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EDITORIAL

The half-life of guidelines for Waldenström's macroglobulinaemia; short stickiness for a sticky disease?

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Waldenström's macroglobulinaemia (WM) is a B-cell lymphoproliferative disorder that has been challenging clinicians since it was first recognised by Dr. Waldenström in the mid-forties of the last century. Several reasons contribute to the difficulties that clinicians face with the management of patients with this disease. First, WM is a very rare entity. The overall age-adjusted incidence of WM is just 3.8 per million persons per year. As a comparison, the incidence of amyloidosis is 8 per million persons per year and the incidence of multiple myeloma is 40 per million persons per year.[1] Second, the wide clinical spectrum of the disease makes standardisation in when and how to treat complicated. The most common presenting symptom is fatigue, often related to a normochromic or normocytic anaemia. Organ-specific manifestations of the disorder are seen in less than a quarter of patients and include hepatomegaly, splenomegaly, and lymphadenopathy.[2] A unique characteristic of WM is the presence of monoclonal IgM protein. Due to its pentameric structure, antibody-binding specificity and protein-folding capacity, IgM paraproteinaemia can result in hyperviscosity syndrome, peripheral neuropathy, cold agglutinin haemolytic anaemia, type II mixed cryoglobulinaemia and immune complex vasculitis.[2] Although these syndromes can arise simultaneously, this is rarely the case and often, patients are referred to organ-specific specialists causing significant delay in diagnosis and treatment. Serum levels of IgM, however, have proven to be an unreliable marker for disease symptoms. Patients can present with markedly elevated IgM levels and infiltration of the bone marrow yet still not require therapy because they lack any symptoms. Conversely, patients with minimal clonal marrow infiltration and low levels of monoclonal IgM protein might require therapy for complications associated with IgM paraproteinemia.[3] In general, most patients who fulfil the criteria of WM do not require immediate therapy because many cases are detected before symptoms occur.
A third aspect which contributes to the absence of a clear standard of care for this disease is the fact that the large majority of clinical trials on WM are single-arm phase II studies. Many of such trials have been conducted in small series of patients and many include mixed patient populations including untreated, relapsed and refractory patients. Two smaller randomised trials have been published so far. One trial compared single-agent chlorambucil as continuous or pulse therapy. The other trial studied the addition of rituximab to the CHOP regimen in lymphoplasmacytic leukaemia of whom two thirds of the patients fulfilled the criteria of WM. Only very recently the results of a large phase III trial in 339 previously untreated patients with WM were published.[4] This study showed superiority of fludarabine as compared with chlorambucil as first-line treatment of WM. Although this study sets a benchmark for any future phase III trials its applicability to the current therapeutic options of patients with WM is limited since rituximab, now considered standard of care, was not part of the study regimen. Different phase II studies and the above-mentioned phase III study (CHOP vs R-CHOP) have shown that rituximab added to chemotherapy significantly enhances responses and progression-free survival without added major toxicity.

The above-described challenges in the clinical care of patients with WM are clearly reflected in a survey that has been carried out amongst Dutch Haemato-Oncologists, the results of which are published in this issue of the *Netherlands Journal of Medicine*.[5] The main results of this survey indicate that although diagnostic methods and

available treatment options for WM are generally well known, uncertainty exists on when to initiate treatment and how to deal with disease-specific and treatment-specific side effects such as hyperviscosity syndrome and the 'IgM flare syndrome'. Also, the survey clearly shows the lack of uniformity in the choice of treatment.

The lack of clinically applicable randomised trials and the overwhelming amount of novel agents highlights the need for guidance on the management of WM. A collaborative effort of the HOVON myeloma working party and the HOVON lymphoma working party resulted in the first Dutch guideline for the diagnosis and management of WM, which is also published in this issue of the *Netherlands Journal of Medicine*.[6] This practical guideline nicely integrates data from published trials with expert-based experiences and will improve standardisation of care for patients with WM in the Netherlands.

Although this guideline is currently up-to-date, its half-life is expected to be limited for two main reasons. First, treatment modalities for multiple myeloma and lymphoma are rapidly expanding and include novel agents with expected potency, also in WM, such as second-generation proteasome inhibitors and monoclonal antibodies, and third-generation immunomodulatory agents. Second, and probably more important, recent discoveries have unravelled the molecular mechanisms that are fundamental in the biology of the disease, paving the way for targeted therapies. Whole genome sequencing of lymphoma cells of patients with WM showed a recurring sequence variant resulting in the single nucleotide change L265P in the MYD88 gene in 90% of the WM samples. This activating mutation via the activation of IRAK kinases ultimately results in activation of NF-kB, a protein that is essential for the growth and survival of Waldenstrom's tumour cells.[7]

Activation of the IRAK-mediated NF-kB pathway by mutated MYD88 (L265P) depends on upstream activation of Bruton's tyrosine kinase (BTK).[8] Specific inhibitors of these kinases, including BTK inhibitors and IRAK inhibitors, have been and are being developed and clinically tested. Long-term effectiveness of these classes of drugs in WM is highly expected and clinical trials with the BTK-inhibitor ibrutinib have already been started. It is therefore anticipated that authors of this guideline need to reconvene in the near future in order to implement these promising new drugs in the management of patients with this still incurable disease.

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REVIEW

Guideline for diagnosis and treatment of Waldenström's macroglobulinaemia

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On behalf of the HOVON Multiple Myeloma Working Party and the HOVON Lymphoma Working Party

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ABSTRACT

On behalf of the lymphoma and multiple myeloma working parties of the Dutch/Belgian Haemato-Oncology Foundation for Adults in the Netherlands (HOVON), we present a guideline for diagnosis and management of Waldenström's macroglobulinemia (WM). Considering the indolent behaviour and heterogeneous clinical presentation of WM, it is crucial to determine the right indications for treatment, as well as to individualise therapeutic options. There are significant differences from the approach to multiple myeloma or the treatment of other indolent non-hodkgin lymphomas, and these results cannot always be extrapolated. There is a lack of large clinical trials due to the low incidence of WM.

Based on the available data, we provide a practical diagnostic classification, as well as recommendations for first-line therapy and options for treating relapsed disease. Some typical clinical features of WM, such as autoimmune phenomena and "IgM flare" after rituximab treatment, are highlighted.

A more elaborate version of this guideline was published in the "Nederlands Tijdschrift voor Hematologie" (Dutch Journal for Hematology) September 2012.

KEYWORDS

Guideline, lymphoma, MGUS, Waldenström, lymphoplasmacytic lymphoma

INTRODUCTION

In the latest World Health Organisation (WHO) classification (2008), Waldenström's macroglobulinaemia (WM) is defined as a neoplasm composed of small B lymphocytes, plasmacytic lymphocytes and plasma cells, accompanied by a paraproteinaemia of the IgM type, while not meeting diagnostic criteria for other small-cell B-cell malignancies. The term lymphoplasmacytic lymphoma (LPL) refers to WM plus those rare cases lacking the IgM M-protein (more than 95% of all LPLs are WM). In this guideline we will use the term WM, but it is also applicable to all cases of LPL.[1-4]

The Swedish physician Jan Gösta Waldenström (1906-1996) described this disease for the first time in 1944. It is a rare type of lymphoma: worldwide the incidence is approximately 3 per million persons per year. The average age at diagnosis is 65 years.

Although there are a wide range of therapeutic options, WM still remains incurable. Therefore, in asymptomatic patients, a 'wait and see' policy is advocated, such as in other indolent lymphomas. The prognosis is very variable with survival ranging from five to more than ten years. Because WM occurs mainly in the elderly, combined with its indolent course, half of the patients die of a cause other than the lymphoma.

This guideline includes recommendations on the diagnosis as well as the treatment of WM.

DIAGNOSIS AND DIAGNOSTIC CLASSIFICATION

For the diagnosis of WM, an IgM M-protein needs to be present in blood, as well as histological proof of a lymphoplasmacytic infiltrate, virtually always localised in the bone marrow. Depending on whether or not there are lymphoma-related symptoms, this can be classified as a symptomatic WM (with treatment indication) or asymptomatic WM (the latter has a higher risk of progression than IgM monoclonal gammopathy of unknown significance (MGUS)). (See *tables 1, 2* and *3*). Of importance, an IgM paraprotein of any level is not sufficient for the diagnosis WM, since there are several other lymphoproliferative disorders that produce IgM (see differential diagnosis). The IgM level is not predictive for the onset of symptoms, and is also not necessarily a reliable marker for tumour burden.[1-4]

CLINICAL SYMPTOMS, HISTORY TAKING AND PHYSICAL EXAMINATION

Approximately one third of the patients are asymptomatic at diagnosis. The symptomatology of WM is determined by both the tissue infiltration and immunological activity of the lymphoma cells, as well as by the physico-chemical properties and immunological specificity of the monoclonal IgM protein. The clinical presentation of WM is therefore often very different from other malignant lymphomas.

In the workup of WM patients, a thorough review of the systems during medical history taking is very important, as well as a complete physical examination, with special attention for lymphadenopathy, hepatosplenomegaly, neuropathy, autoimmune phenomena and signs of hyperviscosity (for a comprehensive list of symptomatology: see *table 1*).[5]

Table 1. *Symptomatology, frequency and mechanism*

Symptom or sign	Percentage at first diagnosis	Mechanism and/or specific recommendations
Fatigue	±70%	Anaemia Constitutional Consider amyloidosis
Constitutional symptoms (night sweats, weight loss)	20-25%	Constitutional
Lymphadenopathy, hepatosplenomegaly	15-25%	Tumour infiltration
Anaemia	40%	Bone marrow infiltration Haemolysis (cold or warm AIHA) Iron deficiency due to gastrointestinal bleeding
Hyperviscosity: Headaches, blurry vision or visual loss, confusional episodes, epistaxis	15%	Emergency: Get an ophthalmology consult for fundoscopy Consider emergency plasmapheresis When measured serum viscosity is >4.0 cp there is a high risk of hyperviscosity-related events
Bleeding tendency	20-30%	Thrombocytopenia i.e. ITP Acquired von Willebrand disease Amyloidosis
Neurological (mainly polyneuropathy)	20-25%	IgM antibodies against myelin-associated glycoprotein (MAG), ganglioside M1 (GM1) or myopathy (antidecorine antibodies) Amyloidosis
Bing-Neel syndrome: impressive neurological symptoms accompanied by WM localisation in CSF and/or abnormalities on MRI	Rare	Aetiology is uncertain: tumour infiltration or local IgM deposition in cerebro are possible causes
Raynaud's phenomenon (11%), acrocyanosis	Up to 20% cryoglobulinaemia, yet only <5% with associated symptoms 5-10% cold agglutinins	Cryoglobulinaemia Cold agglutination Reminder: immunoglobulins should be obtained in a warm bath to avoid cryoprecipitation and false lowering of serum IgM levels
Gastrointestinal symptoms	Unknown	Amyloidosis or IgM deposition Local tumour infiltration Autonomic neuropathy
Hearing loss	Unknown	Hyperviscosity, sensorineural neuropathy, Tumour localisation, thrombosis
Thrombosis	Unknown	Antiphospholipid syndrome via IgM antibodies
Dermatological: urticaria, papules, dermatitis, vasculitis	<5%	Schnitzler syndrome (nonpruritic urticaria), local tumour infiltration, amyloidosis, cold agglutination/cryo
Renal failure	Rare	Specific IgM-mediated glomerulonephritis, amyloidosis, vasculitis. Consider renal biopsy
Osteolytic lesions	Should not be present	When osteolytic lesions are present consider IgM multiple myeloma as a diagnosis
Recurrent infections	Unknown	Hypogammaglobulinaemia, consider antibiotic prophylaxis or IVIG suppletion

Table 2. *Diagnostic workup (Items in bold font should be done in each new patient)*

Test	Remarks
Serum electrophoresis incl. quantative M-protein; immunofixation **Immuunglobulines IgA, IgM, IgG**	Light chain assay in serum/urine: not indicated Reminder: immunoglobulins should be obtained in a warm bath to avoid cryoprecipitation and false lowering of serum IgM levels in patients with cryoglobulinaemia
Complete blood count, PT, APTT **liver enzymes, renal function**	Iron levels if anaemia is present
Bone marrow biopsy for morphology and immunohistochemistry	Please refer to text Immunophenotyping and cytogenetics are optional, they can be helpful to distinguish WM from other small cell b-NHL
CT thorax/abdomen/pelvis	Recommended for staging before starting treatment
Haemolysis parameters (LDH, haptoglobin, reticulocytes, if positive followed by Coombs test and testing for cold agglutinins)	If anaemia is present
B2-microglobulin and albumin	Prognostic markers
Viscosity measurement and ophthalmology consult	When there is clinical suspicion of hyperviscosity, obtain an ophthalmology consult for fundoscopy Measurement of serum viscosity is useful but the diagnosis of hyperviscosity syndrome can be made on clinical grounds only
Hepatitis C and B serology	Mixed (type II) cryoglobulinaemia is associated with hepatitis C, the association of HCV with WM is unclear Determining HCV and HBV status is relevant before starting therapy.
Myelin-associated glycoprotein (MAG), ganglioside M1 (GM1) antibodies (in PNP) or antidecorine antibodies (in myopathy) neurological evaluation incl. EMG	When polyneuropathy is present, in order to determine its cause (i.e. autoimmune versus amyloidosis)
Targeted biopsy if amyloid is suspected	Abdominal fat aspiration if targeted organ biopsy is difficult. Also test bone marrow biopsy for amyloid

Table 3. *Diagnostic classification*

	IgM MGUS	Asymptomatic WM	Symptomatic WM	IgM-related disorder * refer also to tables 1 and 3
IgM M-protein (serum)	Yes	Yes	Yes	Yes
Lymphoplasmocytic infiltration (bone marrow)	No	Yes	Yes	No
WM-related signs or symptoms*	No	No	Yes	Yes
Approach	Follow-up (infrequently)	Wait and see	Start treatment	Depending on specific manifestation, start treatment if applicable
Risk of progression to WM	1.5% per year	50-60% after 5 year	NA	Unknown

The most common presenting complaint is fatigue, often caused by anaemia. The anaemia is often more pronounced than expected based on the degree of bone marrow infiltration. Haemolysis, 'anaemia of chronic disease' caused by proinflammatory factors, dilution through increased plasma volume, and gastrointestinal bleeding can all contribute to the anaemia.

An increased bleeding tendency (of various origins) occurs in up to 20% of patients. Hepatosplenomegaly and lymphadenopathy occur in only 15-20% of patients. IgM-mediated autoimmune disease is a very distinctive manifestation of WM and can also be the presenting symptom. The most common autoimmune phenomena are neuropathy by anti-MAG (myelin associated glycoprotein) IgM antibodies, and autoimmune haemolysis caused by anti-I or anti-i IgM antibodies with complement activation (cold agglutination).

In about 10% of cases the IgM precipitates when the temperature drops below the 37 °C (cryoglobulinaemia); this is associated with vasculitis, Raynaud's phenomenon and glomerulonephritis. Precipitation of the M-protein into amyloid can lead to organ damage: neuropathy, cardiomyopathy, nephropathy or gastrointestinal problems. It is recommended to pay attention to the family history, since WM is sometimes associated with familial clustering of various lymphoproliferative diseases.[6]

Finally, because of the size of the IgM protein, hyperviscosity syndrome can develop with its typical symptoms: disturbed vision, headache, dizziness, heart failure and neurological complications. When hyperviscosity is suspected, an ophthalmological consult should be obtained to aid in diagnosis.

DIAGNOSTIC EVALUATION

Please refer to *table 2* for recommended diagnostic studies in WM patients.

Bone marrow evaluation

Bone marrow examination (biopsy) is required for the diagnosis, since this lymphoma is preferably, and often exclusively, located in the bone marrow. The infiltration typically consists of small B lymphocytes, plasmacytic lymphocytes and plasma cells (see histology illustrations in *figure 1*).

The typical immunophenotype is: expression of B-cell antigens (CD19/20/22/79a), membrane-bound IgM, and cytoplasmatic IgM in the plasma cells, with absence of IgD, CD23, CD103, and CD10. CD5 is usually negative, if positive chronic lymphocytic leukaemia (CLL) and mantle cell lymphoma should be excluded. Additionally, CD38 and CD27 are often positive. The plasma cells are CD138 positive.[1,4,7]

When immunohistochemistry is performed on biopsy material, immunophenotyping of the bone marrow aspirate is not mandatory. However, in some cases it can be helpful to discriminate WM from other low-grade B-cell lymphomas.

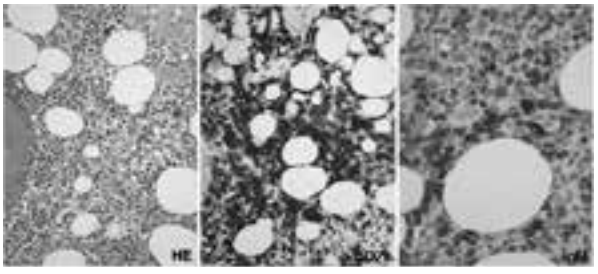
Cytogenetics/molecular markers

Several cytogenetic abnormalities have been described in WM, such as 6q-deletion, and t(9, 14) (p13; q32) both in 40-50% of patients. Recently, Steve Treon *et al.* found a specific point mutation (L265P) in the gene MYD88 (involved in the NFκB cascade) in 27 of 30 patients.[8] However, at this point these findings do not have solid prognostic or therapeutic value. Routine cytogenetic or molecular testing is therefore currently not indicated. Targeted fluorescent in situ hybridisation (FISH) analysis can be helpful if multiple myeloma or follicular lymphoma is suspected; see also the section on differential diagnosis.

Imaging studies

For staging purposes, i.e. before starting treatment, a conventional CT scan of the neck, thorax and abdomen is recommended. A PET scan is not necessary, unless transformation is suspected.

Figure 1. *Bone marrow biopsy with localisation of lymphoplasmacytic lymphoma*



Diffuse infiltration of bonemarrow by small lymphocytes (left). These cells are CD20 positive (middle). Some show plasmacellular differentiation with intracytoplasmatic expression of IgM (right).

CLASSIFICATION OF IGM-RELATED DISORDERS

As for diagnostic classification, WM is somewhere in between non-Hodgkin's lymphomas (NHL) and plasma cell dyscrasias. The WHO classification and important international guidelines are not entirely in agreement.[1,4] For the clinician, it is particularly relevant that in some cases a wait-and-see policy can be applied (IgM MGUS, asymptomatic WM), while in other cases therapy is needed (symptomatic WM, IgM-related conditions) (*table 3*).

IgM MGUS / asymptomatic WM

The risk of progression of IgM MGUS to WM is approximately 1.5% per year. If clear bone marrow infiltration was present (classification: asymptomatic WM) then a treatment indication arose in 6% of patients after one year, 59% after five years, and 68% after ten years in one recent retrospective study.[9,10]

Symptomatic WM and IgM-related disease

In patients with IgM M-protein, bone marrow infiltration and WM-related symptoms, the diagnosis 'symptomatic WM' can be made and treatment is indicated.

Sometimes a very small amount of IgM without evidence of LPL in the bone marrow can cause symptoms, for example amyloidosis, or autoimmune-mediated neuropathy. These patients do not meet the criteria for WM, but do have relevant symptomatology. For these cases, the term 'IgM-related disorder' was introduced. Despite a small tumour load, a treatment indication can occur in these cases, and the choice of therapy is determined mainly by the specific disease manifestation.[4]

DIFFERENTIAL DIAGNOSIS

In the rare cases of LPL without IgM paraprotein (5%), diagnosis can be challenging and depends on the exclusion of other small-cell b lymphomas, such as the marginal zone lymphoma or CLL. Sometimes no definitive diagnosis can be made. When prominent paratrabecular infiltration is found, follicular lymphoma must be considered, which is usually CD10 positive and associated with t(14,18) translocation. When LPL is accompanied by an IgA or IgG type paraprotein, distinction from a multiple myeloma is difficult. Vice versa, multiple myeloma can be accompanied with an IgM paraprotein, although rare. IgM multiple myeloma, unlike WM, usually carries translocation t(11,14), and often features osteolytic bone lesions.

PROGNOSIS

Survival is between five and ten years with a wide range, and there is small group of patients who remain asymptomatic without treatment for a long time (>10 years). The International Prognostic Scoring System for WM (IPSS-WM) gives some idea about the individual prognosis (*table 4*). This scoring system is based on pre-rituximab patient data, but has since been validated in at least one study. The IPSS score does not help in making treatment decisions, but can be important when comparing outcomes of patients in clinical trials.[11]

TREATMENT

Because WM is rare and randomised studies are mostly lacking, it is not easy to establish evidence-based guidelines for treatment. This also applies to the choice of first-line therapy. Some recently published reviews summarise the published data (mostly retrospective series) on effectiveness.[2,3,5,12-14]

When choosing treatment it is important to realise that there are many options available, and that therapy needs to be adapted to the individual patient, taking into account age, life expectancy, (co)morbidity (i.e. neuropathy) and clinical need for rapid response (i.e. hyperviscosity).

