight months. This case highlights the combination of endovascular thrombectomy and intensive plasma exchange for limb salvage in such cases.

KEYWORDS

Acute limb ischaemia, antiphospholipid syndrome, endovascular thrombectomy, plasma exchange

What was known on this topic?
The frequency of arterial thrombosis is low in patients with antiphospholipid syndrome (APS). Surgical intervention, either bypass surgery or thrombectomy, is the current treatment option in these patients. However, the prognosis is poor after surgery, with higher rates of repeat arterial thrombosis and limb loss.

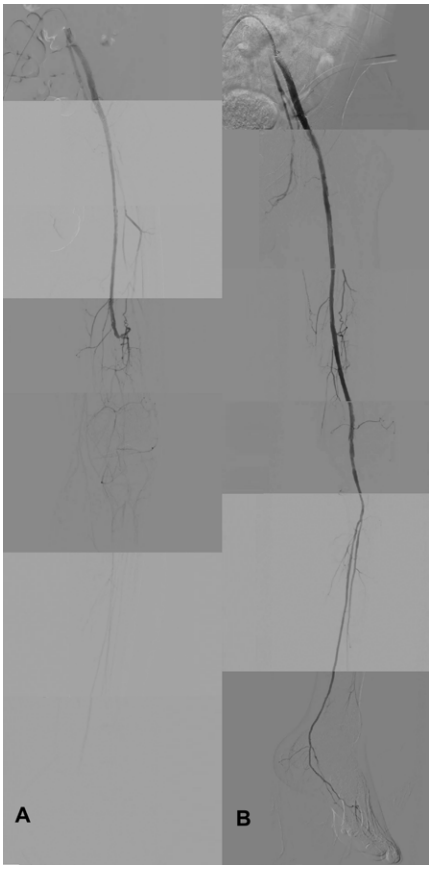
What does this add?
Limb salvage can be achieved with timely endovascular thrombectomy coupled with intensive immune clearance by plasma exchange in primary APS patients presenting with acute limb ischaemia.

INTRODUCTION

Antiphospholipid syndrome (APS) is an autoimmune disease characterized by cardinal manifestations of venous or arterial thrombosis and/or obstetric complications in the presence of antiphospholipid antibodies known as lupus anticoagulants (LAC), anticardiolipin antibodies, and antibodies against β2-glycoprotein 1.[1,2] Although the frequency of arterial thrombosis in the lower limbs is low in APS patients,[3] limb prognosis is poor, and the majority of patients eventually require minor or major amputations to prevent disease progression and mortality.[4] Surgical intervention, either bypass surgery or thrombectomy, has been the treatment option for acute limb ischaemia (ALI) in APS patients for the past few decades. However, reported rates of arterial thrombosis and limb loss post-operation were 50-91% and 43%, respectively.[5-7] Recently, endovascular therapy (EVT), with either catheter-directed thrombolysis (CDT) or thrombectomy devices, has become the first-line therapy for ALI patients. We report a case of APS complicated by repetitive arterial

Figure 1.
(A). *Baseline arteriography of the left leg at first admission showed thrombotic occlusion from the external iliac to the anterior tibial artery and the patent posterior tibial artery.*
(B). *Arteriography after successful recanalization and plasma exchange showed reappearance of left lower limb arteries*

Figure 2.
(A). *Baseline arteriography of the left leg at second admission showed thrombotic occlusion from the distal superficial femoral artery to the proximal posterior tibial artery.*
(B). *Arteriography, after successful management, demonstrated the recanalization of the left distal superficial femoral artery to the posterior tibial artery*



thrombosis, and demonstrate the efficacy of combined EVT and plasma exchange in rescuing the threatened limb.

CASE REPORT

A 42-year-old man was referred to our institution for resting pain and coldness in his left leg, which had persisted for several days. He was a non-smoker with no known risk factors for atherosclerosis, including diabetes mellitus, hyperlipidaemia, hypertension, or a premature family history of cardiovascular events. On admission, physical examination revealed coldness, livedo reticularis, the absence of pulsation in the left infra-inguinal arteries, and left foot drop. Duplex ultrasound showed thrombotic occlusion of the common femoral artery (CFA) to the anterior tibial artery, and patent left posterior tibial artery (PTA). Serologic tests were negative for all of the following: antinuclear antibody, anti-dsDNA antibody, anti-ENA antibody, antineutrophil cytoplasmic antibody, rheumatoid factors, complement C3 and C4, cardiolipin immunoglobulin G and immunoglobulin M, and anti-β2-glycoprotein 1 antibody. The test-to-control plasma ratio of LAC was positive in both screening (4.19; ≤ 1.20 for negative test) and confirmatory tests (2.45; > 1.2 for a positive test), as determined by the dilute Russell's viper venom time (dRVVT). Antithrombin III, protein C, and protein S levels were normal.

