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ISSN: 0300-2977

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# Treatment considerations for primary myelofibrosis

B.J. Biemond

Department of Haematology, Academic Medical Centre, University of Amsterdam, the Netherlands, e-mail: b.j.biemond@amc.uva.nl

Primary myelofibrosis (PMF) belongs to the group of Philadelphia chromosome negative (Ph<sup>-</sup>) chronic myeloproliferative neoplasms caused by a clonal stem cell disorder presenting with myeloproliferation and fibrosis. The clinical presentation is characterised by anaemia, marked hepatosplenomegaly, leuko-erythroblastosis, constitutional symptoms and finally leukaemic transformation. Myelofibrosis may also develop as a secondary complication of two other myeloproliferative neoplasms, essential thrombocytosis (ET) and polycythemia vera (PV).

The median survival of patients with MF is less than five years; this is, however, strongly dependent on risk factors that have been included in several prognostic scoring systems. The International Prognostic Scoring System (IPSS) for PMF uses five risk factors consisting of age (>65 years), anaemia (Hb <10 g/l), leucocyte count (>25 × 10<sup>9</sup>/l), circulating blasts (>1%) and the presence of constitutional symptoms to categorise patients in either a high (≥3 risk factors), intermediate-high (2 risk factors), intermediate-low (1 risk factor) or low (no risk factors) risk group with a median survival of 27, 48, 95, or 135 months, respectively. Besides these risk factors the prognosis of patients with MF is also strongly dependent on the presence of cytogenetic abnormalities, transfusion dependency and the presence of comorbidity. The pathophysiology of MF is not fully elucidated but is characterised by a disturbed bone marrow physiology with expression of multiple growth factors, the release of cytokines, enhanced neoangiogenesis and profound fibrosis in which megakaryocytes, monocytes and clonal stem cells play an important role.<sup>1</sup>

Traditionally, patients with MF are treated with supportive care to reduce anaemia, generally by transfusion and the administration of prednisone, danazol or erythropoietin. Splenectomy, splenic radiation or administration of hydroxyurea has been used to treat the often massive splenomegaly in patients with MF. Although sometimes helpful, these palliative treatments usually only have a limited effect on the clinical presentation with a relatively short duration. The only curative option in patients with

MF is allogeneic stem cell transplantation, which has been demonstrated to result in five-year survival rates of up to 50 to 65% depending on the age, risk factors and comorbidity of the patient. Allogeneic stem cell transplantation is, however, frequently complicated by unacceptable treatment-related mortality (up to 30%) and morbidity in the form of graft versus host disease and graft rejection, which seems to be more frequent in patients with MF than allogeneic stem cell transplantation in other haematological malignancies. Furthermore, the median age patients are diagnosed with MF is 67 years, which limits the possibility to use allogeneic stem cell transplantation as a curative option in a substantial number of the patients.<sup>2</sup>

New therapeutic options represent forms of targeted therapy aiming at the disturbed physiology of the bone marrow in these patients. One of such therapies is the immune-modulating drugs (IMiDs) such as thalidomide and lenalidomide, which act by downregulating the pro-inflammatory response and inhibition of neo-angiogenesis, which have been in particular successful in the treatment of multiple myeloma. In this issue of the Netherlands Journal of Medicine, Holle *et al.* compare the efficacy of both drugs in patients with PMF.<sup>3</sup> The data consist of a retrospective analysis of patients with PMF treated with thalidomide or lenalidomide in two hospitals in the Netherlands. In line with previous observations the authors have shown that treatment with thalidomide induces a clinical response in almost half of the patients but appears to be poorly tolerated due to side effects such as neuropathy and constipation. As discussed by the authors, others found this regime to be more tolerable in combination with prednisone.<sup>4,5</sup> Lenalidomide, which is characterised by a more potent immune-modulating effect than thalidomide and is better tolerated, also proved to produce a clinical response in half of the patients in the study by Holle *et al.* similar to previous observations by others.<sup>6,7</sup> Interestingly, a new IMiD, pomalidomide, either alone or in combination with prednisone, was

recently demonstrated to be effective in reducing anaemia in patients with PMF without serious side effects such as neuropathy or severe myelosuppression.<sup>8</sup>