Plasmapheresis and prevention of IgM flare

When a patient presents with hyperviscosity syndrome, there is an indication for plasmapheresis, and treatment with a rapid-acting agent should be started in order to halt the production of the M-protein. When serum viscosity is >4.0 cp, there is a high risk of hyperviscosity-related events. Plasmapheresis may also be applied as a preventive measure when rituximab is started in a patient with a total IgM of >40 g/l, to prevent hyperviscosity due to IgM flare. A practical alternative in this situation is to start chemotherapy and only add rituximab once IgM levels are lower.

Table 4. *IPSS-WM]*

Item		Score
Age >65 years		1
Hb <7.2 mmol/l		1
Thrombocyte count <100 x 10^9/l		1
β2-microglobulin >3 mg/l		1
IgM M-protein >70 g/l		1
Risk group	**Score**	**Median survival (months)**
Low	0-1 (except age)	143
Intermediate	2, or age >65 years	99
High	≥3	44

CHOICE OF SYSTEMIC TREATMENT

First-line therapy

Immunochemotherapy: In one of the few randomised studies conducted in patients with WM, Buske *et al.*[15] showed that the addition of rituximab to chemotherapy leads to higher response rates and a longer time to progression in patients with WM. Immunochemotherapy is considered the standard for first-line treatment.

The combination of alkylating agents such as cyclophosphamide with rituximab and steroids is usually a well-tolerated regime. A comparative review article (no randomised studies are available here) showed that the response rates and duration for rituximab-cyclophosphamide prednisone *vs* addition to this regimen of adriamycin and/or vincristine (R-CHOP or R-COP) seem similar, while the last two regimens tend to give more toxicity (mainly neutropenic fever and neuropathy).[16]

A good alternative is DRC: dexamethasone, rituximab, cyclophosphamide, which is less myelosuppressive due to the lower dose of cyclophosphamide, while effectiveness remains high.[17] DRC will be used as the standard arm of the first-line study by the European Myeloma Network.

Also chlorambucil, whether or not in combination with rituximab, is still an option when there is no hyperviscosity or other need for rapid response. Chlorambucil and combination chemotherapy have never been compared head to head. In younger patients, who are potential candidates for autologous stem cell transplantation, the long-term use of alkylating agents such as chlorambucil is not recommended, also because of concerns about the risk of developing myelodysplastic syndromes/acute myeloid leukaemia (MDS/AML).

Rituximab combined with purine analogues (fludarabine, cladribine) with or without cyclophosphamide (e.g. FC-R) is very effective, and often leads to fast responses (median after 2.5 months). In a large randomised study by Leblond *et al.*, which will be published soon, monotherapy fludarabine seems to be more effective than monotherapy with chlorambucil.[18]

Regarding purine analogues, there are concerns about a higher risk of MDS/AML and transformation to aggressive NHL; however, data in WM are somewhat conflicting. It is still advised to give a maximum of 4-6 courses. There are fewer data on cladribine, but it seems equally effective. Purine analogues must be avoided in patients who are candidates for autologous stem cell transplantation.

Every rituximab-containing therapy can cause an IgM flare. Please refer to the plasmapheresis section.

Rituximab single agent and IgM flare: In patients with contraindications for chemotherapy, for example cytopenia or severe neuropathy, single agent rituximab may be an option. However, the chances of response are lower and

responses are very slow compared with the combination of rituximab with (mild) chemotherapy and steroids.[5] Again, the phenomenon 'IgM flare' is of importance: in about half of the patients the IgM rises first, and only starts to drop after four months. The initial rise of IgM after rituximab should not be interpreted as progressive disease! Whether this phenomenon has prognostic implications is unknown.

Treatment of IgM-related disease: polyneuropathy and haemolytic anaemia

These patients do not meet the criteria for WM but have WM-related symptoms and an IgM paraproteinaemia.[4] There is a wide array of rare syndromes, ranging from acquired haemophilia to vasculitis, and they all deserve their own therapeutic approach, although little clinical data are available. We will only discuss the two most common presentations of IgM-related disease, namely polyneuropathy (PNP) and cold autoimmune haemolytic anaemia (AIHA).

Approximately 50% of patients with demyelinating polyneuropathy related IgM paraproteinemia carry anti-MAG antibodies (amyloidosis should also be considered as a cause of PNP in WM patients). Treatment is not always necessary because these PNPs usually progress very slowly. Single-agent rituximab is considered the treatment of choice with responses and symptom relief seen in up to 50% of patients. For patients with rapidly progressive and/or recently diagnosed PNP, immunochemotherapy could be considered because reduction of the IgM will be achieved much faster.[19]

In a cohort of 66 patients with cold agglutination, 50% met the criteria for WM. Almost all of the remaining patients had an IgM paraprotein and in most of them cold agglutination was diagnosed as an IgM-related disorder. Treatment is difficult and again not always necessary. Rituximab monotherapy yields a response in about 50% of patients. When there is no response to single agent rituximab, or when rapid response is needed, there are several options, such as combining it with fludarabine but other combinations of immunochemotherapy could also be considered.[20]

Salvage therapy

The same indications for treatment are applicable (treat only if there is WM-related symptomatology and not just based on a rising IgM) as in the first-line setting. If the response after first-line treatment lasts for more than two years the same treatment can be repeated. If not, one of the other options can be chosen, as mentioned above, or alternatively consider novel agents or transplantation as discussed below.

Bortezomib

Proteasome inhibitors prove very effective in the treatment of WM. Monotherapy with bortezomib gave response rates of approximately 80% in three studies with 64 mostly pretreated patients (e.g. Kastritis *et al.*[12]). The response was quick (1.5 months) while with most classic therapies responses only start after four months. The progression-free survival was at least one year. In a small first-line study using the combination of bortezomib, rituximab, dexamethasone (BRD) even higher response rates were seen, and after two years 80% of patients had no signs of progression.[21]

The main adverse effect is neuropathy (about 70% of patients, up to 30% grade 3, often reversible after dose reduction) and this seems to occur more frequently compared with the use of bortezomib in multiple myeloma. Perhaps using once weekly and subcutaneous dosing may reduce rates of neuropathy. Of course, it is important to pay close attention to neuropathic symptoms and adjust or withhold therapy when necessary. Clinical studies in WM patients are underway with the new proteasome-inhibitor carfilzomib, which seems markedly less neurotoxic. The European Myeloma Network (EMN) is preparing an international study for first-line treatment randomising between DRC and DRC + bortezomib.

Bendamustine

In a large study by Rummel *et al.*, which was presented at the American Society of Hematology Congress in 2009 but has not yet been published, 546 patients with various low-grade lymphomas were randomised to first-line treatment with rituximab-bendamustine versus rituximab-CHOP, including 40 patients with WM. The response rate in patients with WM was very high in both arms (96% and 94%). However, bendamustine was less toxic (fewer infections, no alopecia, and less neuropathy) while the progression-free survival was significantly longer.[22]

In a series of 30 patients with relapsed or refractory WM published by Treon *et al.* bendamustine with rituximab resulted in response in 83% of cases; the median progression-free survival was 13 months. The therapy was well tolerated, the main side effect being myelosuppression, especially in patients who were previously treated with purine analogues. [23]

Based on a small group of patients in two studies bendamustine, combined with rituximab, seems a very effective option with relatively little toxicity. Little is known about the long-term side effects of bendamustine in WM (secondary MDS, stem cell toxicity).

Therapeutic options currently not recommended

There is very little experience with alemtuzumab, and it seems to give much toxicity in patients with WM.[24] Thalidomide has too little effectiveness to be applied as monotherapy. But, it is not so myelosuppressive and when combined with rituximab, results are slightly better. However, there is little experience with this combination

and because of its neurotoxicity thalidomide is not an attractive option in WM. Lenalidomide should not be used, because in WM patients deep anaemia has been described, which also persisted after dose reduction or cessation. (e.g. Kastritis *et al.*[12])

Stem cell transplantation

The majority of patients are not candidates for stem cell transplantation due to age and comorbidity. For a select group of younger and fit patients who relapse quickly after immunochemotherapy, autologous stem cell transplantation can be considered. In a recent series in heavily pretreated WM patients, the non-relapse mortality was 4-6% in the 1st year. The median progression-free survival was about 4 years and after 5 years 60% of patients were still alive.[25, 26]

After allogeneic stem cell transplantation, non-relapse mortality is very high in WM patients: in the largest series 23-40% after 1 year, depending on the conditioning (reduced-intensity or myeloablative, respectively).[27] The five-year progression-free survival was about 45-50% and the overall survival 50-60%. Chronic graft versus host disease was associated with higher non-relapse mortality and lower relapse rates. Considering the available alternatives and the high treatment-related mortality, allogeneic stem cell transplantation should only be considered in the rare young patient with a very aggressive disease course.

Maintenance therapy

There are no prospective studies on the role of maintenance therapy in WM. In a retrospective series 86 of 248 WM patients received maintenance therapy with rituximab: a median of eight doses in the two years after induction therapy with a rituximab-containing regimen. Both the progression-free survival (56 *vs* 29 months) and the overall survival (not reached *vs* 116 months) were better in the maintenance group.

Maintenance therapy with rituximab in WM can be considered after second-line treatment, similar to the approach in other indolent lymphomas.[28]

RESPONSE ASSESSMENT

At the 3rd International WM workshop uniform response criteria were established,[29] which are summarised in *table* 5.

Because the M-protein sometimes responds very slowly, and the level of the M-protein is an unreliable indicator of tumour mass, it is recommended to repeat bone marrow examination when in doubt about the response. Depending on the agent used, lowering the IgM level can be faster (purine analogues, proteasome inhibition) or slower (chlorambucil, rituximab). After chlorambucil therapy, there is often a reduction of clonal B lymphocytes, but the plasma cells remain and may be the source of the persisting M-protein.[30]

Table 5. *Response criteria*

Response	Criteria
Complete response (CR)	Disappearance of monoclonal protein by immunofixation; no histological evidence of bone marrow involvement, and resolution of any adenopathy/organomegaly (confirmed by CT scan), along with no signs or symptoms attributable to WM; reconfirmation of the CR status is required at least 6 weeks apart with a second immunofixation
Partial response (PR)	A >50% reduction of serum monoclonal IgM concentration on protein electrophoresis and >50% decrease in adenopathy/organomegaly on physical examination or on CT scan; no new symptoms or signs of active disease
Minor response (MR)	A >25% but <50% reduction of serum monoclonal IgM by protein electrophoresis; no new symptoms or signs of active disease
Stable disease (SD)	A <25% reduction and <25% increase of serum monoclonal IgM by electrophoresis without progression of adenopathy/organomegaly, cytopenias, or clinically significant symptoms resulting from disease and/or signs of WM
Progressive disease (PD)	A >25% increase in serum monoclonal IgM by protein electrophoresis confirmed by a second measurement or progression of clinically significant findings resulting from disease (i.e., anaemia, thrombocytopenia, leukopenia, bulky adenopathy/organomegaly) or symptoms (unexplained recurrent fever >38.4 °C, drenching night sweats, >10% body weight loss, or hyperviscosity, neuropathy, symptomatic cryoglobulinaemia, or amyloidosis) attributable to WM

CONCLUSIONS AND RECOMMENDATIONS

Regarding symptomatology, pathophysiology as well as treatment, WM holds a special position within the low-grade malignant lymphomas. There is definitely room for improvement in treatment results in this relatively rare disease, both regarding effectiveness and toxicity, and therefore it is important to treat patients in clinical trials when possible, stratifying for WM-IPSS score and using uniform response criteria. One of the challenges for the future, as in many non-Hodgkin's lymphomas, is to determine response to treatment faster and more accurately, in order to identify high-risk patients sooner and adjust therapy accordingly.

Because of treatment toxicity, which can be very disease-specific (deep anaemia after lenalidomide, higher risk of neuropathy with proteasome inhibition), clinical experience in treatment of other indolent lymphomas cannot always be extrapolated to WM.[31]

RECOMMENDATIONS FOR CLINICAL PRACTICE

1. WM has a wide range of symptomatology that is partly unique to the disease, and careful history taking, complete physical examination and a targeted diagnostic workup is crucial.
2. Symptoms are not just related to lymphoma infiltration, but often result from the specificity of the IgM paraprotein causing autoimmune phenomena.
3. The level of the M-protein is no reason for treatment if the patient is asymptomatic. Vice versa, a small amount of M-protein can give rise to symptoms and thus be a reason for starting treatment.
4. In patients with hyperviscosity syndrome, plasmapheresis should be started immediately. In addition a rapidly acting therapy to halt IgM production should be instituted. Wait until the IgM has dropped before giving rituximab because of potential IgM flare.
5. As a first-line treatment combination immunochemotherapy is recommended, such as the dexamethasone, rituximab, cyclophosphamide (DRC) regimen, or rituximab plus cyclophosphamide/prednisone (R-CP). Chlorambucil, combined with rituximab and/or steroids, is a good alternative, especially in the older patient.
6. In younger patients (and/or patients who are potential candidates for autologous stem cell transplantation): prolonged use of purine analogues and alkylating agents should be avoided.
7. When starting a WM patient on rituximab, be aware of the phenomenon 'IgM flare'.
8. When a treatment indication emerges two years or longer after first-line treatment, the same treatment can be repeated.
9. When a treatment indication emerges within two years after first-line treatment there is a wide range of active agents and treatment modalities, including autologous stem cell transplantation. Age, life expectancy and comorbidities such as cytopenia and/or neuropathy, will determine which is the preferred choice of salvage therapy in each individual.

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REVIEW

Chocolate/cocoa and human health: a review

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ABSTRACT

Chocolate/cocoa has been known for its good taste and proposed health effects for centuries. Earlier, chocolate used to be criticised for its fat content and its consumption was a sin rather than a remedy, associated with acne, caries, obesity, high blood pressure, coronary artery disease and diabetes. Therefore, many physicians tended to warn patients about the potential health hazards of consuming large amounts of chocolate. However, the recent discovery of biologically active phenolic compounds in cocoa has changed this perception and stimulated research on its effects in ageing, oxidative stress, blood pressure regulation, and atherosclerosis. Today, chocolate is lauded for its tremendous antioxidant potential. However, in many studies, contradictory results and concerns about methodological issues have made it hard for health professionals and the public to understand the available evidence on chocolate's effects on health. The purpose of this review is to interpret research done in the last decade on the benefits and risks of chocolate consumption.

KEYWORDS

Chocolate, cocoa, Theobroma cacao, polyphenols, flavonoids

INTRODUCTION

Chocolate came to Europe in the 16th century. Since then, the modern chocolate industry has developed, and cocoa seeds are now processed in different ways. Chocolate is the most commonly craved food in the world.[1] Initially it was thought of as a luxury item, but now it is considered to be a medicine.

HISTORY OF CHOCOLATE

Chocolate originated from Mexico where the Mayas, Incas, and Aztecs cultivated the cacao tree. At first, it was seen as an aphrodisiac, accessible only for the affluent and rich. Later on, because of its high price, chocolate was replaced by coffee and tea as the main drink. However, ultimately, chocolate did become a favourite confection in most developed countries including Europe and North America. Nowadays cocoa is grown mainly in West Africa, Indonesia, and Sri Lanka.

In the past, due to its health effects, it was considered the drink of Gods,[2] an association that gave rise to the scientific name of the cocoa tree, *Theobroma cacao,* from the Greek words theo (God) and broma (drink). This attribution was given to the tree by a Swedish naturalist Carl Von Linné (1707–1778). In fact, this name is symbolic of the social, religious, and economic importance of chocolate in both New and Old World cultures.

The tree and its dried seeds prior to processing are called 'cacao' in American English; after processing, i.e. roasting and grinding, the term 'cocoa' is used. 'Chocolate' is the food prepared from roasted cacao seeds.

CHEMICAL COMPOUNDS IN CHOCOLATE THAT MAY AFFECT HUMAN HEALTH

The nutritional qualities of chocolate have been acknowledged by several authors and some people have called it a complete food. Important chemicals found in chocolate are as follows.

Fats

The fat predominantly found in dark chocolate is cocoa butter[3] which contains approximately 33% oleic acid (monounsaturated), 25% palmitic acid (saturated), and 33% stearic acid (saturated).[4] Oleic acid has a positive effect on lipid levels.[5] Saturated fats adversely increase the total cholesterol and low-density lipoprotein levels.[6] However, regardless of being one of the saturated fats, stearic acid may not have any effect on lipid levels,[7,8] or it

may increase lipid levels.[9] The reason of this discrepancy regarding stearic acid may be the different nature of cocoa-derived stearic acid, from animal derived sources; or less absorption of stearic acid.[10,11] However, few studies have negated this possibility.[12,13]

Antioxidants

Cocoa contains large concentrations of flavonoids, epicatechin, catechin, and procyanidins.[14] Cocoa has the maximum levels of flavonoids, greater than even tea and wine.[15] Dark chocolate contains considerably higher amounts of flavonoids than milk chocolate.[16] Moreover, the biological effects of flavonoids may also be greater in dark chocolate because the milk in milk chocolate may slow down the intestinal absorption of flavonoids.[17] Finally, chocolate is also rich in procyanidin flavonoids, comparable with levels in procyanidin-rich apples.[18]

Nitrogenous compounds

The nitrogenous compounds of cacao include both proteins and the methylxanthines theobromine and caffeine. They are central nervous system stimulants, diuretics, and smooth muscle relaxants.

Minerals and other properties

Cocoa mass also contains minerals such as potassium, phosphorus, copper, iron, zinc, and magnesium[5,19] which potentiate the health benefits of chocolate. Chocolate also contains valeric acid which acts as a stress reducer despite the presence of the stimulants caffeine and theobromine in the chocolate.[20]

EPIDEMIOLOGICAL EVIDENCE ABOUT HEALTH EFFECTS OF CHOCOLATE

Epidemiological evidence about beneficial effects of chocolate came from the Kuna Indian population of the islands of Panama. This population is characterised by a low prevalence of atherosclerosis, type 2 diabetes, and hypertension. The 'secret' behind this is the daily intake of homemade cocoa drink by indigenous Kuna Indians. These traits disappear after migration to urban areas on mainland Panama and subsequent changes in diet (i.e. consumption of much less cocoa, which is commercially processed), hence negating the genetic nature of the traits.[21]

Further epidemiological evidence has come from a longitudinal study looking at the lifestyle and cardiovascular risk in a cohort of older men.[22] This study found cocoa intake to be inversely related to blood pressure. Even after multivariate adjustment, mean systolic blood pressure was 3.8 mmHg lower in the highest cocoa intake group compared with the lowest intake group. In a perspective analysis, higher cocoa intake was associated with a reduction in cardiovascular and all-cause mortality.[23] These studies show that chocolate may not only be bad for us; some forms of chocolate may actually be good for us.

POTENTIAL HEALTH BENEFITS OF CHOCOLATE CONSUMPTION

Interestingly, regular cocoa ingestion is claimed to be inversely associated with the risk of cardiovascular disease. The past decade has seen an increasing interest in potential anti-pathogenic properties of cocoa. Although still debated, a range of potential mechanisms through which cocoa might exert its beneficial effects on health have been proposed. Some of them are discussed here.

Cocoa and cardiovascular diseases

Numerous studies have suggested beneficial effects of cocoa in cardiovascular diseases (CVD). Most recently, Zomer *et al.*, have concluded that the daily consumption of dark chocolate could be an effective cardiovascular preventive strategy in patients with metabolic disease.[24] The potential mechanisms involved in these beneficial effects of cocoa are as follows.

Rich source of antioxidants

Oxidative stress and reduced antioxidant defences play a pivotal rule in the pathogenesis of atherosclerosis. Chocolate is the third highest daily source of antioxidants for Americans.[25] Antioxidants found in chocolate have been shown to inhibit plasma lipid oxidation.[26] However, there is a study negating the direct antioxidant potential of chocolate, documenting that the large increase in plasma total antioxidative capacity observed after the consumption of flavonol-rich food is most likely not due to flavonols but probably is a consequence of the increased uric acid levels resulting from fructose metabolism.[27]

Blood pressure lowering effects

A large-scale, longer duration study in the Netherlands recruited men aged 65-84 years. The subjects were asked about their dietary intake when they enrolled in the study and again at five-year intervals. Over the next 15 years, men who consumed cocoa regularly had significantly lower blood pressure than those who did not.[22] Consumption of dark chocolate bars for 15 days has been reported to reduce systolic blood pressure in healthy subjects[28] as well as in young[29] and elderly[30] hypertensive patients.