We performed diagnostic angiography via the right CFA with the cross-over approach, which showed complete thrombotic occlusion from the left external iliac artery (EIA) to the foot vessel with patent PTA only (figure 1A). After wire crossing, we performed pre-dilatation with a low-profile balloon followed by intravascular ultrasound assessment (Volcano, Visions PV 0.018, CA, USA), which was aligned with a thrombus-containing lesion between the EIA and the proximal PTA. Mechanical thrombectomy was performed using a Rotarex®S thrombectomy system (Straub Medical AG, Wangs, Switzerland), followed by balloon angioplasty from the EIA to PTA. Continued urokinase was administered via infusion catheter to treat residual thrombus, but the vessel repeatedly occluded. We performed another mechanical thrombectomy using the AngioJet thrombectomy system (Bayer Medical, Minneapolis, MN, USA) to treat the distal popliteal artery and proximal PTA. After successful restoration of direct flow to the foot, hydroxychloroquine and steroid therapy with methylprednisolone (80 mg per day) were administered to achieve immunosuppression of APS. In addition, four sessions of plasma exchange with fresh frozen plasma (FFP) as the replacement fluid were performed on alternate days for immune clearance. This reduced the thrombogenicity of LAC. The patient was successfully discharged one week later with a patent vessel after the treatment mentioned above (figure 1B). The discharge medications included dabigatran (150 mg) and hydroxychloroquine (200 mg) twice a day.

Two weeks post-discharge, symptoms in the left leg reoccurred, and angiography showed occlusion of the left distal superficial femoral artery and popliteal artery (figure 2A). We repeated EVT and administered 11 sessions of plasma exchange with FFP (consecutive sessions for the first three days, followed by eight sessions, performed on alternative days) to salvage this leg. Reintervention and removal of the LAC by plasma exchange freed the patient from recurrent thrombosis and restored direct flow to the left foot (figure 2B).

After discharge, we administered a total dose of 1000 mg rituximab (Roche, Basel, Switzerland) over a 2-week period, and maintained the following medications: dabigatran, ticagrelor, hydroxychloroquine, and prednisolone. The repeated test-to-control plasma ratio of LAC performed was still positive in both screen and confirmatory tests four months after the first episode, which confirmed the diagnosis of antiphospholipid syndrome according to the revised Sapporo criteria.[8] The patient was free from further thrombotic events over the next eight months, and duplex ultrasound showed sustained patency from the iliofemoral artery to the lateral plantar artery.

DISCUSSION

Although the incidence of ALI in patients with primary APS is rare, the prognosis for limb salvage is poor, with the majority of patients eventually undergoing major or minor amputations.[4] Previous studies reported a higher rate of postoperative thrombotic complications in patients with APS who underwent surgical treatment, especially for vascular procedures.[5,6] Ciocca et al. confirmed this extreme postoperative thrombotic rate.[7] Of 19 patients positive for antiphospholipid antibodies and undergoing surgical procedures for cardiovascular disease, 16 had postoperative complications, in particular, arterial thrombosis, and 12 died of complications related to surgery. Some authors postulated that damage to the endothelial cells of the vessels during manipulation might precipitate or aggravate the pre-existing susceptibility to thrombosis.[9]

EVT, a minimally invasive and reproducible procedure, has recently become the treatment of choice for patients with ALI. In this case, timely EVT averted irreversible tissue damage due to acute arterial occlusion. Plasma exchange was performed soon after restoration of the blood flow, aiming to remove antiphospholipid antibodies, cytokines, and complement to achieve a clean slate status. Nevertheless, repetition of thrombotic occlusions occurred two weeks post-discharge, suggesting that inadequate autoantibody clearance and procedure-related endothelial damage might induce further thrombus formation in patients with hypercoagulability status. During the second episode, removal of autoantibodies by intensive plasma exchange attenuated the hyper-response of the coagulation cascade. In addition to steroid and hydroxychloroquine treatment, rituximab was used to further suppress the hypercoagulability status.