Besides these immune-modulating drugs other forms of target therapy have been studied in MF. Following the successful introduction of epigenetic therapy in the treatment of myelodysplastic syndrome and acute myeloid leukaemia, hypomethylating agents, such as 5-azacitidine, and histone deacetylase inhibitors, have been demonstrated to be effective in MF and are currently being evaluated in clinical trials.<sup>9,10</sup> Finally, much research is aimed at the JAK2 pathway since half of the patients with MF appear to have the JAK2V617F mutation, which is also found in 50% of the patients with ET and in almost all patients with PV. JAK2 inhibitors are currently being tested in patients with MF and in small studies JAK2 inhibition appeared to reduce splenomegaly and constitutional symptoms but seems to be less effective in reducing anaemia and transfusion dependency.<sup>11</sup>

In conclusion, multiple therapeutic options will soon become available for the treatment of MF patients when allogeneic stem cell transplantation is not feasible. Given the results presented and discussed by Holle *et al.* and the extensive experience with these agents in the treatment of multiple myeloma, treatment with thalidomide or lenalidomide should be considered in patients with PMF or post ET/PV MF who are not considered to be candidates for allogeneic stem cell transplantation or have no donor.

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# Thalidomide and lenalidomide in primary myelofibrosis

N. Holle<sup>1</sup>, T. de Witte<sup>1</sup>, C. Mandigers<sup>2</sup>, N. Schaap<sup>1</sup>, R. Raymakers<sup>3\*</sup>

<sup>1</sup>Department of Hematology, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands, <sup>2</sup>Department of Internal Medicine, CWZ Hospital Nijmegen, Nijmegen, the Netherlands, <sup>3</sup>Department of Hematology, University Medical Center Utrecht, Utrecht, the Netherlands, \*corresponding author: tel.: +31 (0)88-755 65 52, +31 (0)88-755 72 30, fax: +31 (0)88-755 54 89, e-mail: r.raymakers@umcutrecht.nl

## ABSTRACT

Primary myelofibrosis is a clonal haematopoietic stem cell disease, characterised by marrow stromal fibrosis, extramedullary haematopoiesis, splenomegaly, hepatomegaly and progressive cytopenia. Therapeutic options once cytopenia has developed are limited to supportive care, such as erythrocyte transfusions and growth factors. The aetiology has become more clear, especially since JAK-2 mutations were found, resulting in increased production of cytokines. The immune-modulating drug thalidomide and its derivative lenalidomide have shown to be effective in reducing cytopenia, most probably by inhibiting the cytokine responses. In some patients the bone marrow fibrosis disappears. We describe the experience with these drugs in a cohort of 14 patients for thalidomide and seven for lenalidomide (in six patients lenalidomide was given after thalidomide and one patient received lenalidomide upfront). Thalidomide gave clinical improvement in 6/14 patients, but its use was limited mainly due to toxicity, especially the development of neuropathy. The drug could be given for a median period of 15.5 months in responding patients. Lenalidomide was effective in 4/7 of the patients, in some patients with no response on thalidomide. Due to the more favourable toxicity profile, the median duration of therapy was 19 months, with 3/4 patients on therapy longer than 19 months. These data are discussed in view of the clinical studies published. We conclude that lenalidomide is preferred in myelofibrosis, given a higher response rate and more favourable toxicity profile. If no response the addition of prednisone can be considered. In some patients it can normalise haemoglobin and make them transfusion independent.