The exact mechanism behind antihypertensive effects of chocolate is not known but this may involve increased nitric oxide (NO) bioavailability, flavonol-induced inhibition of angiotensin converting enzyme,[31] and stearic acid-based reduction of diastolic blood pressure.[32]

Contrary to that, a few studies showed no effect on blood pressure with chocolate/cocoa ingestion.[33,34] Van den Bogaard *et al*. in fact put in question the blood pressure lowering effects of cocoa. They concluded from their randomised controlled trial that natural cocoa drinks did not significantly change either 24-hour ambulatory or central systolic blood pressure.[35] Alonso also found no association between chocolate consumption and incidence of hypertension in a Cohort study.[36] The reason for these inconsistencies may relate to a number of factors, including the study design. Since these studies were performed in a rather small number of normotensive individuals and with a lower chocolate intake for a shorter period, an antihypertensive effect may have been missed as a consequence of their study design. Moreover, use of different dietary intake questionnaires and food consumption tables, differences in the levels and the types of chocolates/cocoa studied and differences in the populations investigated, might also account for inconsistencies. Most of the studies supporting antihypertensive effects of chocolate used chocolate bars, whereas the negative studies used cocoa drinks. Perhaps the chocolate matrix is necessary for the antihypertensive effect, acting either directly or synergistically with flavonols.

Effects on blood vessels and nitric oxide

Numerous studies have reported that cocoa causes significant vasodilatation by increasing serum NO levels[37] and endothelial NO bioavailability.[38] The underlying molecular mechanism is the ability of flavonols to increase the NO in the endothelial cells via their capacity to activate vascular endothelial NO synthase[39] and their antioxidant actions which lead to diminished inactivation of NO by free radicals through inhibition of NADPH oxidase.[33]

Inhibits platelet activation

Platelet dysfunction is another characteristic feature of atherosclerotic lesions. Cocoa has aspirin-like effects on platelet function,[40] and the joint effects of the cocoa and aspirin are additive in nature, suggesting improved clot prevention afforded by cocoa.[41] Chocolate has a dual effect on platelets. It not only decreases platelet aggregation[42] but also reduces platelet adhesion.[43]

Consumption of chocolate with high procyanidin content significantly lowered the levels of leukotrienes and increased the levels of prostacyclin when compared with a group consuming a low-procyanidin chocolate.[44]

Antidiabetic effects

Numerous approaches have been tried to improve insulin sensitivity in diabetics.[45] Insulin sensitivity partially relies on NO bioavailability in endothelial cells.[46] Hence flavonol may reduce insulin resistance by ameliorating NO bioavailability. A reduction in insulin resistance and an increase in insulin sensitivity were observed after ingestion of flavonol-rich chocolate in healthy subjects[28] and hypertensive patients.[29] Another study demonstrated a positive impact on glucose and insulin responses to an oral glucose tolerance test, in hypertensive adults with impaired glucose tolerance following flavonol-rich chocolate ingestion.[47]

Antistress effects

There are several bioactive compounds in chocolate that promote alertness.[48] A study in Switzerland also confirmed that chocolate alleviates stress. Following 14 days of dark chocolate ingestion, stress parameters in the adults exhibiting high anxiety profiles became comparable with the low-stress subjects.[42]

Chocolate affects stress levels by prompting serotonin production which is a calming neurotransmitter.[49,50]

Anti-obese effects

Obesity is one of the major risk factors in the development of CVD. In a study an identical high fat diet, with or without cocoa, was fed to rats for three weeks. Cocoa consumption led to a significant decrease in total body weight, mesenteric white adipose tissue weight and serum triglycerides. When DNA analysis was carried out on liver and mesenteric fat tissue samples, the results showed a reduction in expression of various genes associated with fatty acid transport and synthesis in liver and mesenteric fat and increased expression of genes associated with thermogenesis.[51]

Effects on the neurons

A recent study of young, healthy subjects using magnetic resonance imaging found that cocoa intake results in increased cerebral blood flow,[52] suggesting that cocoa might play a role in treatment of cerebral conditions such as dementia and stroke. Nurk *et al*. also reported better cognitive performance with chocolate intake.[53] Larsson *et al*., investigated the association between chocolate consumption and risk of stroke in men and concluded that daily chocolate consumption reduces the likelihood of a stroke attack.[54] Walters *et al*., showed that chocolate acutely improves cerebral blood flow.[55]

Antitumour effects

A few *in vitro* studies suggest that cocoa inhibits the growth of cancerous cells.[56,57] The exact anticancer mechanisms are not clearly understood at this stage. On the other hand, some studies suggest that excess chocolate intake makes a person more prone to develop cancers.[58,59] Further preclinical and clinical trials are needed to investigate the mechanisms involved in cocoa actions and to justify cocoa's usage as a therapy for the prevention and treatment of cancer.

Anti-inflammatory effects

Chocolate inhibits lipoxygenase pathways, by directly binding to the active sites of the enzymes lipoxygenases.[60]

Cocoa and exercise recovery

It has been documented that chocolate supplementation before exercise results in rapid recovery of post-exercise physiological and metabolic changes. Plasma glucose levels of subjects increased significantly at 15 minutes after chocolate intake and stayed at moderately high levels until 30 minutes after an hour's running when compared with the glucose levels of placebo supplemented group.[61]

POTENTIAL HEALTH RISKS OF CHOCOLATE CONSUMPTION

Surprisingly, literature on adverse effects produced by chocolate is scarce when compared with the plenty of studies touting the benefits of chocolate. Chocolate is believed to cause heartburn because of one of its constituents – theobromine, which relaxes the oesophageal sphincter muscle – hence permitting stomach acidic contents to enter into the oesophagus.[62] A few studies have documented allergic reactions with chocolate in children.[63,64]

COULD CHOCOLATE BE A NOVEL THERAPY FOR TREATMENT OF HEALTH DISORDERS?

There appears to be some scientific evidence to justify eating a moderate amount (approximately 2 oz) of dark chocolate daily. However, the major criticism against the consumption of chocolate for therapeutic benefit is the high amount of sugar and triglycerides that needs to be consumed to reach what has been demonstrated to be a potentially therapeutic dose and a person must then compensate for the additional calories by increasing the amount of daily exercise or reducing caloric intake of other fats, sweets, or carbohydrates to prevent obesity and the metabolic and cardiovascular risks related to it.

The current evidence suggests that the beneficial effects of chocolate are attributed mainly to its flavonol content, especially epicatechin. Hence, direct dietary supplementation with flavonol instead of whole chocolate consumption deserves further study.

CONCLUDING REMARKS

The majority of studies claiming the benefits of chocolate are small-scale, sponsored/carried out by the chocolate manufacturers whose personal interests cannot be ignored. This warrants due consideration in implications of the results as there may be potential for research bias.[65] Additional large-scale observational and/or interventional studies from non-biased sources are needed before clinicians can absolutely recommend 'a chocolate a day' to their patients.

In addition, the products used in controlled studies often contain much higher polyphenol contents than most of the commercially available products.[22,66] Since flavonols exhibit a bitter taste, manufacturers have established processing techniques for cocoa which eliminate the bitterness together with flavonoids.[67] As much as 90% of the flavonoids may be lost due to cocoa processing.[68] Thus, it needs to be established whether the consumption of products with lower polyphenol content are associated with any health benefits in humans.

Conflict of interests: none.

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REVIEW

MiRNAs in oesophageal squamous cancer

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ABSTRACT

Oesophageal cancer is a common cancer worldwide with a very poor prognosis. Oesophageal squamous cell carcinoma (OSCC) is the major subtype of oesophageal cancer but also one of the least studied cancers worldwide. Although the molecular genetics of OSCC have been widely studied, the molecular mechanism of OSCC carcinogenesis is not completely understood. MicroRNA (miRNA) is now essential to understanding the molecular mechanism of cancer progression. Recent findings include the following: 1) recent findings regarding the functions of miRNA; 2) some of the latest findings on expression profile of miRNA involved in OSCC; 3) miRNAs and their target genes and molecular mechanisms in OSCC; and 4) the therapeutic-clinical potential of miRNAs in OSCC. We can make full use of this knowledge to guide us to evaluate and improve the patient's condition and choose the most fitting medical treatment or explore new approaches to improve the survival ratio of OSCC patients.

KEYWORDS

MicroRNA, oesophageal cancer, oesophageal squamous cell carcinoma

INTRODUCTION

According to statistics, oesophageal cancer, which occurs worldwide with a variable geographic distribution, is the sixth leading cause of death from cancer and one of the least studied cancers worldwide.[1,2] It has two main forms, each with distinct aetiological and pathological characteristics: oesophageal squamous cell carcinoma (OSCC) and oesophageal adenocarcinoma (OAC). OSCC is the most frequent subtype of oesophageal cancer; it is often diagnosed at an advanced stage and its prognosis remains poor. Although preoperative chemotherapy and chemoradiation therapy are currently used for patients with advanced stage OSCC, their effectiveness is unsatisfactory. Consequently, it is important for targeted prevention and early detection in the control of this disease. MicroRNAs (miRNAs) are a species of small noncoding single-stranded RNA of about 19 to 24 nucleotides that through partial sequence homology may interact with the 3′-untranslated region of target mRNA molecules. MiRNA inactivates multiple target genes by sequence-specific binding-mediated destabilisation of mRNA or inhibition of translation.

In this review, we highlight four issues of current relevance to this field and discuss 1) the recent findings regarding the functions of miRNA; 2) some of the latest findings on molecular mechanisms of miRNA involved in OSCC; 3) miRNAs and their target genes in OSCC; and 4) the therapeutic-clinical potential of miRNAs in OSCC.

PREVALENCE AND AETIOLOGY OF OESOPHAGEAL CANCER AND OESOPHAGEAL SQUAMOUS CANCER

According to statistics, oesophageal cancer is the sixth most common cancer worldwide and the third malignancy of the gastrointestinal tract with a very poor prognosis.[3] Oesophageal cancer has two major histological types: adenocarcinoma (OAC) and squamous cell carcinoma (OSCC); OAC predominantly occurs in Western societies, but OSCC is the predominant histological type of oesophageal cancer worldwide.[4] Incidence rates vary across the world by nearly 16-fold, with the highest rates found in Southern and Eastern Africa and Eastern Asia, and lowest rates observed in Western and Middle Africa and Central America in both males and females.[3] OSCC is also one of the least studied cancers worldwide but one of

the most lethal malignancies, with a five-year survival rate of less than 10%, and occurs at a relatively high frequency in certain areas of China.[5,6] Thus, OSCC is a very deadly disease in strong need of new, effective, therapeutic approaches.

Major risk factors for OSCC are not well understood, but are thought to include tobacco smoking, alcohol consumption, poor nutritional status and low intake of fruits and vegetables.[7-11] In some low-risk areas such as the United States and some Western countries, cigarette smoking and excessive alcohol consumption account for about 90% of the total cases of OSCC.[12] But the rates in these areas have been steadily declining because of long-term reductions in smoking and alcohol consumption.[13] However, OSCC has been increasing in certain Asian countries, probably as a result of increases in tobacco use and alcohol consumption.[14] The distinct risks exhibited by individuals exposed to similar known risk factors implied that genetic predisposition might play an important role in oesophageal cancer aetiology.[7,8] The exact causes and the mechanism of OSCC, which appear to be multifactorial, have not yet been fully appreciated. Thereby there is no effective strategy for treatment and prevention nowadays.

OSCC is often not diagnosed until a late stage when it is incurable and the prognosis of affected patients is unsatisfactory. Consequently, the dismal outcome of oesophageal cancer patients highlights the need for novel prognostic biomarkers to allow a tailored multimodality approach with increased efficacy, such as microRNAs (miRNA).

MiRNAs AND THEIR FUNCTIONS

MicroRNAs are small noncoding RNAs of 19-24 nucleotides in length that are important in the regulation of several crucial biological processes, such as cell growth,[15] apoptosis,[16] development,[17] differentiation,[18] and endocrine homeostasis.[19] The first miRNA was described in 1993, in which the C. elegans heterochronic gene lin-4 encoded small RNAs with antisense complementarity to lin-14.[20] MiRNAs play important roles in the aetiology of many human diseases by post-transcriptionally regulating the expression of approximately one third of all human genes.[21,22] It has been investigated in a variety of diseases, including diabetes, heart diseases, Alzheimer's disease, and viral infections.[23] The most active area and the starting point for the pathogenetic role of miRNAs lies in cancer research.[24,25] MicroRNAs are known as gene silencers and their expression profiles have been reported to be negatively correlated with those of their target genes.[26] The biological role of miRNA is to inactivate single or multiple target genes by sequence-specific binding-mediated destabilisation of mRNA or inhibition of translation mechanisms.[27] The degradation or inhibition of specific mRNA translation could decrease the expression of the resulting protein, and their role in disease development would presumably be through the regulation of their target protein gene.[28,29] Recently, genome-wide expression of miRNAs, which could be examined by microarray and on a more limited miRNA set by microbead hybridisation or reverse transcription-PCR (RT-PCR), can provide increasing information in miRNA function studies of many diseases.

MiRNAs AND CANCERS

Growing evidence has indicated important roles for different miRNA species in the development of different cancers, perhaps being involved in the pathophysiology of all human cancers.[30] MiRNA expression profiles have frequently been reported to be correlated with the aetiology, classification, progression, and prognosis of multiple human cancers.[1,21,29,31-33] Recent studies indicate that numerous miRNAs have been identified as tumour-related and can be categorised in two groups based on their functional relevance, tumour suppressors and oncogenes, and the miRNAs with roles in cancer are designated as oncogenic miRNAs (oncomiRs).[34] MiRNAs can act as oncogenes or tumour suppressors and modulate the expression of approximately one-third of all human genes.[35] In normal cells, tumour suppressor miRs are highly expressed and downregulate the expression of oncogenic proteins, whereas in tumour cells they are silenced leading to upregulation of oncogenic proteins. Conversely, oncomiRs are upregulated in tumour cells, downregulating the expression of tumour suppressive proteins. Both tumour suppressor miRs and oncomiRs are involved in biological and pathological processes including cell differentiation, proliferation, apoptosis and metabolism, and are emerging as highly tissue-specific biomarkers with potential clinical applicability for defining cancer types and origins.[36]

MiRNAs IN OSCC

In recent years, the study of miRNAs in OSCC has been expanded and is producing new knowledge on the molecular basis of this disease. MicroRNA expression profiles are important diagnostic and prognostic markers of OSCC. We summarised the miRNAs that have been reported in recent years[1,6,32,37-66] (*table 1*) and discovered that more upregulated miRNAs species than downregulated species have been found. In other words, this means that we have found more oncomiRs than tumour suppressors

Table 1. *MiRNAs in oesophageal squamous cell carcinoma that have been reported in the literature*

	Upregulation			Downregulation	
Tissue	miR-9	miR-10b	miR-15b	let-7	
	miR-16	miR-17-5p	miR-20a	miR-29c	miR-30a-3p
	miR-20b	miR-21	miR-23a	miR-100	miR-106a
	miR-25	miR-26a	miR-27b	miR-125b	miR-133a
	miR-31	miR-34b	miR-34c	miR-133b	miR-139
	miR-92a	miR-93	miR-96	miR-143	miR-145
	miR-103	miR-106a	miR-107	miR-148a	miR-203
	miR-127	miR-128b	miR-129	miR-205	miR-210
	miR-130a	miR-130b	miR-132	miR-375	
	miR-134	miR-137	miR-138		
	miR-142-3p	miR-151	miR-192		
	miR-194	miR-205	MiR-223		
	miR-296	miR-373	miR-1322		
Cell	miR-16	miR-21		miR-10a	miR-29c
	miR-30a-5p	miR-141		miR-99a	miR-100
	miR-200c	miR-205		miR-125b	miR-146b
	miR-429			miR-153	miR-210
				miR-376a	miR-379
				miR-593	miR-651

correlated with OSCC. Furthermore, increasingly relevant research is being performed. For example, some studies have revealed that functional single nucleotide polymorphisms (SNPs) rs2910164 in pre-miR-146a and rs11614913 in pre-miRNA-196a could contribute to OSCC susceptibility and clinical outcome in Chinese Han.[67,68] Zhang *et al.*[58] identified a profile of seven serum miRNAs (miR-10a, miR-22, miR-100, miR-148b, miR-223, miR-133a, and miR-127-3p) as OSCC biomarkers. And Chen *et al.*[69] concluded that CpG island methylation of miR-34a, miR-34b/c and miR-129-2 occurs frequently and is an important mechanism, for their low expression in OSCC and the high methylation ratio of miR-129-2 indicated its potential as a methylation biomarker in the early diagnosis of OSCC.

MiRNAs AND THEIR TARGET GENES IN OSCC

Altered miRNA expression has been found to promote carcinogenesis, but little is known about the role of miRNAs in oesophageal cancer. Under these circumstances, accumulating research concerns the target genes of specific miRNAs in OSCC to elucidate the exact mechanism of OSCC carcinogenesis. The target genes of these causal miRNAs may be tumour suppressor genes or other genes related to oncogenes, such as growth factors, growth factor receptors, signal transducers, transcription factors, programmed cell death regulators, genes that control cell division, or genes that repair DNA.[1]

OncomiRs are presumed to function by downregulating tumour suppressor genes. Several reports in the literature have mentioned the target genes of several oncomiRs in OSCC (*table 2*). Many studies described that miR-21 targets phosphatase and tensin homolog (PTEN),[70] tumour suppressor gene tropomyosin 1 (TPM1),[71] programmed cell death 4(PDCD4),[43,72] Sprouty2,[73] B-cell CLL/lymphoma 2(BCL2), programmed cell and Maspin,[70,74-76], thereby demonstrating its involvement in tumour growth, invasion, and metastasis.[50] Zhang *et al.*[63] revealed that miR-31 promoted OSCC colony formation, migration and invasion that may regulate three tumour suppressor genes, namely epithelial membrane protein 1 (EMP1), kinase suppressor of ras 2 (KSR2) and regulator of G-protein signalling 4

Table 2. *MiRNAs and their target genes in oesophageal squamous cell carcinoma*

	MiRNAs	Target gene	Effect of OSCC
OncomiRs	miR-10b	KLF4	Promotes cell migration and invasion
	miR-19a	TNF-α	Promotes cell growth
	miR-21	PTEN, TPM1, PDCD4, Sprouty2, BCL2, maspin	Promotes cell growth, invasion, and metastasis
	miR-31	EMP1, KSR2, RGS4	Promotes tumour colony formation, migration and invasion
	miR-34b	p53	DNA damage and oncogenic stress
	miR-92a	CDH1	Promotes cell migration and invasion
	miR-205	ZEB2	Promotes cell invasion and migration
	miR-373	LATS2	Affects cell cycle progression or apoptosis
	miR-296	Cyclin D1, p27	Affects tumour cell growth
Tumour suppressor	let-7	HMGA2	Inhibits cell proliferation, lymph node metastasis
	miR-29c	Cyclin-E	Inhibits tumour cell growth
	miR-133a/b	FSCN1	Inhibits cell proliferation and invasion
	miR-145	FSCN1	Inhibits cell proliferation and invasion
	miR-203	ΔNp63	Inhibits cell proliferation
	miR-210	FGFRL1	Inhibits cell proliferation

(RGS4). Hong *et al.*[55] found that miR-296 might cause the growth of OSCC *in vitro* and *in vivo* through regulation of cyclin D1 and p27. He *et al.*[40] demonstrated that miR-34b was the direct transcriptional target of protein 53 (p53) in human and mouse cells and that its induction by DNA damage and oncogenic stress was p53 dependent. Matsushima *et al.*[51] showed that miR-205 was likely to control cell invasion and migration in OSCC cells through its repression of zinc finger E-box-binding homeobox 2 (ZEB2), a repressor of E-cadherin expression resulting in dysregulating cellular processes which may ultimately lead to tumourigenesis of OSCC. Tian *et al.*[42] described that miR-10b overexpression promoted cell migration and invasion in human OSCC cell lines by regulating the Krüppel-like factor 4 (KLF4), a zinc finger protein of the Krüppel-like factor family that plays a role in cell cycle regulation, differentiation, and rises in response to DNA damage.[77, 78] Lee *et al.*[49] described that the miR-373 expression was found to be inversely correlated with large tumour suppressor homolog 2 (LATS2) expression in the OSCC cell lines and OSCC patients. After detecting 109 consecutive OSCC samples, Kurashige *et al.*[66] found that high expression of miR-223 had a significant adverse impact on the survival of OSCC patients through repression of the function of FBXW7.