This case highlights the feasibility of timely endovascular thrombectomy, coupled with intensive immune clearance after post-plasma exchange, for limb salvage in primary APS patients presenting with ALI. However, effective immunosuppression and appropriate anticoagulation are essential to prevent recurrent thrombotic events.

DISCLOSURE

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

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PHOTO QUIZ

Traffic jam and collateral pathways

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Figure 1A. *Abdominal varices*

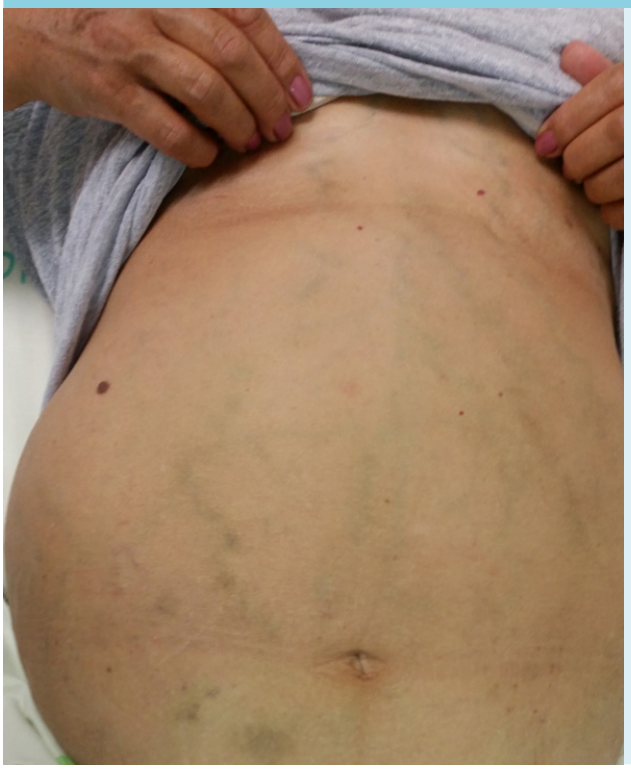
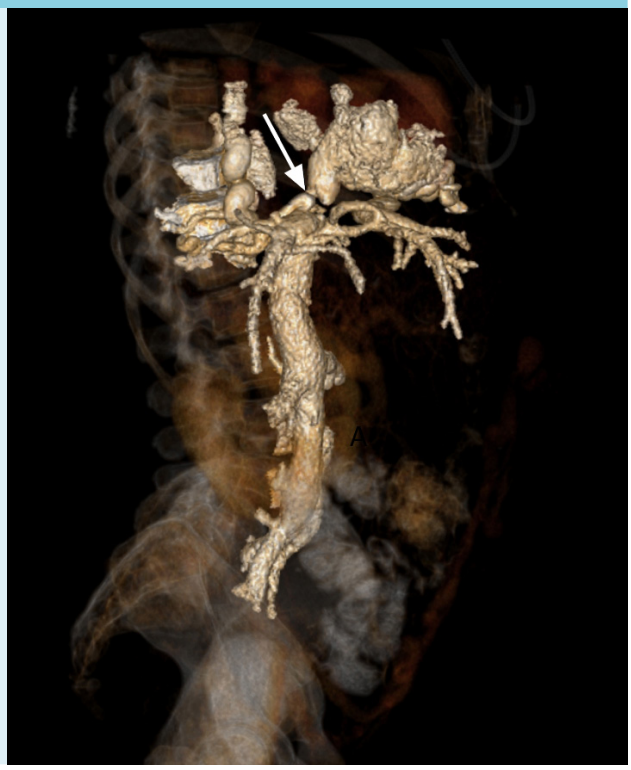


Figure 1B. *Stenosis of the inferior vena cava (arrow)*



CASE REPORT

A 64-year-old female presented at our center with diffuse abdominal pain. Since her second decade of life, her medical history has been significant for abdominal (figure 1A) and leg venous varicosities with spontaneous recurrent skin bleeding episodes. Endoscopy showed esophageal varices. Laboratory values revealed a low platelet count of 73,000 x 10^3/l, but were otherwise unremarkable with normal albumin level and (active) partial thromboplastin time serology results for hepatitis A, B, and C were negative. Abdominal ultrasound demonstrated normal liver size and texture, without evidence of ascites. Splenomegaly was found measuring 13.6 cm. Computed tomography (CT) angiography revealed a narrowing of the suprahepatic portion of the inferior vena cava (IVC) (figure 1B) with venous collaterals, but no evidence of thrombosis. Percutaneous balloon angioplasty with stent placement to the stenotic suprahepatic inferior vena cava was performed. The one-year follow-up visit revealed an increase in platelet count to 100,000 x 10^3/l, a decrease in spleen size to 10.5 cm, no episodes of leg varicosity bleeding, as well as an improvement in the esophageal varices grade.