## KEYWORDS

Myeloproliferative neoplasm, myelofibrosis, thalidomide, lenalidomide

## INTRODUCTION

Primary myelofibrosis (PMF), also known as idiopathic fibrosis or myelofibrosis with myeloid metaplasia, is a clonal haematopoietic stem cell disorder characterised by intense bone marrow stromal reaction including collagen fibrosis, osteosclerosis and neoangiogenesis. Typical clinical features include extramedullary haematopoiesis with marked splenomegaly, hepatomegaly and progressive cytopenias in advanced stages.<sup>1,2</sup>

The median survival is estimated to be five to seven years, but exceeds ten years in younger patients with good prognostic features.<sup>3,4</sup> Older age, anaemia, leukocytosis, constitutional symptoms and peripheral blast are the major risk factors.<sup>1</sup> Current therapy for patients with PMF such as hydroxyurea, interferon or corticosteroids has not shown to improve the overall survival and as such, these drugs serve palliative purposes. The only possibility to cure the disease is a haematopoietic stem cell transplantation, but this is limited to younger patients and even then associated with a relatively high morbidity and mortality.<sup>6</sup>

Apart from primary myelofibrosis, fibrosis may also develop in later stages of other myeloproliferative neoplasms such as polycythemia vera (PV) and essential thrombocytosis (ET). These patients, who initially show high blood counts and need cytoreductive therapy, eventually develop cytopenia, bone marrow fibrosis, splenomegaly and become transfusion dependent, often referred to as 'spent phase'.

The pathogenesis of PMF has not been fully elucidated, but there is increasing evidence that various cytokines such as transforming growth factor (TGF), platelet derived growth factor (PDGF), basic fibroblast growth factor (bFGF) and tumour necrosis factor (TNF) contribute to the changes in the microenvironment of the bone marrow that lead to collagen fibrosis, osteosclerosis and angiogenesis.<sup>1,4,7</sup> The JAK-2 mutation, found in about 50% of the PMF and ET patients and almost all PV patients, results in constitutively activated JAK-2 signalling and enhanced cytokine production. Due to JAK-2 mutations in the pseudokinase domain of the gene, the kinase inhibitory part of the gene is lost, resulting in enhanced signalling and increased production of cytokines. Interestingly, patients diagnosed with the JAK-2 mutation seem to be characterised by a more symptomatic myeloproliferative disorder. However, the prognostic relevance of the JAK-2 mutation has not been elucidated yet.<sup>8</sup>

Thalidomide, showing potent antiangiogenic and cytokine-modulating activity, has proved to be effective in patients with myelofibrosis.<sup>6,9,11-15</sup> The thalidomide analogue, lenalidomide, has also been studied for its effect in myelofibrosis.<sup>7</sup> *In vitro* cell line models show a much more potent activity for lenalidomide than thalidomide.<sup>16</sup> The experience with these drugs for treatment of myelofibrosis in the Netherlands is limited. Nevertheless, we observed clinical effects in some patients. In this report we summarise the experience with thalidomide and the switch of a number of patients to lenalidomide in a university (UMC St Radboud, Nijmegen) and a general hospital (CWZ Nijmegen). Based on the literature and our own data, we give recommendations on the use of thalidomide and lenalidomide in myelofibrosis.

## PATIENTS AND METHODS

We reviewed clinical data from all patients in our database who have been treated with thalidomide and/or lenalidomide for chronic idiopathic myelofibrosis, either primary or secondary after PV or ET. The first patient started treatment in February 2004, the follow-up was until evaluation in February 2009. For thalidomide, patients of the UMC Radboud and the Canisius Wilhelmina Hospital, both located in Nijmegen, were included. The indication for treatment was myelofibrosis and cytopenia, in most cases anaemia. The initial treatment in 14/15 patients was thalidomide; based on toxicity or no response 6/14 switched to lenalidomide, one patient started upfront on lenalidomide. In some patients prednisone was also given next to thalidomide or lenalidomide. Lenalidomide for compassionate use was provided by Celgene, the Netherlands.