Conversely, it can be postulated that re-introduction of tumour suppressor miRs into tumour cells will result in upregulating tumour suppressor genes leading to the downregulation of target oncogenes and tumour suppression (*table 2*). Liu *et al.*[59] demonstrated that let-7, a tumour suppressor, was expressed lower in OSCC and might repress cell proliferation, and correlated with lymph node metastasis of OSCC patients by negatively regulating high mobility group AT-hook 2 (HMGA2) at the post-transcriptional level. Ding *et al.*[6] reported that miR-29c, a potential tumour-suppressing miRNA in OSCC development, could influence the activity of cyclin E-CDK2 complexes by inhibiting the expression of cyclin E, one of the human G1 cyclins that binds to and activates its catalytic partner cyclin-dependent kinase 2 (CDK2) which phosphorylates a number of downstream substrates.[79-82] Yuan *et al.*[83] reported that miR-203 can significantly inhibit the proliferation of OSCC cells through the fjNp63-mediated signal pathway. fjNp63, an alternative splice variant of p63 gene lacking TA domain,[84] is the major isotype expressed in a variety of human squamous cell carcinomas including OSCC,[85] and the expression level of fjNp63 in tumour tissues was significantly higher than in the matched normal tissues.[85,86] Chen *et al.*[56] revealed that miR-92a promotes OSCC cell migration and invasion at least partially via suppression of CDH1 expression, and patients with upregulated miR-92a are prone to lymph node metastasis and thus have a poor prognosis. Kano *et al.*[47] found that miR-145 and miR-133a/b directly regulate FSCN1 and contribute to cellular proliferation and invasion in OSCC. Tsuchiya *et al.*[57] showed that miR-210 mediated mainly by the targeting of fibroblast growth factor receptor-like 1 (FGFRL1), inhibiting the proliferation of OSCC cells by inducing cell cycle arrest and apoptosis. Kong *et al.*[87] reported that downregulation of miR-375, which is mainly caused by promoter methylation, is one of the molecular mechanisms involved in the development and progression of OSCC through inhibiting the expression of IGF1R. Zhang *et al.*[65] found that miR-1322 can regulate oesophageal cancer-related gene 2 (ECRG2) in an allele-specific manner.

MiRNAs AND THEIR TARGET GENES AFTER MEDICAL TREATMENT

Several studies have focused on the alteration and mechanism of miRNAs in the medical treatment of OSCC patients. It was shown that some miRNA alteration was closely related to some medical treatment. For example, Hummel *et al.*[88] revealed that 13 miRNAs (miR-199a-5p, miR-302f, miR-320a, miR-342-3p, miR-425, miR-455-3p, miR-486-3p, miR-519c-5p, miR-548d-5p, miR-617, miR-758, miR-766, miR-1286) were deregulated after 24- and/or 72-hours of treatment (Cisplatin or 5-fluorouracil) in OSCC cell lines. Additionally, many researchers appeared to notice the roles and possible mechanisms of miRNAs in the medical treatment of OSCC. For example, Hong *et al.*[55] described that downregulation of miR-296 could confer sensitivity of both P-glycoprotein-related and P-glycoprotein-nonrelated drugs on OSCC cells, and might promote ADR-induced apoptosis, accompanied by increased accumulation and decreased release of ADR. Imanaka *et al.*[45] showed that miR-141 negatively regulates the expression of YAP1 and conferred cisplatin resistance in OSCC. Hummel *et al.*[48] revealed that miR-148a upregulation sensitises chemotherapy-resistant variants of OSCC cell lines, to cisplatin and 5-FU *in vitro*, and further improves sensitivity in the corresponding chemotherapy-sensitive maternal cell lines. Mitogen and stress-activated protein kinase 1 (MSK1), *de novo* DNA methylation and pregnane x receptor (PXR) were potential mediators of these observations. Hamano *et al.*[89] demonstrated that overexpression of miR-200c induced chemoresistance in OSCC cell lines mediated through activation of the AKT signalling pathway. Hummel *et al.*[61] also suggested that miR-21, miR-106a, and miR-148a correlate with tumour location, distant lymph node metastases and outcome in patients with locally advanced OSCC, and might inform the initial assessment of these patients and predict those at higher risk of postsurgical recurrence. Consequently, it can provide very valuable information to guide us to

evaluate the patient's condition and choose the most fitting medical treatment methods in individual OSCC patients. Furthermore, it could mean that a specific miRNA could have a treatment effect in itself.

CLINICAL APPLICATION OF MiRNAs

Improvement in the survival rate of patients with OSCC will most likely result from new therapeutics based on an increased understanding of the tumour biology and identification of biomarkers for earlier detection. The differential miRNA expression of OSCC may lead to identification of specific markers for progression, molecular classification of carcinoma lesions. Both oncomiRs and tumour suppressor miRs have the potential to serve as molecular therapeutic targets. The inhibition of oncomiRs and the promotion of tumour suppressor miRs should result in increased levels of tumour suppressor proteins or decreased oncogene-induced proteins. For example, the inhibition of miR-21 may possibly regulate PTEN, TPM1, PDCD4, Sprouty2, BCL2, programmed cell and maspin and their corresponding proteins and may inhibit tumour growth, invasion, and metastasis as a result. Upregulating the expression of miR-29c may inhibit the tumour growth through inhibition of cyclin-E. Additionally, we could choose the best medical treatment for individuals by detecting and evaluating specific miRNA. Furthermore, we can change the expression of specific miRNA to improve the treatment effect for some medical methods. For example, the OSCC patients of overexpression of miR-200c may lead the chemoresistance, and inhibition of miR-200c may possibly promote the chemotherapy-sensitivity and improve the effect of chemotherapy if there is no other method to choose.

Consequently, a better understanding of changes in miRNA expression and their functions during OSCC carcinogenesis might lead to possible improvements in the diagnosis and treatment of OSCC.

At present, there are no reports on the use of miRNA for anticancer therapy in the clinical field. However, miRNA therapy provides an attractive antitumour approach for integrated cancer therapy.

CONCLUSION

To the best of our knowledge, miRNA expression and its role in OSCC have considerably advanced our understanding of the molecular mechanisms of OSCC. Emerging reports on miRNAs in OSCC have suggested that miRNAs are promising in stratifying the risk of susceptibility to developing and diagnosing OSCC, and developing future therapeutic targets in OSCC. Furthermore, we can make full use of this knowledge to guide us to evaluate and improve the patient's condition and choose the most fitting medical treatment or explore new approaches to improve the survival rate of OSCC patients.

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ORIGINAL ARTICLE

Educational disparities in mortality among patients with type 2 diabetes in the Netherlands (ZODIAC-23)

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ABSTRACT

Background: Relative mortality differences between educational level in mortality have been reported among diabetic as well as among non-diabetic subjects in Europe, but data on absolute differences are lacking. We studied the effect of educational disparities on mortality in a Dutch prospective cohort of type 2 diabetes mellitus (T2DM) patients.

Methods: This study was part of the ZODIAC study, a prospective observational study of patients with T2DM. Data on educational level were first collected on 19 May 1998, and from this date on, 858 patients were included in 1998; educational level was known for 656 patients. Vital status was assessed in 2009. The relationship between mortality and educational level was studied using a Cox proportional hazard model, the relative index of inequality (RII), slope index of inequality (SII) and the population attributable risk (PAR). Educational level was divided into four categories; the highest educational level was used as reference.

Results: After a median follow-up time of 9.7 years, 365 out of 858 patients had died. The hazard ratio of primary education for total mortality was 3.02 (95% CI 1.44-6.34). The RII was 2.85 (95% CI 1.21-6.67), the absolute difference in the risk for mortality (SII) was 384 deaths (95% CI 49-719) per 10,000 follow-up years. PAR for patients with the lowest level of education was 51.4%.

Conclusions: A low educational level had a higher impact on mortality than having a macrovascular complication. Given the substantial differences in mortality between educational levels in T2DM, more understanding of underlying (modifiable) mechanisms is necessary.

KEYWORDS

Educational disparities, type 2 diabetes, mortality

INTRODUCTION

In many countries, socioeconomic position and educational level are inversely related to unhealthy behaviour and to lesser access to high quality care. Socioeconomic position (SEP) refers to an individual's position within a hierarchical social structure and is influenced by many social, societal, and economic factors, such as educational level, income, or wealth. Social disparities in mortality can theoretically be expected to be amplified among patients with diabetes, compared with those without diabetes.[1-5] In Europe, socioeconomic disparities and educational disparities in mortality have been reported among diabetic as well as among non-diabetic subjects.[6-10] Mortality differences between social classes have always been present in the general population; but it was not until the 1990s that widening socioeconomic mortality disparities were also observed among diabetic patients.[8,9]

Most data on SEP and educational disparities and the relationship with mortality are based on cross-sectional data, retrospective data or record linkage studies,[8,10,11] making it difficult to determine the exact impact of educational level on the risk for mortality. Furthermore, all previous studies performed in Europe looked at relative measures. Two large record linkage studies found that the effects of social economic position (SEP) and educational level on survival were weaker in people with diabetes than in the general population.[8,11] Eastern European countries

have higher relative disparities in mortality by SEP.[10] A recent study performed in the US looked at both relative as well as absolute educational disparities in mortality in patients with type 2 diabetes mellitus (T2DM).[12] And although the relative effects of educational disparities on mortality were weaker in adults with diabetes, the absolute impact on mortality was far greater in adults with diabetes. Given the increasing burden of T2DM and the observed increase in social and educational inequalities in the prevalence of T2DM and its complications, further efforts to quantify these effects are urgently needed.[13] The aim of this study was to estimate relative and absolute educational disparities in mortality in a Dutch cohort of adults with T2DM.

MATERIALS AND METHODS

Study population

This study was part of the ZODIAC (Zwolle Outpatient Diabetes project Integrating Available Care) study.[14] In this project, general practitioners are assisted in their care of T2DM patients by hospital-based nurses specialised in diabetes. At baseline, patients with a very short life expectancy (including patients with active cancer) or insufficient cognitive abilities were excluded from this study. ZODIAC started in January 1998, but data on educational level were not collected until 19 May 1998. From this date on, 858 patients were included in 1998, and educational level was known for 656 (76%) patients. Vital status was recorded in January 2009. The ZODIAC study was approved by the medical ethics committee (reference number 03.0316).

Educational level was divided into four categories: primary education, lower secondary education, higher secondary education and tertiary education (bachelor's degree or higher). We categorised patients who went to high school into two groups (lower secondary education and higher secondary education) in accordance to the Dutch school system. Working status was classified as employed (yes) or unemployed (no).

Statistical methods

The effects of relative educational disparities on total mortality were measured using Cox regression models, tertiary education was used as the reference group. We used two different models. In model 1, age and gender were included as possible confounders. In model 2, we adjusted for age, gender, body mass index (BMI), smoking status (smoker/non-smoker), macrovascular complications (yes/no), diabetes duration and working status. We selected these confounders based on their possible relationship with both education as well as mortality.

Furthermore, the relative index of inequality (RII) and the slope index of inequality (SII) for assessment of educational disparities in mortality were used. Both the RII and the SII are generally accepted measures for assessing relative and absolute mortality risk.[15]

Although the interpretation of hazard ratios (HRs) is straightforward, the interpretation of the impact of educational level on mortality by comparing HRs across various groups is hampered by differences in the distribution, by factors such as for example smoking. Measures such as the RII and the SII can overcome this problem.[15,16] Educational level is transformed into a continuous measure in which the rank of education is calculated as the mean proportion of the population having a higher level of education.[16] The RII is the ratio between the estimated mortality prevalence among persons at rank 1 (the lowest education level) and rank 0 (the highest level). In other words, the RII is the predicted ratio of mortality at the two extremes of the educational scale. The RII was calculated with the use of binary logistic regression analysis. The SII measures absolute differences in rates (e.g., in deaths per 100,000 person-years) between the lowest and the highest ends of the educational scale. The SII is the predicted difference in mortality rates between the two extremes of the educational scale.

The SII is computed as the slope of the regression of mortality on the indicator of relative educational position in a generalised linear model using the identity link. Confidence intervals of RII and SII were estimated using a bootstrap procedure.

Based on the hazard ratios of the analyses with educational level as a categorical variable, we also calculated the population attributable risk percentage (PAR%) for all-cause mortality.[17] In our analyses, the PAR% can be interpreted as the percentage by which mortality rates could be reduced if the risk factor of interest was eliminated. PAR% can be calculated by using the following formula: *prevalence of risk factor among decedents x [(HR-1) / HR]*. The PAR% was also calculated for macrovascular complications. Statistical analyses were performed using SPSS version 15.0 and Stata 11.

RESULTS

Baseline data are presented in *table 1*. After a median follow-up time of 9.7 years, 365 out of 858 patients had died. The absolute mortality rate was 441 deaths per 10,000 follow-up years.

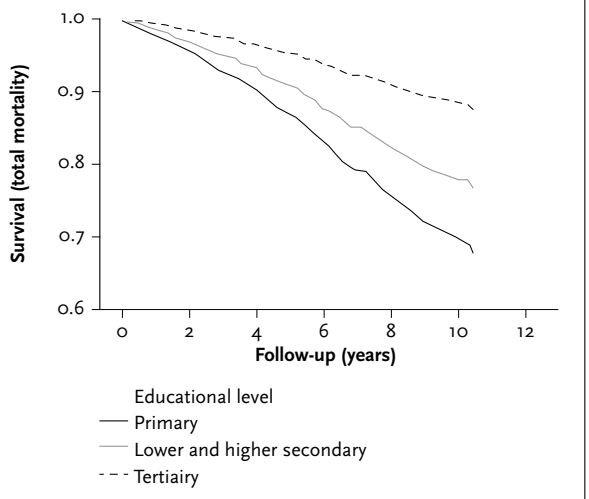
The HRs of primary education, lower secondary education and higher secondary education, compared with tertiary education, for total mortality were 2.53 (95% CI 1.23-5.19), 1.74 (95% CI 0.81-3.72), and 2.31 (95% CI 0.84-6.39), respectively, as calculated with model 1. Using model 2, HRs for total mortality were 3.02 (95% CI 1.44-6.34), 2.01 (95% CI 0.93-4.37), and 2.59 (95% CI 0.92-7.28), respectively. Also see *figure 1*.

Table 1. *Baseline characteristics*

Characteristic	Total *n=858*	Deceased patients *n=365*	Surviving patients *n=493*
Age (years)	67.8 (11.7)	75.4 (8.7)	62.3 (10.8) ***
Female (%)	58.0	55.9	59.6
Diabetes duration (years)	6.0 (3-11)	7.5 (4-13)	5.0(2-9)***
Smoking (%)	21.0	17.3	23.7*
BMI (kg/m²)	28.9 (4.9)	28.4 (5.0)	29.4 (4.7)**
Systolic blood pressure (mmHg)	152 (25)	154 (27)	151 (24.)
HbA1c (%)	7.5 (1.3)	7.4 (1.3)	7.4 (1.2)
Total cholesterol/HDL	5.3 (1.6)	5.2 (1.6)	5.4 (1.5)
Macrovascular complications (%)	35.8	50.4	24.9***
% Primary school (N)	68.1 (447)	76.9 (186)	63.0 (261)
% Lower secondary education (N)	23.0 (151)	16.9 (41)	26.6 (110)
% Secondary education (N)	3.2 (21)	2.9 (7)	3.4 (14)
% Tertiary education (N)	5.6 (37)	3.3 (8)	7.0 (29)
Working status	13.6	4.8	18.9***
Age >75 years (%)	27.9	53.5	9.3***

Data are presented as means SD for normally distributed data and median with interquartile range for non-normally distributed data or %. *P<0.05, **P<0.01, ***P<0.001 for differences between deceased and survived patients. The sum of patients in the different education categories does not correspond to 858 due to missing data.

Figure 1. *Survival curve for total mortality (model 2) according to the different educational levels*



Total mortality risk was nearly three times higher in T2DM patients with the lowest versus the highest position on the education scale (RII of 2.85, 95% CI 1.21-6.67). The absolute difference in the risk for total mortality between T2DM patients with the lowest versus the highest position on the educational scale, as measured with the SII, was 384 deaths (95%CI 49-719) per 10,000 follow-up years. The PAR for total mortality for patients with the lowest level of education was 51.4% (as calculated with the HR from model 2).

DISCUSSION

Disparities in educational level were related to substantial differences in mortality risk. In relative terms, the mortality risk after ten years was almost three times higher in the lowest educational level group compared with patients with the highest educational level. The impact of a low educational level was far greater than having a macrovascular complication. The population attributable risk (PAR) can give insight into the contribution of a risk factor to total mortality. The PAR of having a low educational level was 51%. Notably, the PAR of having a macrovascular complication was 25%. In absolute terms, patients with the lowest educational level suffered the greatest mortality burden with an absolute difference of 384 deaths per 10,000 follow-up years. The absolute increase in mortality is even more striking when compared with the absolute expected number of deaths in healthy subjects from the general population with a mean age of 68 years: 139 deaths per 10,000 follow-up (data available at www.cbs.nl).

Our study confirms the large absolute educational disparities in mortality in patients with T2DM, as observed in a recent study from the US.[12] Even after correction for important behavioural factors such as BMI, working status and smoking status, there remained a high contribution of having a low educational level to total mortality. The slope index of inequality in the US study was 503 deaths (95% CI 302-697) per 10,000 follow-up years compared with 384 deaths (95% CI 49-719) in our study. Whilst the US study adjusted for age, gender, race and survey year in their Cox proportional hazard analyses, we also adjusted for working status and clinical variables reflecting unhealthy behaviour, in this case smoking and BMI.

Health behaviour and BMI explain only partly the association between socioeconomic status and educational level and incidence of T2DM.[13] The factors that explain the higher mortality in patients with lower educational levels are probably related, at least for a large part, to differences in unmeasured healthy behavioural factors, for example exercise, eating habits and health seeking behaviour.[18,19] But also access to care, financial coverage of care, quality of care, and even different communication styles of the physician have been implicated to influence health behaviour.[20,21] We acknowledge that it would be very interesting to investigate these underlying factors in future studies. However, these data were not available in the ZODIAC study. Our study was specifically designed to estimate the contribution of educational disparities on total

mortality and not for studying the underlying mechanisms explaining this difference.

Several different indicators of SEP have been used in previous studies, including the amount of education, employment grade, income and indices based on residential area characteristics. For example in the US, educational level is most often used as a proxy for SEP.[22] Fortunately, different socioeconomic indicators show strong mutual associations.[23] However, the associations between health and the different socioeconomic indicators could have different implications and causes. For example, the educational level achieved by an individual patient in our cohort could have been influenced by other socioeconomic factors, such as family income and school costs at the time of starting his or her education. Whether, and to what extent, the relationship between educational level and mortality will be applicable to next generations needs to be determined.

There were more limitations to our study. Because of the small sample size the confidence intervals of our results were wide. Therefore, our results should be interpreted with caution. Secondly, selection bias could not be excluded, since data on educational level were not available for one quarter of the participants in the original ZODIAC study. For this reason, we calculated the hazard ratio for missing values on education for total mortality (HR= 1.25, 95% CI 1.04–1.50, adjusted for age, gender, BMI, smoking status, macrovascular complications, and diabetes duration), an outcome that even suggests an underestimation of the relationship observed. Also, the HR for mortality in patients with lower secondary education was lower than patients with higher secondary education; however, CIs overlapped substantially. We also did not correct for race or ethnicity, although most of our cohort (>98%) were Caucasians and the relative risks were comparable with other European studies.[8,11] Neither did we make a formal comparison with the Dutch population because in the ZODIAC study these data are not available. Although we adjusted for working status, no information was available on income level or working status before retirement. Furthermore, the *a priori* selected variables for model 2 and their role as confounders can be debated. As the differences between the HRs between model 1 and 2 were small, the impact of this potential methodological problem will probably be small.

Although regarded as more appropriate, previous studies did not use RII and SII.[15,16] Other strengths are its prospective design, the follow-up period of ten years, and the number of clinical variables available in the ZODIAC study.

In conclusion, we were not able to confirm the 'reassuring' small effect of educational level on mortality in diabetes patients.[9,11] As a matter of fact, relative as well as absolute risks were high in patients with T2DM with a low educational level. A low educational level had a higher impact on mortality than having a macrovascular complication. Further investigation should focus on modifiable factors that underlie these inequalities.