WHAT IS YOUR DIAGNOSIS?

See page 269 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 268)

TRAFFIC JAM AND COLLATERAL PATHWAYS

DIAGNOSIS

Congenital stenosis of the IVC. This is characterized by narrowing of the IVC, with or without a web formation, mostly at the diaphragmatic level or hepatic segment.[1,2] The reported prevalence rate of interrupted IVC with azygos or hemiazygos continuation is 0.6%.[1,3] If a well-developed azygos or hemiazygos continuation is present, the patient will most likely be asymptomatic. If it is absent, this type of anomaly would be expected to be symptomatic.

Presence of acute or recurrent deep vein thrombosis, diffuse varices, varicocele, hemorrhoids venous aneurysm, or venous collaterals (including the abdominal wall) in a relatively young patient can indicate interruption or congenital stenosis of the IVC. Noninvasive imaging modalities such as multidetector row computed tomography (MDCT) and magnetic resonance imaging (MRI) are the most reliable methods for identification of these anomalies.[3]

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DISCLOSURES

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

PHOTO QUIZ

Haemoptysis: why is the etiological investigation important?

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Figure 1A. *CT Scan of the chest showing an image originating from the left inferior lobe with a contrast material of central zone (arrows)*

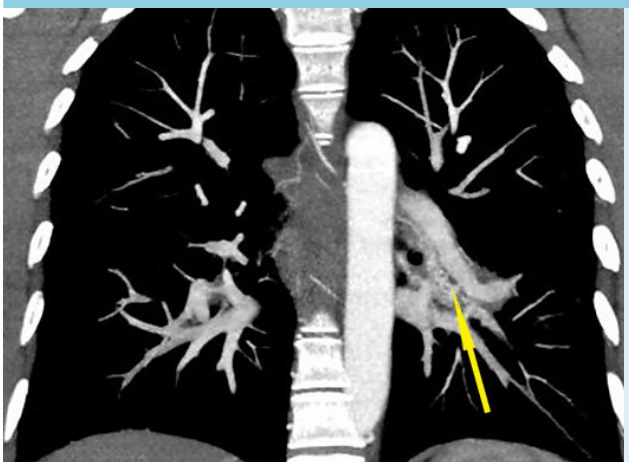
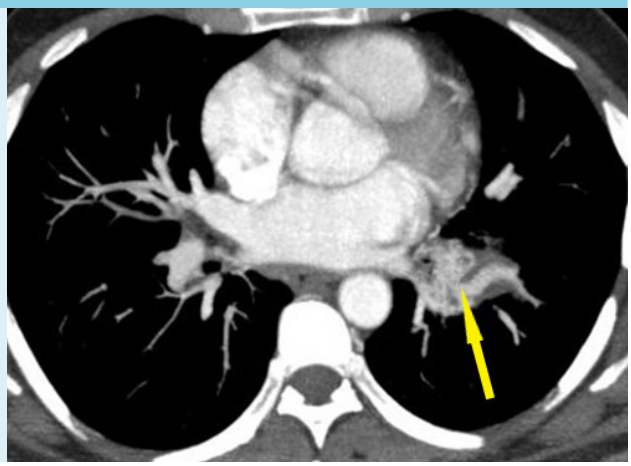


Figure 1B. *CT Scan chest axial view*



CASE REPORT

A 45-year-old man, working as a police officer presented with a 3-day history of intermittent haemoptysis. On physical examination, his blood pressure was about 130/70 mmHg with a heart rate of 100 bpm. He had no signs of heart failure. Blood tests showed a white blood cell count of 9000/µl, C-reactive protein of 38 mg/l, and erythrocyte sedimentation rate of 80 mm. Chest examination was normal. Chest computed tomography (CT) showed a lesion originating from the left inferior lobe with a contrast material of the central zone (figure 1A, B).

WHAT IS YOUR DIAGNOSIS?

See page 271 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 270)

HAEMOPTYSIS: WHY IS THE ETIOLOGICAL INVESTIGATION IMPORTANT?