We defined a major response as a normalisation of blood counts and for patients who were transfusion dependent to become transfusion independent. If there was an increase in blood counts or a decrease in spleen size but no normalisation we designated the response as limited. In individual patients bone marrow biopsy was done before and after treatment to show changes in marrow fibrosis.

## RESULTS

### Thalidomide treatment

Fourteen patients were treated with thalidomide at a dose of 50 to 100 mg for a median of eight months, varying between one and 37 months (*table 1*). Of these 14 patients

**Table 1.** Patient data before and after treatment with thalidomide

UPN	Sex	Jak2	TD	Dose (mg)	Duration of thalidomide therapy (months)	Before-after				
						Hb (mmol)	WBC (10 <sup>9</sup> /l)	PLT (10 <sup>9</sup> /l)	Spleen (cm)	Response
1	M	Neg	No	50-100	11	5.4-4.9	6.1-2.8	35-92	Stable	Limited
2	F	Neg	TD	100	3	6.7-TD	12.8-2.8	463-373	No change	No
3	M	Pos	No	100	4	7.4-6.7	11.7-12.3	515-815	No change	No
4	M	Nd	No	50	7	7.4-6.2	6.0-3.8	36-63	Stable	No
5	M	Nd	TD	100	20	3.8-9.5	9.2-9.5	42-226	Decreased to normal	Major
6	M	Pos	No	50	15	5.4-7.4	9.6-8.2	62-113	NA	Limited
7	F	Pos	TD	50	3,5	4.4-5.2	19.8-5.8	958-763	NA	No
8	M	Pos	No	50	1	6.2-5.5	19.0-9.2	177 - 299	Decrease	Limited
9	M	Nd	No	50	16	6.4-TD	35.8-3.8	423-184	NA	No
10	M	Pos	No	100-50	37	5.7-6.5	7.0-4.4	86-227	Splenectomy	Limited
11	F	Neg	No	50	4	5.7-4.7	7.5-2.5	60-45	NA	No
12	F	Nd	TD	100	16	5.2-5.8	8.4-6.6	380-570	Stable	Limited
13	M	Nd	TD	50-100	8	4.1-4.0	18.7-6.9	491-563	NA	No
14	M	Nd	TD	100-150	8	5.0-4.8	2.0-1.6	97-89	Increase	No

UPN = unique personal number; Hb = haemoglobin; WBC = white blood count; PLT = platelets; TD = transfusion dependent; NA = not available; NE = not evaluable; Nd = not determined.

the JAK-2 mutation was determined in eight: five were positive and three were negative. The major reason to start treatment was anaemia. In case of anaemia the only treatment option is supportive care, including blood transfusions and erythropoietin. Two patients are still on thalidomide after 10 and 20 months, respectively. In 12 patients the thalidomide treatment was stopped. The reason to stop treatment was due to side effects such as constipation, tiredness and concentration weakness in three patients at 1, 7 and 15 months, neuropathy in four patients after 1, 4, 10 and 37 months and neuropathy as well as therapy resistance in one patient at 20 months. In three patients thalidomide was stopped due to lack of effect after 3, 4 and 4 months, respectively. One patient received an allotransplant after eight months of thalidomide treatment, with no significant improvement at that time. One patient (UPN 7), already in a poor clinical condition at the start of treatment, died after a hip fracture. Five out of the 14 patients who started on thalidomide showed an increase in haemoglobin level of >1 mmol/l. Three of six patients who were dependent on erythrocyte transfusions at the start of thalidomide became transfusion independent, also designated as a major response. The median treatment duration was 15.5 months in responding patients (between 1 and 37 months). One out of 14 patients showed a normalisation of the haemoglobin, increasing from 3.8 mmol/l to normal levels.