Conflicts of interest: none

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CASE REPORT

Unresectable pancreatic tumour? The issue is tissue

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ABSTRACT

The majority of tumours in the pancreas are adenocarcinomas for which therapeutic options are limited and which are associated with an unsatisfactory prognosis. However, alternative diagnoses may result in other therapeutic approaches with often a more favourable outcome. Hence, it is crucial to obtain a histological diagnosis before a definitive therapeutic plan can be devised. In this manuscript, a small series of pancreatic tumours other than adenocarcinoma are described.

KEYWORDS

Adenocarcinoma, histology, pancreatic cancer

What was known on this topic?
In rare cases, well treatable malignancies may present as an unresectable pancreatic adenocarcinoma. Tumour markers may help monitoring response to therapy but should not be used to confirm a diagnosis.

What does this add?
This article emphasises the importance of pathological confirmation of one's diagnosis in the case of an unresectable pancreatic tumour.

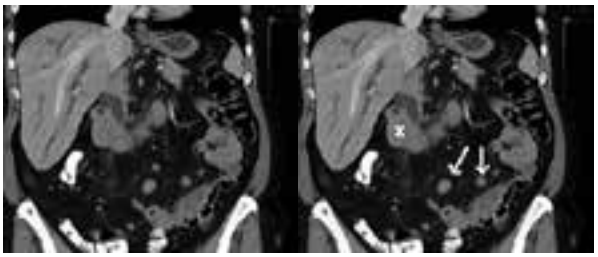
INTRODUCTION

Patients presenting with obstructive jaundice are often diagnosed with a tumour in the pancreas. Unresectable pancreatic adenocarcinoma can not be cured and has a dismal prognosis. In daily practice, the typical combination of clinical features and radiological findings, together with the lack of curative options, may result in refraining from pathological confirmation.
We describe three patients with similar complaints of obstructive jaundice and an unresectable pancreatic tumour who, after pathological examination, turned out to have a more favourable prognosis with therapeutic options.

CASE REPORTS

A 65-year-old woman presented with weight loss, fatigue, dark-coloured urine and itching. Laboratory results showed a total bilirubin of 164 µmol/l (normal 2-20) and conjugated bilirubin of 106 µmol/l (normal <5), an alkaline phosphatase level of 381 U/l (normal <120), and a γGT of 600 U/l (normal <38). The tumour marker CA 19.9 was high: 655 U/l (normal <37). An abdominal computed tomography (CT) scan revealed a tumour in the pancreatic head (3 x 3.7 cm) together with dilated intra- and extrahepatic bile ducts and locoregional lymphadenopathy. There were no signs of distant metastasis (*figure 1*).
An ultrasound-guided lymph node biopsy was carried out which revealed no metastasis. We referred our patient to an academic centre to investigate possible resection of the tumour. However, because of the enlarged lymph nodes the tumour was considered to be unresectable. To treat the obstructive jaundice a wall stent was placed during endoscopic retrograde cholangiopancreatography (ERCP). Of notice, a cytological examination of a bile duct brush revealed a low-grade B-cell non-Hodgkin lymphoma. This diagnosis was confirmed after external revision of the lymph node biopsy. A bone marrow aspiration revealed no localisation of the lymphoma.
Chemotherapy was started to treat a stage III follicular lymphoma using rituximab, cyclophosphamide, vincristin and prednisone (R-CVP). However, during the first course

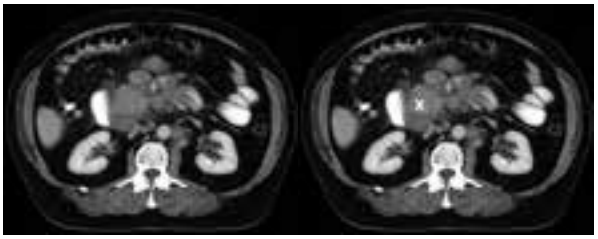
Figure 1. *Abdominal CT scan showing the tumour in the pancreatic head (marked by X in figure 1a), dilated intra- and extrahepatic bile ducts as well as loco regional lymphadenopathy (marked by arrows in figure 1a)*



the patient noticed a lump in her right breast (which in retrospect could not be seen on the CT scan), which turned out to be a diffuse large B-cell non-Hodgkin lymphoma.
To treat this transformed high-grade lymphoma chemotherapy was continued using R-CVP plus doxorubicin (R-CHOP). After three courses a PET/CT scan showed complete remission of the lymphoma.
After a total of six courses, because transformed lymphomas have a high chance of recidivating after just R-CHOP therapy, the lymphoma is currently consolidated with an autologous stem cell transplantation after 90Y-ibritumomab tiuxetan (Zevalin®) and carmustine, etoposide, cytarabine and melphalan (BEAM) chemotherapy and the patient is doing well.

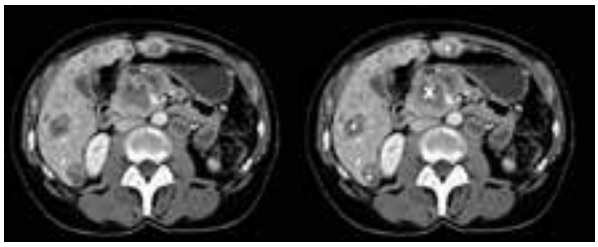
A 59-year-old man presented with abdominal pain, dark-coloured urine and light coloured stools. Laboratory results showed a total bilirubin of 67 µmol/l (normal 2-20) and conjugated bilirubin of 45 µmol/l (normal <5). An abdominal CT scan showed an unresectable pancreatic tumour invading the surrounding tissues (*figure 2*).
Histology of ultrasound-guided biopsies of the tumour revealed a small-cell neuroendocrine carcinoma. Serum chromogranin A, a marker for neuroendocrine tumours, was slightly elevated: 130 ng/l (<100). After six courses of etoposide/cisplatin a control CT scan showed complete remission. After a follow-up of five years the patient is still doing well.

Figure 2. *Abdominal CT scan showing the pancreatic tumour invading surrounding tissues (marked by X in figure 2a)*



A 56-year-old woman presented with abdominal pain and weight loss. On physical examination the liver was enlarged with an irregular surface. The tumour marker CA 19.9 was elevated: 81 U/l (<37).
An abdominal CT scan showed a tumour in the pancreatic head with liver metastases and peripheral lymphadenopathy (*figure 3*).

Figure 3. *Abdominal CT scan showing the tumour in the pancreatic head (marked by X in figure 3a) and liver metastases (marked by * in figure 3a)*



Histological examination of a liver biopsy showed a large cell carcinoma with neuroendocrine differentiation, suggesting an atypical carcinoid. This diagnosis was confirmed by high uptake on an octreotide scan together with an elevated serum level of chromogranin A: 354 ng/l (<100).
We referred our patient to an academic centre where she was treated with radioactive labelled octreotide (Lutetium-177-ocreotate). A control CT scan after four courses showed regression and the patient is doing well after a follow-up of almost two years.

DISCUSSION

Pancreatic adenocarcinoma is diagnosed in over 90% of all pancreatic malignancies. Unresectable pancreatic adenocarcinoma is a diagnosis with few therapeutic options and a very poor prognosis, with a median survival of only 3 to 6 months.[1,2]
However, not all unresectable malignant pancreatic tumours prove to be adenocarcinoma. A pancreatic tumour may be the first presentation of a malignant lymphoma.[3,4]
A tumour size of more than 6 cm at presentation, as well as peripheral lymphadenopathy, are clues that may lead to this diagnosis.[3,5]
In 1-2% of all cases the tumour appears to be a neuroendocrine tumour.[6,7] Pancreatic neuroendocrine tumours (PNETs) are rare and have different treatment options and a longer median survival.[6,8] The incidence of these tumours seems to be increasing, possibly due to improving radiological and pathological techniques such as endoscopic ultrasound fine-needle aspiration (EUS-FNA).[1,9]

Rarely, the tumour appears to be benign, for example concerning (IgG4-mediated) autoimmune pancreatitis.[10]

These cases show that both laboratory and radiological findings may be misleading in the case of an unresectable pancreatic tumour. An elevated CA 19.9 does not exclude a diagnosis other than pancreatic adenocarcinoma, and sometimes an unresectable pancreatic tumour may be well treatable. Given the fact that other, benign conditions such as cirrhosis and cholangitis may also lead to elevated levels, the diagnostic use of CA 19.9 will lead to a high rate of false-positive results. It may provide valuable information on response to therapy but, with a sensitivity of only 80% in symptomatic individuals, should not be used as a diagnostic tool.[11,12]

In order to establish a diagnosis, EUS-FNA has become an important tool. The new guideline 'pancreatic carcinoma' by the Comprehensive Cancer Centre the Netherlands (IKNL) states that, in the case of an unresectable pancreatic tumour, EUS-FNA is superior to ultrasound or CT-guided biopsy.[13] Additionally, in patients with suspected pancreatic malignancy in whom a CT scan is negative or inconclusive, EUS-FNA should be considered to establish a diagnosis, doing so in 88% of cases in a recent case series.[14]

We think that all patients diagnosed with an unresectable pancreatic tumour should have this diagnosis confirmed by pathological examination, ideally by histological biopsy but otherwise by EUS-FNA or ERCP-acquired cytology. This is in line with the IKNL guideline 'pancreatic carcinoma' version 2.0.[13]

CONCLUSION

An unresectable pancreatic tumour is not always an unresectable adenocarcinoma with an infaust prognosis. Pathological confirmation of the diagnosis is always indicated and may be lifesaving.

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PHOTO QUIZ

Acute left-sided abdominal pain

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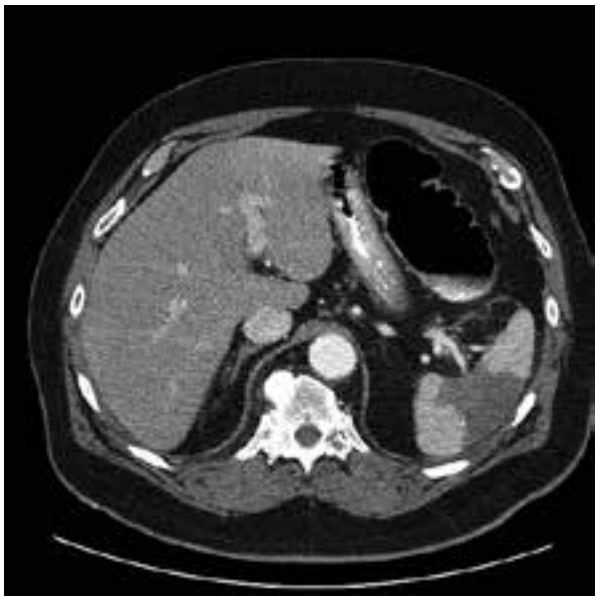
CASE REPORT

A 65-year-old Caucasian male with obesity (BMI 34) and no medical history was referred to our emergency room for acute and continuous left-sided abdominal pain. The pain increased during inspiration. The patient was vomiting and had constipation. He had no motion tendency or fever. The patient was not on any medication, smoked ten cigarettes and consumed three alcoholic beverages each day. On physical examination, the patient was in pain and sweating. He had an irregular pulse and pain on the left-sided hemithorax and left upper abdominal quadrant. No enlarged lymph nodes were palpated.

An ECG revealed atrial fibrillation with a ventricular rate response of 95 beats/min. A thoracic X-ray showed an enlarged heart, with no pulmonary or bone abnormalities. Laboratory tests revealed normal renal and liver function, a negative d-dimer (0.39 µg/ml), a mild leucoytosis (13.4 x 10^9/l), a CRP of 162 mg/l, and a conjugated bilirubin of 29 µmol/l. No protein, leucocytes or erythrocytes were found in the urine dipstick. A pulmonary embolism was unlikely because of a low Wells score and a negative d-dimer. We performed an abdominal ultrasound which revealed an hypo-echogenic lesion in the spleen. Next step was a CT angiography of the abdomen, which showed a wedge-shaped hypodensity in the spleen (*figure 1*).

Figure 1. *Contrast CT abdomen. There is a wedge-shaped hypodensitiy in the spleen*



WHAT IS YOUR DIAGNOSIS?

See page 87 for the answer to this photo quiz.

Netherlands
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PHOTO QUIZ

What's crawling in this sputum?

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CASE REPORT

A 64-year-old Dutch Caucasian male was admitted to our intensive care unit for postoperative care after aortic valve replacement. His previous medical history was relevant for giant cell arteritis for which he was taking 60 mg of prednisone daily. On the sixth postoperative day sepsis developed and a hospital-acquired pneumonia was suspected. Sputum was collected and investigated and is shown in *figure 1*.

Figure 1. *Microscopic analysis of sputum*

WHAT IS YOUR DIAGNOSIS?

See page 88 for the answer to this photo quiz.

Netherlands
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A large soft tissue mass of the chest wall

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CASE REPORT

After collapsing in the street, a 48-year-old African man was presented to our shock room. The patient had been suffering from productive cough, shortness of breath, nightly sweating and weight loss for five months. He arrived from a trip to Nigeria three days before. He was previously diagnosed with type 2 diabetes mellitus and hypertension. Physical examination showed a 20 cm large subcutaneous swelling on the right chest wall, diminished breath sounds over the right lung, tachypnoea of 29 breaths/min, tachycardia of 110 beats/min, normal blood pressure (145/90 mmHg), and a temperature of 38.0 °C. Oxygenation was 100% with 15 l O_2. The abdomen was not tender, traumatological and neurological examinations were normal. Laboratory findings: haemoglobin 4.6 mmol/l, leucocytes 4.9 x 10^9/l , platelets 325 x 10^9/l, serum creatinine 163 µmol/l, sodium 126 mmol/l, albumin 28 g/l and C-reactive protein 42.5 mg/l. Liver-associated enzyme levels were normal. Blood gases showed pH of 7.51 with PCO_2 4.0 kPa and HCO_3 24.4 mmol/l.

Anteroposterior chest X-ray showed a large soft tissue shadow overlying right-sided consolidations and pleural fluid (*figure 1*). Chest CT showed a large intra- and extrathoracic fluid collection (*figure 2*).

Figure 1. *Chest X-ray showing a large right-sided soft tissue shadow, pulmonary consolidations and pleural fluid*

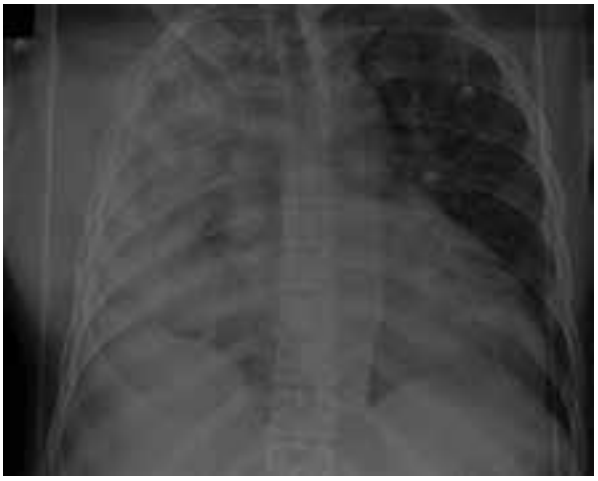
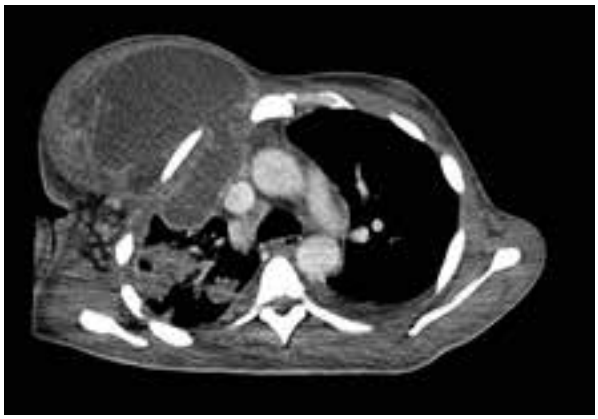


Figure 2a. *Chest CT in mediastinal setting (2a) showing a right-sided pleural collection with rim enhancement with a breakthrough fistula into a large prepectoral subcutaneous collection*



WHAT IS YOUR DIAGNOSIS?

See page 89 for the answer to this photo quiz.

DIAGNOSIS

The CT angiography of the abdomen clearly showed a wedge-shaped hypodensity in the spleen indicating a splenic infarction (*figure 1*). Contrast CT scan of the abdomen is currently the best non-invasive test available to diagnose splenic infarctions. It has the advantage of showing the infarction in the spleen and other target organs, while the extent of the thrombosis and sometimes the source of the infarction can be clarified. Ultrasonography has a low diagnostic yield for acute splenic infarction, however, ultrasonography can be useful in the follow-up of patients with acute splenic infarction, for detection of possible complications such as peritoneal haemorrhage, or pseudocyst or abscess formation.[1]

Different causes of spleen infarction are thromboembolic causes (such atrial fibrillation (AF), patent foramen ovale, endocarditis), hypercoagulable disorders, haemoglobinopathy, myeloproliferative diseases, acute infection, splenomegaly, trauma or obstruction of the spleen artery.

Infarction is caused by total occlusion of the splenic artery or one of the side branches due to thrombi or emboli. The incidence of splenic infarction found in the literature is very low.[2] Single patient case reports are still published in peer-reviewed medical journals,[3,4] emphasising that the diagnosis of splenic infarction is far from obvious at the emergency department. In this case, AF was probably the cause of the splenic infarction. The mechanism of thrombus formation in AF patients is due to: 1) Stagnation of blood flow in the left atrium, visible on an echocardiogram as spontaneous echo-contrast; 2) Anatomical cardiac wall defects, such as progressive atrial dilatation; and 3) Abnormal platelet activation and changes in coagulation factors, contributing to an increased propensity for blood clot formation.[5] In the literature, the incidence of splenic infarction due to AF is unknown.

In summary, this patient presented with acute abdominal pain, worsening during inspiration based on a splenic infarction due to thromboemboli formed during AF. The patient received analgesics, anti-emetics, and intravenous fluids. For his AF he was treated with anticoagulants (acenocoumarol after fraxiparin) and rate control with beta blockers. He recovered fully with this conservative treatment.

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ANSWER TO PHOTO QUIZ (PAGE 85)

WHAT'S CRAWLING IN THIS SPUTUM?

DIAGNOSIS

The sputum was investigated and surprisingly high numbers of mobile *Strongyloides stercoralis* larvae were observed. The same day blood cultures grew *Escherichia coli* and *Enterococcus faecium* and a diagnosis of *Strongyloides stercoralis* hyperinfection syndrome was made. Broad-spectrum antibiotics and ivermectin (12 gram orally, once daily) were started. During the first few days of treatment the clinical situation progressively deteriorated and severe multiorgan failure developed. Due to gastroparesis insufficient ivermectin uptake was suspected and subcutaneous ivermectin therapy, which is an off-label use of the compound, was started. Thereafter the number of live larvae in sputum samples decreased massively in a few days. After two weeks of intensive treatment the patient died due to intractable severe multiorgan failure. An evaluation of risk factors for *Strongyloides stercoralis* revealed that the patient had lived in Indonesia until the age of nine.

Strongyloides stercoralis is a helminthic parasite which can complete its lifecycle entirely within the human host.[1] Infection with the parasite is highly prevalent in developing countries. Together with depressed cell-mediated immunity, autoinfection can give rise to potentially fatal hyperinfection with disseminated disease.[2] Clinical findings in hyperinfection syndrome may be attributable to the direct consequences of organ invasion or to secondary Gram-negative bacteraemia, pneumonia or meningitis due to bloodstream seeding.[3] This dissemination of filariform larvae from the gastrointestinal tract to lungs, liver, heart, central nervous system and endocrine glands often results in severe and ongoing septic shock. *Strongyloides stercoralis* hyperinfection syndrome is a rare clinical entity in the Western world for which mortality rates exceeding 80% have been reported. The likelihood of developing the hyperinfection syndrome is increased if cell-mediated immunity is impaired and strongly associated with the use of corticosteroids.[4] In retrospect, a full travel history revealed that the patient had spent several childhood years in Indonesia, which should have led to testing and treatment of *Strongyloides stercoralis* prior to the implementation of steroid therapy. We here report a case of *Strongyloides stercoralis* hyperinfection syndrome and disseminated polymicrobial sepsis after cardiac surgery, in a patient who was on steroids. This report illustrates that *Strongyloides stercoralis* should be excluded in any patient with a history of travel to endemic areas and/or gastrointestinal symptoms before induction of immunosuppressive therapy. Hyperinfection syndrome with disseminated disease should be suspected in immunosuppressed patients with polymicrobial sepsis who are at risk for *Strongyloides stercoralis* infection.