Figure 2A. *After medical therapy, pulmonary artery aneurysm regression (arrows)*

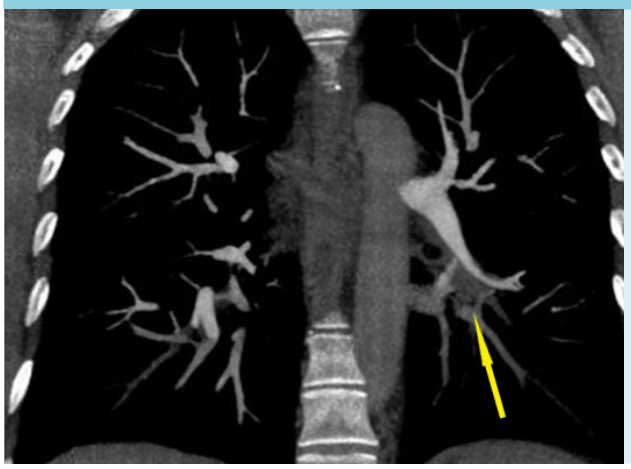
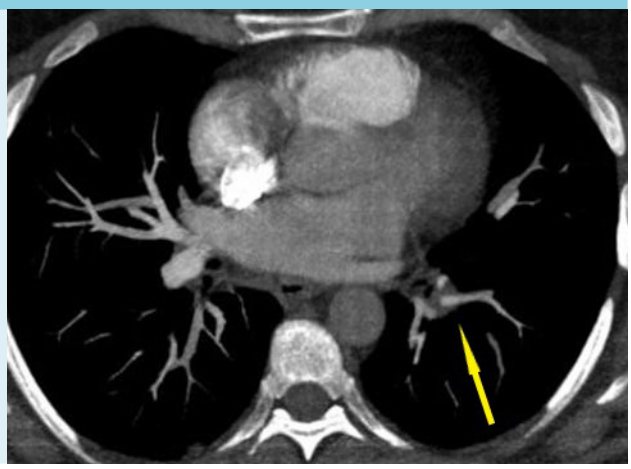


Figure 2B. *Axial view*



DIAGNOSIS

Review of the history of the patient showed that he was diagnosed six years ago with Behçet's disease (BD). The diagnosis was made according to International Criteria for Behçet's Disease based on the combination of oral and genital ulcers and acneiform lesions. He was treated with colchicine for recurrent oral and genital ulcers.

The diagnosis of BD, the inflammatory activity in his blood tests, and the CT interpretation by the radiologist suggested a pulmonary artery aneurysm in the left inferior lobe which was partially thrombosed. Other causes of haemoptysis particularly, tuberculosis, pulmonary malignancies, and anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis were considered unlikely. Screening of tuberculosis in sputum samples was negative, CT showed no signs suggestive of pulmonary malignancies and myeloperoxidase anti-neutrophil cytoplasmic antibody (MPO-ANCA) and proteinase 3 (PR3)-ANCA were both negative.

Intravenous methyl prednisolone 1 g/d for three days followed by oral prednisone 60 mg/d (1 mg/kg/d) was administered; the prednisone dose was subsequently tapered. Concomitantly, cyclophosphamide at a dose of 15 mg/kg was given every four weeks over six months and then switched to oral azathioprine (3 mg/kg/d). Within one month, haemoptysis disappeared and six months later, a chest CT showed resolution of the pulmonary aneurysm and thrombosis (figure 2A, B).

Pulmonary artery aneurysm is a life-threatening condition. Congenital vascular diseases are the main etiology. Others causes include infections, mainly tuberculosis and syphilis, pulmonary arterial hypertension, and chronic pulmonary embolism. BD can cause inflammation of the pulmonary arteries that result in aneurysms. Surgical or endovascular therapies are the commonly used treatment options for pulmonary artery aneurysm.

Our case illustrates the importance of considering the etiology of an arterial aneurysm before choosing the treatment option. Vascular surgery in BD is associated with a high risk of occlusion, recurrence, and false aneurysm formation.[1] Medical therapy combining corticosteroid and immunosuppressive agents should be considered in patients with arterial aneurysm due to BD.[2] No consensual treatment of pulmonary artery aneurysms caused by BD has been developed; the most commonly administered therapy is corticosteroid combined with cyclophosphamide pulses.[3] In refractory cases, anti-tumour necrosis factor should be considered[4] and surgery is discouraged due to the high risk of complications.[4] In our patient, a therapy consisting of combined corticosteroid and cyclophosphamide pulses followed by a switch to oral azathioprine therapy was effective.

DISCLOSURES

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

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