In 6/14 patients a thrombocytosis was present at the start of thalidomide, in seven patients a low platelet count. Among the seven patients with a platelet count below  $100 \times 10^9/l$ , five showed an increase, from a median of 42 to a median of 113. The response in spleen size was not evaluated in five patients. Only two out of nine patients showed a decrease in spleen size. The spleen size stabilised in three patients under thalidomide therapy. Three out of the 14 patients showed a response in both haemoglobin and platelets; in one of these the spleen size normalised (one was splenectomised, the third showed a stable spleen size). In two of these three responding patients the improvement in

myelofibrosis was confirmed by a decrease in fibrosis in the marrow biopsy. In one of these three responding patients thalidomide was given in combination with prednisone.

#### Lenalidomide treatment

Seven patients received lenalidomide, 6/7 had been treated before with thalidomide (all except patient 15, table 2). The starting dose was 10 mg in five patients, 15 mg in one patient and 25 mg in another patient. In one patient lenalidomide 10 mg had to be stopped after three weeks due to pancytopenia, in a second patient after three months due to diarrhoea, despite lowering the dose. Patient 8 could not be evaluated, because lenalidomide therapy was only given for six days. Patient 3 stopped after six months of treatment because he underwent a haematopoietic stem cell transplantation (SCT); at the time of SCT the platelets had already normalised and the spleen was reduced in size. A response was also observed in three other patients, all of whom have been on lenalidomide treatment for more than 19 months.

Among the five patients with symptomatic anaemia, three patients responded. A reduction in spleen size occurred in 3/4 patients. One patient had a platelet count below  $100 \times 10^9/l$ , which normalised after lenalidomide treatment. Patient 1 was red blood cell transfusion dependent during lenalidomide therapy, but he became transfusion independent when he received a combination of lenalidomide and prednisone (25 mg). Interestingly, patient 3 did not respond to thalidomide, but he did respond to lenalidomide. In two patients thalidomide neuropathy was the reason to switch to lenalidomide. To summarise, lenalidomide had to be stopped due to side effects in 2/7 patients, was effective in 4/7 patients, in one patient only after the combination with prednisone.

#### DISCUSSION

Our results on thalidomide treatment for myelofibrosis show improvement in haematopoiesis in 6/14 patients, a

Table 2. Patient data before and after treatment with lenalidomide

Patient	Sex	Dose (mg)	Duration of lenalidomide therapy (months)	Before-after				
				Hb (mmol/l)	WBC ( $10^9/l$ )	PLT ( $10^9/l$ )	Spleen (cm)	Response
1	M	10-15	>48	5.4-4.4	5.5-1.4	35-152	Decrease	Major
2	F	10	3	4.6-TD	3.6-1.8	222-455	No change	No
3	M	10	6	7.0-6.6	11.7-5.2	702-299	Decrease	Major
8	M	15	6 days	6.6-7.3	5.5-39.4	57-84	No change	NE
9	M	10	3 weeks	4.4-4.2	3.9-1.4	246-44	NA	NE
10	M	25	>19	7.2-8.0	5.8-4.8	216-194	NA	Major
15	M	10	>19	6.3-8.2	5.6-6.6	430-381	Decreased to normal	Major

Hb = haemoglobin; WBC = white blood count; PLT = platelets; TD = transfusion dependent; NA = not available; NE = not evaluable.

raise in haemoglobin, including transfusion independency, reduction in thrombocytopenia as well as reduction in spleen size and bone marrow fibrosis. The major drawback of thalidomide treatment is the toxicity, constipation, weakness but especially neurotoxicity. Due to this toxicity the mean duration of therapy was only 11 months.

In the literature we found nine studies and one case report evaluating the effect of thalidomide (with or without prednisone) in patients with myelofibrosis (table 3).