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ANSWER TO PHOTO QUIZ (PAGE 86)

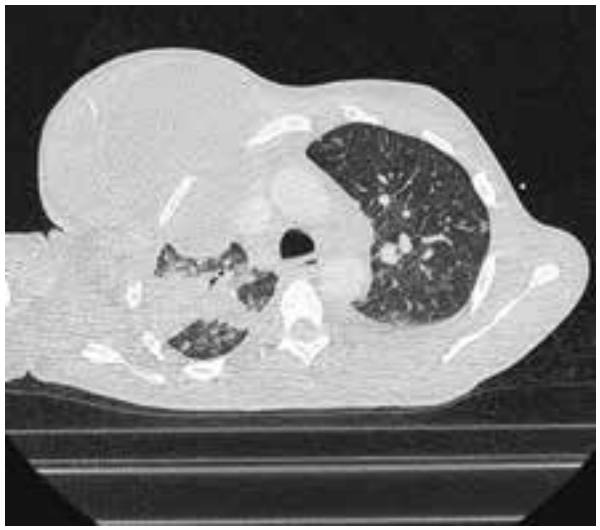
A LARGE SOFT TISSUE MASS OF THE CHEST WALL

DIAGNOSIS

Tuberculous empyema necessitatis.

Contrast-enhanced CT of the chest showed a right-sided empyema with breakthrough into a large prepectoral collection, the 'empyema necessitatis'.[1] Also large consolidations in the right lung, bilateral randomly spread intrapulmonary nodules and mediastinal lymphadenopathy were observed.
Subsequently pus was aspirated from the prepectoral swelling. Culture was positive for *Mycobacterium tuberculosis*, as was a sputum culture. The patient was put into quarantine, a chest tube was inserted for drainage of the abscess, and quadruple therapy in combination with pyridoxine was started.
Eight days later he was transferred to another hospital for further treatment. Mantoux screening ordered by the public health authority was negative for all caretakers involved.

Figure 2b. *In lung setting (2b) large consolidations in the right lung, bilateral randomly spread intrapulmonary nodules and mediastinal lymphadenopathy are observed*



Tuberculosis is a common, world-wide, airborne infectious disease caused by *Mycobacterium tuberculosis*.[2] Following ingestion by alveolar macrophages in the distal airways, the disease is controlled in most immunocompetent people. When not controlled, active disease i.e. primary or 'open' tuberculosis will follow, with accompanying malaise, persistent cough, haemoptysis, fever and weight loss. In a later stage caseinating granulomas can form. Secondary tuberculosis occurs when a silent disease progresses to an active disease, usually in immunodeficient people. In the Netherlands the incidence of tuberculosis nowadays is stable at ± 6.1 per 100,000, mostly in non-native inhabitants.[3] Mortality is around 6%.[4] As symptoms can be insidious, tuberculosis is not always easily recognised.[2]
Our patient showed a large swelling of the chest wall. Differential diagnosis of unilateral chest swelling consists of haematoma, chest wall tumours (lipoma, lymphoma, metastasis, sarcoma) and infectious diseases or abscesses from chest wall, pleural, pulmonary or mediastinal origin. Tuberculosis accounts for most cases of empyema necessitatis, but other organisms such as *Actinomyces* and *Nocardia* can also be responsible.
If a patient presents with insidious pulmonary symptoms, a high index of suspicion for tuberculosis is required and preventive measures against spreading should be taken.

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SPECIAL ARTICLE

A survey on diagnostic methods and treatment strategies used in patients with Waldenström's macroglobulinaemia in the Netherlands

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ABSTRACT

Background: Waldenström's macroglobulinaemia (WM) is defined as a lymphoplasmacytic lymphoma primarily located in the bone marrow, accompanied by an immunoglobulin M (IgM) monoclonal protein in the serum. The symptoms are highly variable, which can sometimes lead to a diagnostic delay. Currently, there is a wide range of therapeutic options used for the management of WM but no approved therapeutic agents are available specifically for this disease.

Methods: An online survey was prepared and sent out to haematologists and haemato-oncologists in the Netherlands, together with an invitational letter to participate. Information was gathered about the preferred methods of diagnosing and treating patients with WM in general, and about the last WM patient diagnosed in their department.

Results: 83 (31.8%) responses were obtained, out of which 68 (81.9%) contained responses to all three parts of the survey. The respondents most commonly used either rituximab-CVP or chlorambucil as first-line treatment, whereas rituximab in combination with purine analogues was the most frequently applied second-line treatment. The prevention of an IgM 'flare' was managed by the respondents in various ways, and rituximab maintenance treatment was not commonly used.

Conclusion: This survey indicates that in general the diagnostic methods and treatment options for WM are well known to a representative number of Dutch haematologists. The areas of uncertainty are knowledge about asymptomatic *vs* symptomatic disease, risk of hyperviscosity in relation to IgM level, and the occurrence and prevention of an IgM 'flare'. These issues should be addressed in clinical research and guidelines to improve care for WM patients in the Netherlands.

KEYWORDS

Waldenström's macroglobulinaemia, M-protein, immunochemotherapy

INTRODUCTION

Waldenström's macroglobulinaemia (WM) is a non-Hodgkin's lymphoma, characterised by infiltration of the bone marrow with small lymphocytes, lymphoplasmacytic cells and plasma cells, accompanied by secretion of monoclonal immunoglobulin M (IgM) protein in the serum.[1] WM is a rare disease, with an overall incidence of approximately 3 per million people per year and about 75-100 newly diagnosed patients in the Netherlands per year. The clinical presentation is variable among patients, and around 30% of WM patients are asymptomatic and do not require therapy at diagnosis.[2]

According to current standards, treatment should be initiated only when lymphoma-related clinical symptoms or at least one of the following parameters are present: haemoglobin <6.2 mmol/l, platelets <100 x 10^9/l, significant organomegaly or adenopathy, hyperviscosity, cryoglobulinaemia, cold agglutinin disease or amyloidosis.[3] Until recently, no treatment recommendations were available for WM patients in the Netherlands. Even internationally, there is no consensus on the standard of

first-line treatment.[4] Additionally, there are no approved therapeutic agents specifically for this disease. The drugs used most often are alkylating agents, such as chlorambucil and cyclophosphamide, purine analogues, rituximab and corticosteroids. Many aspects must be taken into account when deciding on a certain treatment regimen for a WM patient, which may be recognised as quite a complex process by practising clinicians.

In order to examine the currently used diagnostic and therapeutic management of patients with WM, a survey was carried out amongst Dutch haematologists and haemato-oncologists, investigating the strategies used in general as well as the specific methods used in their last patient diagnosed with WM.

METHODS

An online questionnaire in Dutch containing 24 questions was prepared, and a link to this survey was sent out to all known haematologists and haemato-oncologists in the Netherlands (n=261) (see *Appendix*). A reminder was sent after one month. The questionnaires were answered anonymously.

In the first part of the survey, physicians were asked questions relating to the type of hospital (HOVON level, see http://www.hovon.nl/ziekenhuizen/echelonering.html) they work at and the consultation region it belongs to, as well as the availability of various diagnostic methods. HOVON level A hospitals are academic hospitals equipped to perform both allogeneic and autologous stem cell transplants (SCTs). Level B and C hospitals may administer intensive therapy, for example treatment of acute leukaemia, but only level B hospitals perform autologous SCTs, and level D hospitals do not treat patients requiring intensive haematological care.

In the second part the questions were focused on the preferred diagnostic methods for diagnosing WM and the line of treatment preferred for newly diagnosed as well as relapsed patients. In the third part, physicians were asked about their last patient diagnosed with WM, the symptoms that led to the suspicion of WM, the therapeutic management of that patient and the time before a response was detected. Most answers were multiple choice and more than one answer was possible if appropriate.

RESULTS

Eighty-three surveys (31.8% of total) were completed, out of which 15 (18.1% of responses) were incomplete, because the questions in the third part of the survey were left unanswered. All of the percentages are given as a percentage of respondents who answered the question.

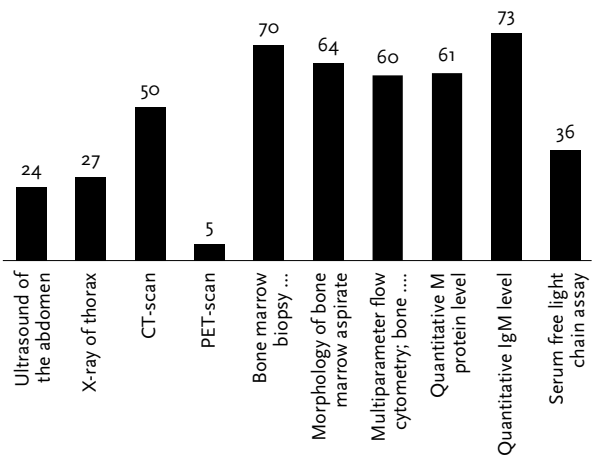
Basic information about the physician and the hospital

The single largest group of respondents was between age 45 and 55 (44.6%), and 62.7% of the respondents were 45 years or older. Most worked at an academic hospital (38.6%) or at a HOVON level C (31.3%) hospital, which represents hospitals equipped to give intensive treatment. Out of the ten referral regions, the responses were mostly gathered from haematologists based in the VU University Medical Center and Isala region (18.1%), University Medical Centre Nijmegen St Radboud region (16.9%), University Hospital Maastricht region (15.7%) and the Erasmus Medical Centre (15.7%). Most of the diagnostic tools, including CT scan, protein electrophoresis and bone marrow morphology and histology were readily available in all Dutch hospitals. Tests for cryoglobulins and cold agglutinins were available to 94 and 92% of respondents respectively. Tests with low availability were serum blood viscosity measurement and anti-MAG antibody titre test, available to 7.5% and 5.6% of respondents respectively.

Diagnostic methods used in patients with WM

The most frequently used diagnostic methods are the level of the M-protein in the serum as assessed by protein electrophoresis (88%), bone marrow biopsy with immunohistochemistry (84.3%), bone marrow aspirate morphology (77.1%), serum total IgM level (73.5%) and multiparameter flow cytometry of the bone marrow aspirate (72.3%) (*figure 1*). Furthermore, 43.4% of respondents reported use of the free light chains (FLC) assay. Imaging techniques were less commonly applied, a CT scan was selected by 60.2% of respondents, followed

Figure 1. *Which diagnostic methods do you use to diagnose WM?*



Commonly used diagnostic methods in Dutch hospitals for diagnosing WM. More than one answer was possible. Numbers are given as absolute value of the responses received.

by chest X-ray and ultrasound of the abdomen, chosen by 32.5% and 28.9% of respondents, respectively. An FDG-PET scan was used by only 4.8% of respondents. For diagnosing and staging of WM a combination of tests is necessary and the respondents used on average seven diagnostic methods (range 2-9). One respondent chose a combination of only two diagnostic methods but no one opted for only one diagnostic method.

Although demonstrating the presence of monoclonal IgM M-protein is essential for the diagnosis of WM, it was not chosen by 12% of respondents. We looked whether these respondents chose the total IgM level as an alternative test, but this was not the case.

Treatment preferences in patients with WM

Two treatment options were preferred in the first line: rituximab-CVP (cyclophosphamide, vincristine, prednisone) in 26 (36.1%) of the respondents, and rituximab in combination with other alkylating agents in 24 (33.3%) (*figure 2*). In total, 81.9% of the respondents chose a combination of rituximab and chemotherapy as the preferred first line of treatment. Monotherapy with an alkylating agent was the preferred first-line treatment in only 10 (13.9%) of the respondents. None of the respondents indicated rituximab monotherapy, bortezomib, bendamustine or thalidomide as their recommended first line of treatment.

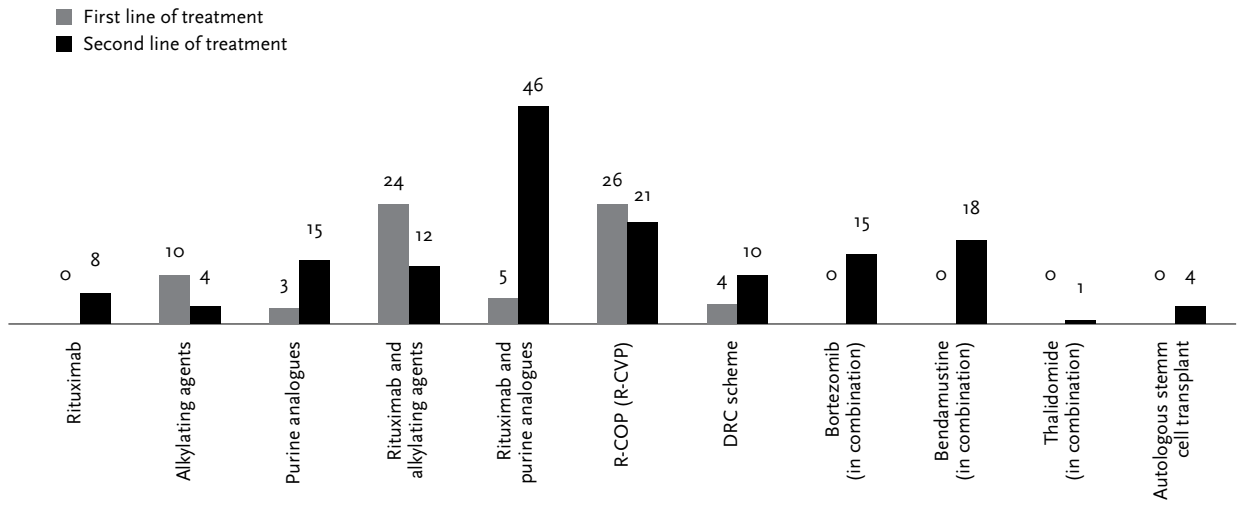
As preferred second-line treatment, respondents indicated the use of rituximab in combination with purine analogues (55.4%). Additionally, rituximab monotherapy (9.6%), bortezomib (18.1%), bendamustine (21.7%), thalidomide (1.2%) and an autologous stem cell transplant (4.8%) were all indicated as possible second-line treatments (*figure 2*). Respondents indicated on average two (range 1-8) possible options for second-line treatment.

Subsequently, respondents were asked about any precautionary actions used to reduce the risk of hyperviscosity syndrome due to an IgM 'flare' reaction when treating patients with rituximab. Twenty-one of the respondents (29.6%) did not use any preventive actions, whereas 22 (31%) stated that as a precautionary measure they avoided using rituximab in the first treatment cycle. The remaining 28 (39.4%) of the respondents stated that in some cases they would use plasmapheresis before starting treatment or do not use rituximab in the first treatment cycle. As a follow-up question respondents were asked at which level of M-protein or IgM they would apply this strategy. The mean reported level was 40 g/l with a range from 20 g/l up to >90g/l (n=15).

When asked about the use of maintenance therapy 52 (74.3%) of the respondents answered that maintenance therapy is not indicated, whereas 16 (22.9%) of the respondents indicated rituximab as best maintenance therapy. One respondent indicated bortezomib and one respondent indicated thalidomide as best option.

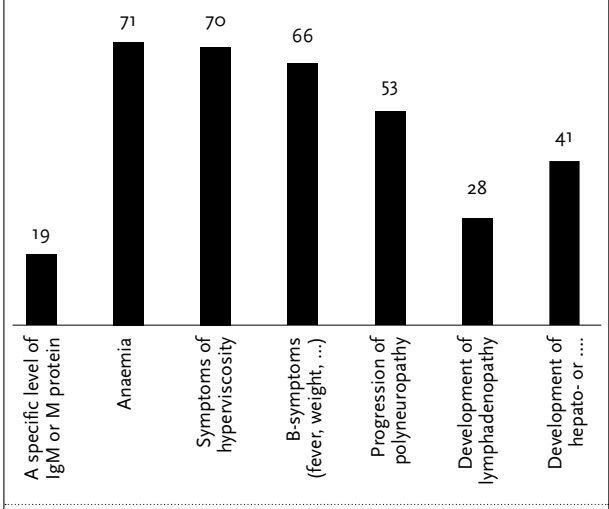
The next series of questions concerned the symptoms which prompted the start of treatment in patients previously diagnosed as asymptomatic. Multiple answers were possible and the most frequent were anaemia (85.5%), symptoms of hyperviscosity (84.3%) and occurrence of B symptoms (79.5%) (*figure 3*). Nineteen respondents (22.9%) reported that a specific level of M-protein or IgM was the

Figure 2. *Which of the given treatment options do you prefer as a first and second line of treatment?*



Preferred first and second line of treatment for WM. More than one answer was possible. Numbers are given as absolute value of the responses received.

Figure 3. *Variables evaluated in follow up of asymptomatic patients*



Variables evaluated in follow up of asymptomatic patients. More than one answer was possible. Numbers are given as absolute value of the responses received.

main reason to start treatment, at a median M-protein level of 30 g/l (range 20 - >60g/l).

Most recent experience with diagnosis and treatment of a WM patient

To this section 68 responses (81.9% of all responses) were gathered. The questions concerned the number of patients the respondents currently have under observation or treatment, and the diagnostic and treatment methods which were used on their last patient with WM.

Twenty-five (36.8%) respondents stated that they currently have 5-8 patients with WM on follow-up or under treatment, whereas 20 (29.4%) stated that they have more than 8 patients. Twenty respondents (29.4%) have between 2 and 4 patients and the remaining 3 (4.4%) respondents stated that they have 1 or no patients under control or treatment. When asked about the number of patients actively being treated the most frequent response was 0-1 patient (48.5%) or 2-4 patients (45.6%). The age of the last treated patient was 'between 60 and 70' in 39.7% and 'more than 70 years of age' in 38.2% of the respondents. Only 4 respondents (5.6%) indicated that the age of the last patient was less than 50 years.

The most common symptoms leading to the diagnosis of WM in their last treated patient were anaemia (51.8%) and weakness and fatigue (15.9%) and many respondents selected both answers.

Anaemia was also the most frequently indicated reason to start treatment in 43 (52.9%) of cases. Other indications for the start of therapy included 'evidence of problems caused by the M-protein'(29.4%) as well as 'evidence of disease progression by increase in the level of IgM or M-protein' (16.2%) and 'development of B symptoms'(9.9%). Ten respondents (15.4%) stated that they began treatment as soon as the patient was diagnosed.

The most commonly used first-line therapy to treat the last patient with WM was R-CVP (33.3%). Alkylating agents such as chlorambucil were also commonly used (30.3%). In total, 56% of respondents used rituximab in combination with any chemotherapy, while 4 (6%) gave rituximab monotherapy as first-line treatment to their last patient. Furthermore, respondents choosing a combination of rituximab and chemotherapy as first-line treatment also more often reported (59.5%) that the patient responded to the treatment (defined as a >25% decrease in the M-protein level) within three months of the initiation of treatment, compared with the other first-line treatment options (41.7%). In general, the maximum response was reported to occur in the first part of treatment (cycle 1 to 3-4) in 17 patients (30.4%), in the last part of treatment (cycles 3-4 to 6-8) in 27 patients (48.2%), and after stopping treatment in12 patients (21.4%). In 38 patients (64.4%) second-line treatment had not yet been necessary, in 14 (23.7%) second-line therapy was started between 1 and 4 years after first-line therapy and in 4 (6%) patients after 4 years. Three patients (5.1%) needed second line of treatment within one year of the last dose of the first-line treatment. Five respondents (8.5%) reported that they used rituximab maintenance therapy for their last patient.

DISCUSSION

The survey had a response rate of 31.8%. Responses were evenly spread among all of the ten haematological consultation regions in the Netherlands. The majority of respondents were experienced haematologists, but selection bias is of course always present in this type of research. Respondents may be haematologists with a specific interest in WM and answers may be partly guessed due to lack of memory of details or answers may be given that are the 'expected correct' answers instead of reflecting daily practice.

Many of the diagnostic methods important for the diagnosis of WM are easily accessible in Dutch hospitals. As expected, most respondents use a combination of tests to confirm the diagnosis. The five methods used most frequently were blood tests assessing the M-protein and total IgM levels, bone marrow biopsy with immunohistochemistry, as well as multiparameter flow cytometry and morphology of the bone marrow aspirate. Surprisingly, many respondents (72.3%) chose multiparameter flow cytometry as one of the diagnostic methods, which is indeed very helpful but is not mentioned in international guidelines as a required test.[5] The FLC assay was chosen

by 43.4% of the respondents, although this method has not shown to have additive value in patients with WM.

The preferred first line of treatment according to most respondents (81.9%) was immunochemotherapy. Although rituximab is registered for treatment of non-Hodgkin lymphomas (NHL), it is not registered specifically for WM treatment. Since WM is considered a type of NHL, and effectiveness has been shown in one randomised trial, its use has become common practice.[6] However, when asked for first-line treatment used in their last diagnosed patient only 56% had prescribed immunochemotherapy, and chlorambucil monotherapy was still used in 30% of the patients. This discrepancy might reflect transition of treatment preferences in the last years, comorbidity of the last treated patient, difference between daily practice and given 'expected' answers or it may be due to the fact that less haematologists responded to the last part of the survey. When deciding on a second-line treatment, many respondents indicated that several options were possible. Rituximab in combination with purine analogues was the preferred second line of treatment (55.4%), and additionally R-CVP, bendamustine, bortezomib and rituximab monotherapy were chosen. Rituximab maintenance therapy is seldom applied but a recent retrospective analysis suggests that it may also be beneficial in WM patients.[7] Results of an ongoing randomised trial performed by the Studiengruppe Indolente Lymphome in Germany are eagerly awaited.

Subsequently, the respondents were asked about the precautions taken to prevent an IgM 'flare' reaction when a rituximab-containing treatment was started. The IgM 'flare' is the occurrence of an initial increase in the IgM level that usually occurs within 15-30 days after initiation of treatment which can cause hyperviscosity syndrome in patients who already had a high IgM level.[8] The increased level of IgM can remain elevated for three to four months and is not an indication of treatment failure. The IgM 'flare' arises in approximately half of the WM patients treated with rituximab monotherapy, and is generally seen less frequently when rituximab is combined with fast-acting chemotherapy such as fludarabine or bortezomib.[9,10] In the DRC (Dexamethasone-Cyclophosphamide-Rituximab) trial, in which no preventive actions were taken, an IgM 'flare' was observed in 32% of patients, and 11% experienced an >25% IgM increase.[11] However, this did not lead to signs or symptoms of hyperviscosity syndrome in any of the patients. Respondents were given three possible options and had a slight preference to select patients in whom they would omit rituximab in the first cycle and/or use plasmapheresis. As a follow-up question, the last group of respondents were asked at which level of IgM they would take preventive measures, which varied from >20g/l up to >90g/l. This disparity in the responses might be an indication of uncertainty and the need for more defined guidelines to prevent an IgM 'flare'.

Indications for starting treatment were mostly aneamia, symptoms of hyperviscosity and B symptoms, such as weakness, fatigue, weight loss and anorexia. Nineteen (22.9%) of the respondents stated that they initiate treatment when a certain level of IgM or M-protein is present, usually between 30g/l and 50 g/l. In general, the level of IgM or M-protein itself is not an indication for treatment initiation, unless symptoms of hyperviscosity are present.

The last part of the survey asked about personal experience with WM patients. As expected for an indolent disease, most patients were not receiving active treatment ('wait and see' policy) and were older than 60 years. The majority of respondents did not have many patients in their practice which may imply they have limited experience in treating WM patients. When deciding on treatment initiation, the most important symptoms were anaemia, development of symptoms of hyperviscosity and progression of disease although an increase in IgM or M-protein levels was also mentioned.

Because WM is a disease in which treatment responses are often delayed, respondents were asked what their experience was with time to first response. Combinations of rituximab and chemotherapy seem to induce the fastest responses. In about 20% of patients responses were only obtained after treatment was stopped. This is also a well-known phenomenon: responses occurring until one year after the end of treatment have been reported and this may be due to longer survival of the clonal plasma cells, which produce the M-protein, after anti B-cell directed therapy.

CONCLUSION

Most oncologists and haematologists participating in this survey showed excellent understanding of the diagnostic methods and treatment options in WM. The level of M-protein at which symptoms may be expected is always a subjective clinical judgement and some physicians wait until symptoms occur while others start treatment if IgM levels increase. The areas of uncertainty mostly concern the risk of hyperviscosity and its relationship with IgM levels, and the occurrence and prevention of IgM 'flare'. These issues, among others, are addressed in the Dutch guidelines for WM, which are published in this same issue of the *Netherlands Journal of Medicine* and hopefully will contribute to improved care for WM patients in the Netherlands.[12]

Grants or conflict of interest: none reported

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APPENDIX

A survey on diagnostic methods and treatment strategies used in patients with Waldenström's macroglobulinaemia

Basic information about the haematologist and the type of work facility

1. What is your age?
a. Younger than 25
b. Between 25 and 30
c. Between 30 and 35
d. Between 35 and 40
e. Between 40 and 45
f. Between 45 and 50
g. Between 50 and 55
h. Older than 55

2. What is the HOVON level ('Echelon') of your hospital?
a. Level A
b. Level B
c. Level C
d. Level D

3. In the Netherlands the haematological care is organised around 10 consultation centres. Which one is your consultation centre?
a. UMCG
b. UMCN St Radboud
c. MST
d. AZM
e. ErasmusMC
f. LUMC
g. Haga Hospital
h. UMCU
i. AMC
j. VUmc/Isala

4. Which diagnostic methods are available in your hospital? *(multiple answers possible)*
a. CT scan
b. PET scan
c. Multiparameter flow cytometry
d. Total IgM levels
e. Total M protein levels
f. Serum free light chain assay
g. Viscosity measurement (centipoise)
h. Cryoglobulin analysis
i. Cold agglutinin test
j. Anti-MAG antibodies test

Diagnosis and treatment of Waldenström's macroglobulinaemia

5. Which diagnostic tools do you use to diagnose WM? *(multiple answers possible)*
a. Ultrasound of the abdomen
b. X-ray
c. CT scan
d. PET scan
e. Bone marrow biopsy with immunohistochemistry
f. Morphology of bone marrow aspirate
g. Multiparameter flow cytometry of bone marrow aspirate
h. Blood tests to determine IgM levels
i. Blood tests to determine M-protein levels
j. Serum free light chain assay
k. Other, namely...

6. What is, in your opinion, the preferred first-line treatment for symptomatic WM patients?
a. Rituximab
b. Alkylating agents such as cyclophosphamide and chlorambucil
c. Purine analogues such as fludarabine and cladribine
d. Rituximab in combination with alkylating agents
e. Rituximab in combination with purine analogues
f. R-COP (R-CVP)
g. DRC regimen (dexamethasone, rituximab, cyclophosphamide)
h. Bortezomib (in combination)
i. Bendamustine (in combination)
j. Thalidomide (in combination)
k. Other, namely...

7. The administration of rituximab is associated with an 'IgM flare' in 50% of the patients. Do you take precautionary measures?
a. No, the IgM flare rarely causes problems
b. Yes, in the first cycle I do not give rituximab
c. In some cases I take precautionary measures such as plasmapheresis or I give the first cycle without rituximab

8. If your answer to question 7 was 'In some cases I take precautionary measures such as plasmapheresis or I give the first cycle without rituximab': at what level of IgM protein do you decide to take precautionary measures? (give your answer in g/l) ...

9. If you decide to follow a wait-and-see policy (no treatment), which of these diagnostic results are an indication for you to start treatment? *(multiple answers possible)*
a. A certain level of IgM or M protein
b. Anaemia or other
c. Symptoms of hyperviscosity
d. B-symptoms
e. Progression of polyneuropathy
f. Development of lymphadenopathy
g. Development of splenomegaly or hepatomegaly
h. Other, namely...

10. If your answer to question 9 was : 'A certain level of IgM or M protein': what level of IgM or M protein is important for you to start the treatment? (give your answer in g/l)...

11. What do you think are preferred second-line treatments? *(multiple answers possible)*
a. Rituximab
b. Alkylating agents such as cyclophosphamide and chlorambucil
c. Purine analogues such as fludarabine and cladribine
d. Rituximab in combination with alkylating agents
e. Rituximab in combination with purine analogues
f. R-COP (R-CVP)
g. DRC regimen (dexamethasone, rituximab, cyclophosphamide)
h. Bortezomib (in combination)
i. Bendamustine (in combination)
j. Thalidomide (in combination)
k. Autologous stem cell transplantation
l. Other, namely...

12. What do you think is the best maintenance therapy?
a. None
b. Rituximab
c. Bortezomib
d. Thalidomide
e. Other, namely...

Most recent experience with the diagnosis and treatment of a patient with Waldenström's macroglobulinaemia

13. Do you treat patients with WM in your clinic?
a. Yes
b. No

14. How many patients are you currently seeing (follow-up and in treatment)?
a. 0-1
b. 2-4
c. 5-8
d. >8

15. How many patients are you currently treating?
a. 0-1
b. 2-4
c. 5-8
d. >8

16. What was the age of the last patient with WM that you treated?
a. Less than 50 years
b. Between 50 and 60
c. Between 60 and 70
d. More than 70 years

17. What was/were the first symptom(s) of this patient, which resulted in the diagnosis of WM? *(multiple answers possible)*
a. None, patient was asymptomatic
b. Weakness and fatigue
c. Bleeding
d. Weight loss and/or anorexia
e. Anaemia
f. Elevated erythrocyte sedimentation rate
g. Lymphadenopathy
h. Hepatomegaly
i. Splenomegaly
j. Neuropathy
k. Vasculitis/skin lesions
l. Haemolysis
m. Other, namely...

18. When did you start treatment? *(multiple answers possible)*
a. As soon as the patient was diagnosed
b. As soon as there was evidence of problems caused by the M protein (hyperviscosity, neuropathy, amyloidosis, cryoglobulinaemia)
c. As soon as there was evidence of disease progression caused by a rise in the M protein or IgM levels
d. As soon as development of B-symptoms occurred
e. Anaemia
f. Not applicable, no treatment given
g. Other, namely...

19. What was the first-line treatment that you used in this patient?
a. Rituximab
b. Alkylating agents such as cyclophosphamide and chlorambucil
c. Purine analogues such as fludarabine and cladribine
d. Rituximab in combination with alkylating agents
e. Rituximab in combination with purine analogues
f. R-COP (R-CVP)
g. DRC regimen (dexamethasone, rituximab, cyclophosphamide)
h. Bortezomib (in combination)
i. Bendamustine (in combination)
j. Thalidomide (in combination)
k. Autologous stem cell transplantation
l. Other, namely...

20. How soon after the start of treatment did the patients show a response (defined as >25% reduction in M-protein levels)?
a. Less than 3 months
b. Between 3 and 6 months
c. Between 6 months and 1 year
d. No response to first line of treatment

21. When was the maximum (best) response achieved?
a. In the first part of the treatment (cycle 1 to 3-4)
b. In the last part of the treatment (cycles 3-4 to 6-8)
c. After the treatment was stopped
d. Insufficient or no response, switched to another treatment, namely...

22. How long after the last dose of the first-line treatment did you begin with second-line treatment?
a. Second line of treatment was not needed
b. Less than 1 year
c. Between 1 and 2 years
d. Between 2-4 years
e. More than 4 years

23. If applicable, what was the second line of treatment that you used?
a. Not applicable
b. Rituximab
c. Alkylating agents such as cyclophosphamide and chlorambucil
d. Purine analogues such as fludarabine and cladribine
e. Rituximab in combination with alkylating agents
f. Rituximab in combination with purine analogues
g. R-COP (R-CVP)
h. DRC regimen (dexamethasone, rituximab, cyclophosphamide)
i. Bortezomib (in combination)
j. Bendamustine (in combination)
k. Thalidomide (in combination)
l. Autologous stem cell transplantation
m. Other, namely...

24. Did you use maintenance treatment in this patient?
a. Yes, in the first line, and I used rituximab
b. Yes, in the first line, and I used bortezomib
c. Yes, in the first line, and I used thalidomide
d. Yes, in the second line, and I used rituximab
e. Yes, in the second line, and I used bortezomib
f. Yes, in the second line, and I used thalidomide
g. No
h. Other, namely...

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SPECIAL ARTICLE

The prevention of contrast-induced nephropathy in Dutch hospitals

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Background: A major adverse effect of intravascularly administrated iodinated contrast medium is contrast-induced nephropathy (CIN). To reduce CIN incidence, two different prevention guidelines have been introduced in the Netherlands.

Objective: Our goal was to assess the use of CIN prevention guidelines at the radiology departments in Dutch hospitals.

Methods: We conducted a survey in all 90 Dutch hospitals with a radiology department. The questionnaire included questions about guideline execution (e.g. which guideline, (compliance) problems).

Results: All responding (67/90) hospitals used a CIN prevention guideline. When asked who was responsible for conducting preventive measures in high-risk patients identified according to either guideline, the referring physician was responsible in 38 hospitals (56.7%); in 23 hospitals (34.3%) there was a specialised CIN outpatient clinic. Renal function was routinely checked after exposure to intravenous iodinated contrast medium in all CIN outpatient clinics (23) and radiology departments (2) when these were responsible for this measurement and in 52.6% (18/38) hospitals when the referring physicians were responsible. When asked if identifying patients at risk caused any problems, 47.8% reported problems.

Conclusion: In all responding Dutch hospitals a CIN prevention guideline was used. There was considerable variation in the execution of the guidelines and there were substantial compliance problems. The follow-up procedure was more consistent in hospitals with an outpatient clinic.

KEYWORDS

Compliance, contrast-induced nephropathy, guidelines, prevention

BACKGROUND

The use of intravascular iodinated contrast media in radiological procedures has increased over the years.[1-3] Unfortunately the use of intravascular iodinated contrast medium is associated with contrast-induced nephropathy (CIN) and is one of the top three causes of acute nephropathy in hospitalised patients.[4] CIN is mostly defined as a rise of serum creatinine (SCr) of at least 25% or 44 μmol/l compared with the baseline with no other explanation for the rise in SCr or nephropathy. CIN usually develops within 24-48 hours after exposure to intravascular iodinated contrast medium and it seems to be transient in most cases. However, in some cases it is associated with long-term adverse events leading to increased morbidity and mortality.[4-9] The precise pathophysiology of CIN is unknown. It is a common theory that CIN is the result of transient vasoconstriction that leads to hypoperfusion of the glomeruli. This generates oxidative stress in combination with the nephrotoxicity of the contrast medium itself.[10-13]

During the past years, it has become clear which patients are at risk to develop CIN and what might be the best means of prevention in the patients at risk.[8,13-19] In general, patients with pre-existent kidney disease in combination with other risk factors (e.g. diabetes, hypertension) seem to be at risk for developing CIN. Oral and intravenous hydration before and after the contrast-enhanced examination in these at risk patients seems to reduce the incidence of CIN.[2,5,11,15-20]

The Dutch hospital patient safety program (Veiligheids Management Systeem: VMS) identified CIN as one of the ten main causes of preventable mortality and morbidity in Dutch hospitals and introduced a CIN prevention guideline in 2009.[21] The VMS prevention guideline for CIN is partly based on the multidisciplinary evidence-based CIN prevention guideline that was developed under the chair of the Radiological Society of the Netherlands (NVvR)

and introduced by the Dutch Institute for Healthcare Improvement (Centraal Begeleidings Orgaan: CBO) in 2007. [21-22]

These guidelines only differ in their strategy to identify patients at risk. The VMS guideline indicates that the estimated glomerular filtration rate (eGFR) should be determined in every patient scheduled for intravascular iodinated contrast medium administration. In case of an abnormal eGFR, other risk factors should be checked to identify patients at risk. The CBO guideline advises that risk factors should be assessed first and if present, eGFR has to be determined to identify patients at risk.

In both guidelines the prevention measures are similar and encompass prophylactic intravenous hydration before and after the procedure and discontinuation of metformin and all nephrotoxic medication. Two to three days after the intravascular contrast medium exposure, renal function should be verified (*see figure 1* and *tables 1* and *2*).

The introduction of these guidelines into clinical practice has led to discussion about the necessity of extensive prevention guidelines, feasibility and associated costs.

General critics were that the identification of high-risk patients and the measures that should be taken according to these guidelines are based on an overestimation of the incidence of CIN (especially for intravenous iodinated contrast medium administration) and therefore cause unnecessary use of medical resources.[23-25] However, others find that there is enough evidence and that the problem is often trivialised. They find these precautions have a great effect on the incidence and adverse outcomes of CIN.[2, 26,27]

Figure 1. *Patient flow in accordance with both guidelines*

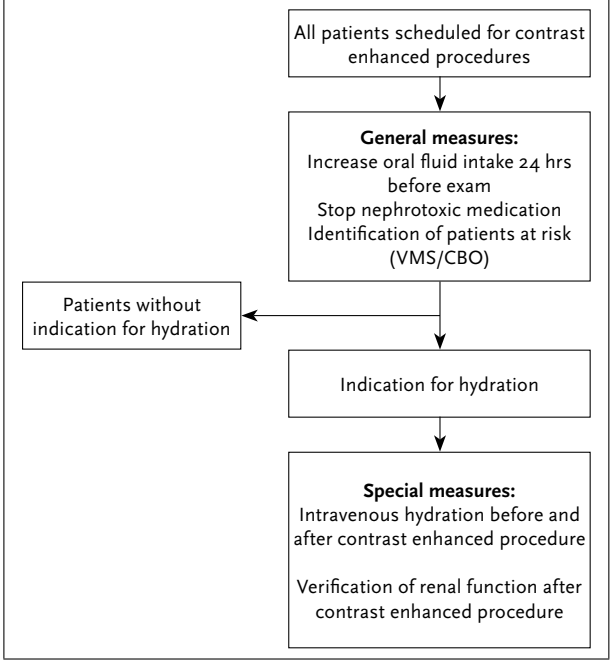

Table 1. *CBO: indication determination eGFR*

Risk factors (CBO)
Age > 60 years
Diabetes mellitus
Cardiovascular disease
Hypertension
History of urological or nephrological disease
Multiple myeloma or Waldenström's disease with small chain proteinuria
Use of nefrotoxic medication e.g. NSAID's*, metformin, aminoglycosides

***Non-steroidal anti-inflammatory drugs.**

Table 2. *Identification of patients at risk to develop CIN according to VMS and CBO guideline*

High-risk patients VMS guideline	**High-risk patients CBO guideline**
eGFR <60 ml/min and Diabetes Mellitus	eGFR <60 ml/min and diabetes mellitus
eGFR 45-60 ml/min and >1 risk factors: Peripheral arterial disease Congestive heart failure Age >75 years Anaemia: haematocrit (0.39 (m) 0.36 (f)[¥] Symptomatic hypotension Contrast volume >150 ml Decreased effective circulating volume Nephrotoxic medication i.e. diuretics and NSAIDs	eGFR <60 ml/min and >1 risk factors: Peripheral vascular disease Congestive cardiac disease Age >75 years Anaemia: (haematocrit <0.39 (m), <0.36 (f)), Symptomatic hypotension High volume (contrast index >1*) Dehydration Use of diuretics and/or NSAIDs, metformin, aminoglycosides
eGFR 45-60 mL/min and multiple myeloma/ Waldenström's disease with small chain proteinuria	eGFR <45 ml/min
eGFR < 45 ml/min	

[¥]**m=male, f=female, *contrast volume in relation to body mass index.**

Given these varying opinions and no uniform national guideline, one might envision a considerable variation in implementation of CIN prevention.

We aimed to assess whether Dutch hospitals used a CIN prevention guideline in contrast-enhanced procedures performed at the radiology departments. If they did, on which of the two Dutch guidelines this was based and if there were problems with the compliance of the prevention guideline. We also tried to assess whether the implementation of the guidelines was the same in every hospital.

METHODS

Questionnaire

A survey was performed using a questionnaire (*see appendix 1*) which was sent by email to radiologists in

all Dutch hospitals. Since radiologists mainly have a central role in the implementation of these guidelines, we addressed radiologists who where specifically involved with the prevention of CIN in their hospital. The questionnaire included questions concerning the CIN prevention guideline that was followed, who (e.g. the referring physician, radiology department) was responsible for the execution of the guideline, whether implementation of the guideline had led to any kind of problems and whether renal function was measured before and after an intravascular iodinated contrast enhanced procedure. Furthermore, we included some general questions, including information about the hospital (*appendix 1*). The survey was performed between May and August 2012.

Response

After two weeks all non-responders were contacted. They initially received a reminder via e-mail that – in case of no response – was followed by a phone call. We aimed to obtain a response rate of at least 70%. Of the hospitals that did not respond after two reminders, we decided post hoc to check whether they used a prevention guideline and which guideline; we did this to ensure that we did not overestimate the use of one of the two CIN prevention guidelines. We checked this by looking up information on the hospital's website. If the information on the website did not give clear details about the CIN prevention in the radiology department (e.g. which guideline was used and if and when eGFR was determined) we contacted the radiology department by phone. We did not try to complete the information of the non-responding hospitals for the other parts of the questionnaire.

Data presentation

We used descriptive statistical analysis to summarise the results. Categorical data were expressed as numbers and percentages. We used IBM® SPSS® statistic data editor version 19 SPSS® inc. to summarise the results.