In the largest trial, by Marchetti *et al.*,<sup>17</sup> 63 patients were enrolled and patients who received more than one month of treatment were evaluated. In this trial anaemia ameliorated in 11 out of 49 (22%) patients, red blood cell transfusions could be stopped in seven out of 18 (39%) transfusion-dependent patients and nine of those transfusion-dependent patients had a 50% reduction of transfusion requirement. Thrombocytopenia improved in 20 patients (41%) and a 50% reduction in spleen size occurred in 19% of the patients. However, 51% of the patients had discontinued thalidomide after six months of treatment. Significant side effects such as paresthesias, tremors, altered hearing, seizures, depression or extrapyramidal symptoms occurred in 38 patients (60%) and four patients had a toxicity grade 3 or more. The median of maximum tolerated thalidomide dose was 100 mg daily; only eight patients (13%) could tolerate higher doses of thalidomide daily (300 mg). The main reasons for discontinuing treatment were pneumonia, neutropenia, rash, leukaemic transformation, neuropathy and venous thromboembolism.

In the study by Thomas *et al.*,<sup>12</sup> 44 patients were treated with thalidomide with an average tolerated dose of 400 mg for the median duration of three months. Forty-one patients were evaluable, because three patients discontinued treatment within 15 days for a grade 3 rash or for rapidly progressive disease with splenic infarction. Also in this study, a significant number of patients showed

improvement of anaemia (20%), thrombocytopenia (21%) and splenomegaly (31%). After thalidomide treatment, five out of 24 patients became transfusion independent. Dose-related toxicities included fatigue (50%), constipation (48%), rash or pruritis (37%), sedation (35%), peripheral oedema (29%), tremors (23%), peripheral neuropathy (22%), and orthostasis. Similar findings have been described in three other studies with lower numbers of patients,<sup>6,9,18</sup> all evaluating the effect of thalidomide monotherapy in patients with myelofibrosis.

Abgrall *et al.*<sup>13</sup> reported in a prospective placebo-controlled trial that thalidomide (400 mg/day) compared with placebo did not demonstrate significant efficacy and was associated with considerable side effects. After two months, 40% of the thalidomide group (26 patients) had discontinued study participation and after four months 56% had discontinued treatment. In the placebo group (26 patients), these figures were 20 and 32%, respectively. Furthermore, only ten patients in the thalidomide group completed the six months of treatment. In most cases, thalidomide therapy was discontinued prematurely due to intolerance related to the initial high treatment dose.

In summary these studies suggest that thalidomide monotherapy in moderate and high doses (200 to 800 mg) produces a response rate of 20 to 50%. However, treatment is poorly tolerated with high dropout rates. Interestingly, three studies<sup>8,11,15</sup> and one case report<sup>14</sup> showed that the combination of low-dose thalidomide (50 mg) and prednisone was better tolerated and more efficacious than thalidomide alone. In the Mesa trial,<sup>8</sup> 21 patients participated and 20/21 patients completed the first three months of therapy. An improvement of anaemia was observed in 13/21 (62%). Transfusions could be stopped in four out of ten transfusion-dependent patients (40%) and in seven patients the need for transfusions decreased. Thrombocytopenia improved in 6/8 patients and 4/21 patients showed a reduction

**Table 3. Thalidomide studies**

Recent studies	No. of patients	Dose (mg)	Pred.	Responders / evaluable patients				TID / TD after therapy
				Hb	PLT	WBC	Spleen	
Weinkove <i>et al.</i> , <sup>15</sup> 2008	15	50	+	5/7	0	0	4/13	2/7
Berrebi <i>et al.</i> , <sup>14</sup> 2008	1	50	+	1/1	1/1	1/1	1/1	1/1
Abgrall <i>et al.</i> , <sup>13</sup> 2006	52	400	-	NS	NS	NS	S	NR
Thomas <i>et al.</i> , <sup>12</sup> 2006	44	100-800	-	7/35	5/24	NR	9/29	5/24
Benatatos <i>et al.</i> , <sup>11</sup> 2005	5	50	+	5/5	2/2	4/5	1/5	3/3
Marchetti <i>et al.</i> , <sup>17</sup> 2004	63	50-400	-	11/49	20/49	0	20/47	7/18
Mesa <i>et al.</i> , <sup>8</sup> 2003	21	50	+	13/20	6/8	NR	4/20	4/10
Piccaluga <i>et al.</i> , <sup>6</sup> 2002	12	100-600	-	3/4	2/2	2/4	7/11	2/4
Elliott <i>et al.</i> , <sup>18</sup> 2002	15	50-400	-	3/13	12/13	NR	3/12	1/5
Barosi <i>et al.</i> , <sup>9</sup> 2001	21	100-400	-	3/13	2/3	4/5	4/13	1/7