RESULTS

General information

Of the 90 hospitals that were contacted, 67 (74.4%) responded, including all eight academic hospitals. The smallest hospital that participated had 140 beds, the largest hospital 1339 beds.

Guideline used

All responding hospitals used a prevention guideline; most hospitals (97.0%; 65/67) used either the VMS or CBO guideline. The VMS guideline had been adopted in the majority of hospitals (70.1%; 47/67) and a minority followed the CBO guideline (18/67; 26.9%). In two hospitals (3.0%; 2/67) a combination of the VMS and CBO guideline was used.

In 23 hospitals an outpatient clinic specialised in CIN prevention had been installed, these mainly (87%; 20/23) concerned hospitals using the VMS guideline.

Non-responders

All 23 non-responding hospitals had a prevention guideline. Most hospitals (65.2%; 15/23) used the VMS guideline, 21.7% (6/23) used the CBO guideline and the remaining (8.7%; 2/23) hospitals used a combination or variation of the two guidelines *(table 3)*.

Estimated glomerular filtration rate

Both VMS and CBO guidelines advise to determine the eGFR by using the Modification of Diet in Renal Disease (MDRD) formula in order to identify patients at risk. Renal function was determined in compliance to the guideline (MDRD-4 point formula) in 76.1% (51/67) of the hospitals, 6.0% (4/67) used the MDRD-6 point formula, 7.5% (5/67) the Cockcroft-Gault and 10.4% (7/67) of the hospitals used another not further specified formula (*table 4*).

Responsibility for general measures

Both guidelines also describe the general measures which are applicable in every patient receiving intravascular iodinated contrast medium. These include advising the patient to drink extra fluids, instructing the patient what to do if dehydration, diarrhoea or hospitalisation occurs in the time between the request for the examination and the examination itself. If patients use diuretics or non-steroid anti-inflammatory drugs (NSAIDs) they should be advised to stop these 24 hours in advance.

In most hospitals, 74.6% (50/67), the referring physician was responsible for the general measures, followed by the

Table 3. *Guidelines used in different Dutch hospitals*

Guidelines used	**N= 67**
Response % ***(n)***	74.4 (67/90)
Hospitals that followed guideline % ***(n):67***	
VMS	70.1 (47)
CBO	26.9 (18)
Other	3.0 (2)
Specialised outpatient clinic % ***(n): 23***	
VMS	87.0 (20)
CBO	13.0 (3)
Other	
Non-responding hospitals % ***(n): 23***	
VMS	65.2 (15)
CBO	26.1. (6)
Other	8.7 (2)

Table 4. *Variation in execution of the guidelines*

Variation in execution	**N=67**
eGFR % *(n)*	
MDRD-4 point	76.1 (51)
MDRD-6 point	6.0 (4)
Cockroft-Gault	7.5 (5)
Other	10.4 (7)
Responsible for implementing general measures % *(n)*	
Radiology/ nuclear imaging department	3.0 (2)
Requesting physician	74.6 (50)
Outpatient clinic	16.4 (11)
Other	6.0 (4)
Responsible for specific patients at risk % *(n)*	
Radiology/ nuclear imaging department	3.0 (2)
Physician	56.7 (38)
Outpatient clinic*	34.3 (23)
Other	9.0 (6)
Determination of renal function % *(n)*	
No determination	28.4 (19)
Determination	71.6 (48)
Determination of renal function % *(n) by department*	
Radiology/ nuclear imaging department	100 (2/2)
Requesting physician	52.6 (20/38)
Outpatient clinic	100 (23/23)
Other	75 (3/4)

***Two outpatient clinics discussed specific measures with requesting physician**

specialised outpatient clinic in 16.4% (11/67), the radiology department in 3% (2/67) and four 6% (4/67) hospitals indicated having a different arrangement (*table 4*).

Responsibility for specific measures in patients at high risk

The responsibility for execution was different with respect to the enforcement of the specific, more intricate measures for patients identified as being at risk (e.g. intravenous hydration, verification of the eGFR after intravascular iodinated contrast medium exposure). The referring physician was responsible in 56.7% (38/67) of the cases and more hospitals indicated to have a specialised outpatient clinic (34.3%; 23/67), two hospitals with outpatient clinics indicated that after the need for specific measures was established the outpatient clinic discussed this with the referring physician. There were two radiology departments (3%) responsible and four other departments conducted these measures in the remaining 6% (4/67) (*table 4*).

Determination of renal function

To identify those patients who developed CIN, renal function has to be determined preferably within 48-72 hours after intravascular iodinated contrast medium exposure as is advised in the guidelines. In 48 hospitals renal function (eGFR) was determined after iodinated intravascular contrast administration. However, in 19 hospitals (28.4%; 19/67) renal function after intravascular contrast administration was not determined. All specialised outpatient clinics (23/23) screened for changes in renal function after iodinated intravascular contrast administration, as did the radiology departments (2/2). In hospitals where the referring physician was responsible for implementing the special measures, the majority (52.6%; 20/38) determined renal function after iodinated intravascular contrast administration. In the group of hospitals that reported having other arrangements regarding the responsibility of the implementation of special measures, most of them (75%; 3/4) determined renal function after intravascular contrast administration (*table 4*).

The time interval between intravascular iodinated contrast medium administration and renal function determination varied from two to seven days. Nine (13.4%; 9/67) hospitals determined renal function within the given interval of 48-72 hours.

Problems concerning selection of high-risk patients

When we asked responders about problems with the identification of patients at risk, 47.8% (32/67) reported that there were process-related problems in their institute. When we divided the hospitals according to which guideline they used, 48.9% (23/47) of the responders using the VMS guideline reported problems with the identification of patients at risk *vs* 50.0% (9/18) of the responders using the CBO guideline (*table 5*).

Most reported comments in the free text box were that the referring physicians did not determine the renal function prior to the requested procedure or did not mention whether other risk factors were present (26.8%; 18/67 responders). About 35% (24/67) of the responders also reported that some physicians might be trying to bypass the guidelines by not mentioning risk factors that

Table 5. *Problems concerning execution of guidelines*

Problems execution/compliance	**N=67**
Problems in selection high-risk patients % *(n/total)*	
All hospitals	47.8 (32/67)
VMS	48.9 (23/47)
CBO	50.0 (9/18)
Problems with general and specific measures % *(n/total)*	
All hospitals	43.3 (29/67)
VMS	48.9 (23/47)
CBO	33.3 (6/18)

later proved to be present or simply forgetting to identify patients at risk to develop CIN. Four responders reported that discontinuation of nephrotoxic medication was a problem in their institutes (*table 5*).

Problems concerning general and specific measures

When we asked about the application of the general and specific measures, 43.3% (29/67) reported that there were problems; this was somewhat higher in hospitals using the VMS strategy compared with the CBO strategy: 48.9% (23/47) *vs* 33.3% (6/18) (*table 5*).

The main problems were that there was no verification if preventive measures were executed (25.4%; 17/67 responders). If no preventive measures had been performed, this was in most cases discovered on the day of the intravascular iodinated contrast medium enhanced examination, leading to ad hoc logistical problems. These 17 responders (25.4%; 17/67) also reported that there was no agreement about who was responsible for determination of renal function after intravascular iodinated contrast medium administration, which made the determination of CIN unclear.

CIN incidence

We asked hospitals to report the CIN incidence in their institute. Forty-three (64.2%; 43/67) responders answered this question. The other 24 (35.8%; 24/67) hospitals did not answer this question.

Thirteen (19.4%; 13/67) hospitals reported that CIN did not occur in their institute, five (38.5%; 5/13) responders stated that this number was measured, the other eight (61.5%, 8/13) indicated this was an estimation.

Six (9%; 6/67) hospitals reported the incidence in percentages, varying from <1% up to 5%; three of the six (50%; 3/6) hospitals based this number on measurements; the other half estimated the incidence (50%; 3/6).

Of the three hospitals that measured the incidence in their institute, one reported that this was the incidence in outpatients who were identified as being at risk (incidence: 3%), one responder stated that the group of patients consisted of outpatients undergoing CT scans (incidence: 1.4%) the other respondent did not specify, one hospital derived their data from emergency department patients undergoing CT-pulmonary angiography who were clinically suspected of pulmonary embolism (incidence: 4%).

The remaining 24 hospitals did not provide exact information from which the incidence in percentages could be derived and compared, but the range varied from 0 up to 29 patients per hospital per year. Seven responders (29.2%; 7/24) declared that these numbers were measured and 17 (70.8%; 17/24) declared the numbers were estimated. The hospital that counted 29 cases in the past 12 months looked for other reasons for nephropathy besides CIN (*table 6*). They found other reasons in 28 cases reducing the cases of CIN to one.

Table 6. *Reported CIN incidence*

CIN incidence	**N=67**
Response % *(n/total)*	
Answered the question	64.2 (43/67)
Did not answer the question	35.8 (24/67)
Hospitals reporting incidence of 0%	19.4 (13/67)
Measured	38.5 (5/13)*
Estimated	62.5 (8/13)*
Hospitals reporting incidence in percentage % (n/total)	9(6/67)*
Range %	<1-5
Measured	50(3/6)*
Estimated	50(3/6)*
Hospitals reporting incidence in absolute data %(n/total)	
Range (n)	1-29*
Measured	29.2(7/24)*
Estimated	70.8(17/24)*
Reason for not answering % *(n/total)*	
Not measured	20.8 (5/24)
Reported as unknown in institute	29.2 (7/24)
Responder had no idea	25.0 (6/24)
No reason given for not responding	25.0(6/24)

***Data from (other) 24 hospitals were not comparable**

In general the CIN incidence was estimated in 28 hospitals, while measured in 15 hospitals.

When the question was not answered responders commented that this was not measured in their institute (20.8%; 5/24), or that this was unknown (29.2%, 7/24) or the responder acknowledged that he or she had no idea (25%, 6/24). The other six (25%, 6/24) hospitals gave no reason as why they were not able to answer the question (*table 6*).

DISCUSSION

Our study shows that all hospitals in the Netherlands use a CIN prevention guideline, consisting of the VMS guideline and/or the CBO guideline. The majority of Dutch hospitals (70.1%) have applied a CIN prevention strategy based on the VMS guideline, implying that renal function is determined in every patient who is exposed to intravascular iodinated contrast medium.

The execution of these guidelines has proven to be cumbersome. Almost 50% of the hospitals experienced problems with the compliance to the guideline in their institution. When we looked at the general (measures for all patients) and specific measures (measures in high-risk patients) there seemed to be fewer problems in hospitals using the CBO guideline than the VMS guideline. Our results show a great variation in the practical implementation of the guideline, concerning

the responsibility, timing and the way renal function was determined. Several hospitals have a specialised outpatient clinic to manage patients undergoing intravascular iodinated contrast enhanced procedures, mostly established to coordinate and execute the special measures in patients at risk. Nearly one third of the hospitals did not determine renal function after intravascular iodinated contrast medium administration. It was only in hospitals with a specialised outpatient clinic that all high-risk patients had a consistent follow-up procedure. When we asked about CIN incidence it was remarkable that the large majority of hospitals did not know the exact incidence in their hospital. Because data were obtained from different groups of patients with different risk profiles and were not always reported as percentages, available data could not be considered comparable.

The above-mentioned problems and differences are well known when it comes to the compliance of guidelines in general.[28] This could be related to the laborious process, especially in the follow-up of patients at risk. The inconsistencies and variation might also to some extent be fuelled by the lack of evidence for the prevention measures for intravenous contrast medium administration which concerns the bulk of the examinations with intravascular contrast medium administration.[23,24,28-30] The effectiveness of the proposed CIN prevention strategy has not been proven in a randomised controlled manner, neither has the special measure of prophylactic intravenous hydration before and after intravascular iodinated contrast medium exposure.[23,24,28,29,31] The absence of CIN registration (in most institutes) underscores this. Because the lack of (uniform) registration before and after the implementation of the guidelines, we do not know if the CIN incidence (in the Netherlands) has diminished as result of the implementation.

Our study has some limitations. Our outcomes are based on a questionnaire instead of patient data collected in a prospective manner. To increase the response, we limited the number of questions, which makes the inventory less detailed. However, responders often used the option to include free comments but it is not known whether the points raised would also be applicable to other departments and institutions. We only asked radiologists to fill in the questionnaire, thus we cannot be certain that the information provided can be generalised for other departments in the same institution where intravascular iodinated contrast media are used (e.g. cardiology department). However, most iodinated intravascular contrast administration takes place in the radiology department. Thereby, it is unpractical to have two different CIN prevention programs in place in an institution as the execution of the guideline involves many departments.[30] Furthermore, not all Dutch hospitals participated in this survey, although the response was substantial (74.4%) and therefore the results are most likely a good reflection of all Dutch hospitals. Selection bias with respect to the use of a CIN prevention guideline was minimised by completing the information by phone and internet. This does not have to reflect the compliance or adherence in these hospitals; therefore, for these data a selection bias might be more prominent.

Based on the inventory of the current practice of CIN prevention in the Netherlands, it may be concluded that most Dutch hospitals use a prevention guideline. There was considerable variation in the execution of the guidelines and there were substantial compliance problems. The experienced problems were similar between the two guidelines.

The follow-up procedure in specialised outpatient clinics was more consistent.

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APPENDIX

Questionnaire: Prevention programme/ guideline contrast-induced nephropathy

Q1: Radiologist since .....(e.g. 2001)

Q2: Name of hospital where you are currently working?

.....

Q3: Selection of high-risk patient according to which of the following guidelines?

◯ VMS guideline
◯ CBO guideline
◯ Other.....

Q4: Formula used for determination of renal function (eGFR)?
◯ MDRD-4 point formula (serum creatinine, age, gender, African European)
◯ MDRD-6 point formula (serum creatinine, urea, albumin, age, gender, African American)
◯ Cockroft-Gault (age, weight, serum creatinine)
◯ Other.....

Q5: Same selection procedure for inpatients and outpatients?

◯ Yes
◯ No

Q6: In normal (low-risk) patients and high-risk patients certain general measures need to be taken, e.g. stopping of diuretics, non-steroid inflammatory drugs if possible 24 hours prior to examination. Who is responsible for conducting the general measures?

◯ Nuclear/ Radiology department
◯ Requesting physician
◯ Outpatient clinic (specialised in preventing CIN)
◯ Other.....

Q7: In patients identified as being at risk, specific measures need to be taken, e.g. stopping metformin, intravenous hydration and verification of eGFR. Who is responsible for conducting the specific measures?

◯ Nuclear/ Radiology department
◯ Requesting physician
◯ Outpatient clinic (specialised in preventing CIN)
◯ Other.....

Q8: One of the guidelines aspects is the verification of renal function until normal (pre-contrast administration) values are reached. Is this verified? If so when?

◯ eGFR is not determined
◯ >2 days
◯ Between 2-3 days
◯ Between 3-5 days
◯ Between 5-7 days
◯ Other.....

Q9: Do problems concerning the logistics/ execution of the identification of patients at risk occur?

◯ Yes
◯ No

Q10: Do problems concerning the logistics/ implementation of the general and specific measures occur?

◯ Yes
◯ No

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LETTER TO THE EDITOR

Acute groove pancreatitis due to isoniazid

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Dear Editor,

We present a case of acute groove pancreatitis due to isoniazid (INH) occurring three months after treatment initiation. A 74-year-old female had tolerated three months of INH monotherapy (300 mg daily) for latent tuberculosis infection before presenting with severe epigastric pain and non-bloody, non-bilious emesis. Her other medications included mirtazapine, simvastatin, tramadol, ranitidine, colace, senna, fluticasone, vitamin D, and ferrous gluconate. She denied any history of alcohol, tobacco, or drug use. On examination, she was afebrile with epigastric tenderness and guarding, but no rebound tenderness, jaundice or organomegaly. Laboratory examination revealed elevated lipase (167 IU/l) and leukocytosis (17.7 K/Ul); amylase, urea, electrolytes, liver function, and IgG4 were normal. Abdominal ultrasound was normal with no gallstones, while abdominal CT revealed inflammatory changes consistent with groove pancreatitis, a rare segmental pancreatitis.[1] INH was discontinued immediately, and with bowel rest, intravenous fluids, and analgesics, the patient's symptoms resolved with lipase normalisation by hospital day 3. At 219 days follow-up, she had remained off of INH and symptom-free.

INH-induced acute pancreatitis has been previously reported occurring within five weeks of treatment initiation and with non-specific radiographic findings,[2-13] including by Chow *et al*. in this journal in 2004.[10] Our report is the first case of groove pancreatitis due to INH, and the first occurring after five weeks of treatment initiation. INH was implicated as the cause based on the lack of more common risk factors, such as alcohol use or gallstone disease; simvastatin was deemed unlikely as the cause because our patient had tolerated it well for several years. Although we did not confirm the diagnosis via INH re-challenge, our patient's rapid resolution of symptoms in response to discontinuing INH strongly suggests it as the culprit. The mechanism of INH-induced acute pancreatitis is poorly understood, possibly via toxic or immune-mediated effects.[4,7,11] Groove pancreatitis is characterised by scarring of the head of the pancreas, the duodenum, and the common bile duct,[14] and is thought due to pancreatic outflow obstruction,[1] which may provide further insight into the mechanism of INH-induced pancreatitis.

Medications are a rare cause of pancreatitis that must be considered when more common causes are ruled out. Contrary to previous reports, our case occurred months after treatment initiation. Practitioners should therefore maintain a high degree of clinical suspicion in patients presenting with abdominal pain in the setting of INH therapy even months after starting therapy.

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LETTER TO THE EDITOR

Acute abdomen in the elderly, still a potential pitfall

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Dear Editor,

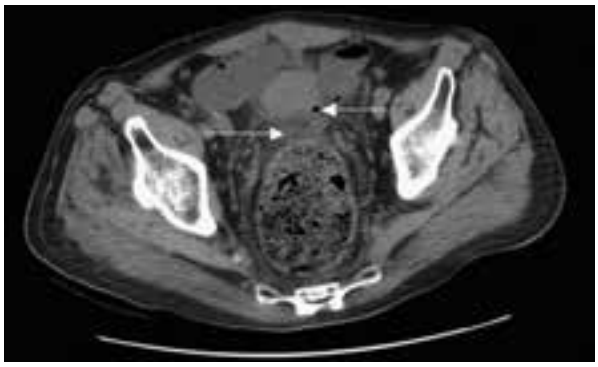
In most developing countries the number of elderly people is rising, which is also reflected by the increased proportion admitted to the emergency department (ED) for abdominal pain.[1] Elderly patients, i.e. those above the age of 65 years, comprise around 12-24% of all ED visits,[2] whilst abdominal pain is the main complaint in 3%-13% of older patients.[2] Approximately one third of these visits lead to a hospital admission,[3] whereas mortality strongly rises in those above the age of 65 years.[1]

An 80-year-old man was referred to the ED with a three-hour history of abdominal pain and nausea. The patient's vital signs were within the normal range. Apart from minimal pressure pain in the umbilical region, physical examination was unremarkable. Two days earlier the urologist had attempted to insert a suprapubic catheter, because of persisting bladder retention, but failed. Laboratory investigations revealed a white blood cell count of 15.9 x 10^9/l (normal range 4.0-10.0), erythrocyte sedimentation rate 98 mm/h (normal range 2-20), C-reactive protein 317 mg/l (normal range <5) and creatinine 148 μmol/l (calculated MDRD: 40 ml/min). Urinalysis showed leukocyturia and haematuria. CT scan of the abdomen showed air and faeces in the peritoneal cavity, indicating a bowel perforation (*figure 1*).

In elderly patients an acute abdomen is often silent and lacks the typical signs on physical examination. Even when reporting abdominal pain, clinical diagnostic accuracy in elderly patients approximates 29% compared with 60% in the young.[1,3]

There are several difficulties complicating the diagnosis of abdominal disorders in the elderly, among which baseline alterations in laboratory parameters and physical findings. Additional barriers are formed by the inability to obtain an accurate history, and pre-existing medical disorders altering clinical manifestation.[3] Moreover, older patients tend to present later in the course of their illness and have more nonspecific symptoms, while alternative diagnoses, e.g. pneumonia or myocardial infection, may present as acute abdominal pain in this population.[4]

Figure 1. *CT scan of the abdomen. The left arrow shows faeces in the peritoneal cavity, the right arrow shows extra-luminal air*



Additionally, several non-medical factors may influence the delay in seeking medical care, including fear of losing independence and lack of transportation.[4]

In conclusion we underscore that acute abdominal pain in the elderly patient is a diagnostic challenge in which a systematic approach is required. We advocate prompt use of additional radiological investigations, especially given the unreliable findings on physical examination in a population where comorbidity often further complicates clinical interpretation.

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