Hb = haemoglobin; WBC = white blood count; PLT = platelets; NR = not reported; NS = not significant with placebo; S = significant with placebo; TID = transfusion independent (Hb); TD = transfusion dependent (Hb); Pred. = prednisone.



of splenomegaly. Especially fatigue, constipation, depression, bradycardia and orthostatic symptoms were significantly reduced by this low-dose thalidomide-prednisone regimen. In agreement with this Mesa trial, the case report<sup>14</sup> and the other two studies<sup>11,15</sup> showed similar responses to the combination therapy. To study the effect of low-dose thalidomide after the discontinuation with prednisone, 12 patients who responded were enrolled in the second phase of the Mesa trial (thalidomide monotherapy 50 mg/day for three months). After discontinuation of prednisone, clinical responses were maintained in eight out of 13 (62%) in terms of anaemia, four out of six (66%) in terms of thrombocytopenia and two out of four (50%) patients in terms of splenomegaly.<sup>8</sup>

We treated seven patients with lenalidomide, six after previous thalidomide treatment. Our results show a response in 4/7 patients, interestingly also in a patient not responding to thalidomide, but more importantly lenalidomide could be continued for much longer. The median time was 19 months instead of 15.5 months for thalidomide. The toxicity in lenalidomide was diarrhoea in one patient and cytopenia, as is known for this drug. Tefferi *et al.*<sup>7</sup> presented two phase II studies (Mayo Clinic Rochester and M.D. Anderson Houston) involving single-agent lenalidomide at a dose of 10 mg for three or four months (table 4). Overall, anaemia ameliorated in 10/68 patients (22%). After the lenalidomide treatment, four patients (12%) became transfusion independent. In eight (17%) of 46 patients, with a baseline haemoglobin level below 100 g/l, the haemoglobin level normalised. Furthermore, thrombocytopenia improved in six patients (50%) and 14 patients (33%) showed a reduction in their spleen size. The most common adverse events in both studies were neutropenia, thrombocytopenia, fatigue and pruritus. Of the eight patients with the major anaemia response, all patients received a maximum of six months therapy. In the Mayo Clinic study, two patients were still in remission two and five months after treatment discontinuation. In the M. D. Anderson clinical trial, three patients have not relapsed after six to 36 weeks. However, the other three patients relapsed off therapy. In our patients we continued lenalidomide as long as a

response was observed. Quintas-Cardama<sup>20</sup> reported 40 patients treated with lenalidomide 10 mg for 21 days in a 28-day cycle and prednisone 30 mg in the first cycle, than 15 mg for two more cycles. They report a 30% response for anaemia and 42% for splenomegaly. These authors also report a reduction in JAK-2 in all eight JAK-2 patients, with 4/8 more than 50% reduction. In this study lenalidomide was continued indefinitely, as in our patients, with a median response duration of 18 months (3.5 to >24 months). The major side effects were haematological, neutropenia (58%), anaemia (42%) and thrombocytopenia (13%), for which reason they advise close monitoring of blood counts. Tefferi *et al.*<sup>21</sup> in a recent trial reported that another immune-modulating drug, pomalidomide, showed increased activity in myelofibrosis compared with prednisone, in a randomised trial. The lowest dose of pomalidomide plus prednisone seemed most effective, showing a 36% response of the anaemia. Haematological toxicity was infrequent; perhaps pomalidomide has a superior toxicity profile compared with lenalidomide but it is not available, even for compassionate use.

In conclusion, thalidomide is effective in about 30% of patients with primary myelofibrosis, especially if given at a low dose of 50 mg and in combination with prednisone. However, the toxicity of the drug limits the duration of therapy and especially the neuropathy will not resolve once established and may disable patients for the rest of their life. Lenalidomide at a dose of 10 mg in combination of prednisone, as used in the trials of Tefferi *et al.* and Quintas-Cardama *et al.* shows similar response rates, but less toxicity, although haematological toxicity may be a concern as reported by Quintas-Cardama *et al.* Based on the literature data and our own limited experience we suggest that in patients with myelofibrosis and cytopenia it is worthwhile to consider treatment with lenalidomide. Other treatment options are limited; perhaps JAK-2 inhibitors may become an alternative in the near future. If lenalidomide treatment is considered it seems rational to start at a low dose (5 to 10 mg), given the cytopenia as side effect, and preferably in combination with prednisone. If a response is seen, prednisone can be stopped in view of the potential side effects of long-term prednisone treatment.

**Table 4. Recent lenalidomide studies**

Recent studies	No. of patients	Dose (mg)	Responders / evaluable patients				TID / TD after therapy
			Hb	PLT	WBC	Spleen	
Tefferi <i>et al.</i> , <sup>7</sup> 2006	68	10	10/46	6/12	NR	14/42	4/34
Quintas-Cardama <i>et al.</i> , <sup>20</sup> 2009	40	10	7/23	0/6	0/2	10/24	NR

Hb = haemoglobin; WBC = white blood count; PLT = platelets; NR = not reported; TID = transfusion independent (Hb); TD = transfusion dependent (Hb).

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# Dilemmas in treatment of women with familial hypercholesterolaemia during pregnancy

D.M. Kusters<sup>1,2\*</sup>, S.J.M. Homsma<sup>1</sup>, B.A. Hutten<sup>3</sup>, M.T.B. Twickler<sup>1</sup>, H.J. Avis<sup>1,2</sup>, J.A. van der Post<sup>4</sup>,  
E.S.G. Stroes<sup>1</sup>

Departments of <sup>1</sup>Vascular Medicine, <sup>2</sup>Paediatrics, <sup>3</sup>Clinical Epidemiology, Biostatistics and Bioinformatics, <sup>4</sup>Obstetrics & Gynaecology, Academic Medical Centre, Amsterdam, the Netherlands, \*corresponding author: tel.: +31 (0)20-566 60 23, fax: +31 (0)20-566 93 43, e-mail: d.m.kusters@amc.uva.nl

## ABSTRACT

Familial hypercholesterolaemia (FH) is a co-dominant monogenic disorder of lipoprotein metabolism, characterised by severely elevated levels of low-density lipoprotein cholesterol (LDL-C) from birth onwards. Treatment of FH patients with cholesterol-lowering medication is mandatory to prevent premature cardiovascular disease (CVD). As a result of a nationwide screening in the Netherlands, a large group of women with FH in the child-bearing age range has been identified. Physicians are faced with a treatment dilemma if these females present either with a wish for pregnancy or an established pregnancy, since all systemically absorbed lipid-lowering medication is contraindicated during pregnancy. Currently, no evidence-based guidelines exist on the optimal clinical approach in these patients. Animal studies have shown conflicting data on potential teratogenicity of statins. In humans, there is no strong adverse safety signal, but prospective studies are lacking. The consequences of maternal hypercholesterolaemia during pregnancy for both mother and child are not well determined, although it has been suggested that it may increase the risk of CVD in the offspring.

This review describes two representative cases from clinical practice, and discusses clinical considerations for treating pregnant FH patients supplemented with what is known from the literature.

## KEYWORDS

Familial hypercholesterolaemia, pregnancy, statins, teratogenicity

## INTRODUCTION

Heterozygous familial hypercholesterolaemia (FH) is a common inherited disorder of lipid metabolism with a prevalence of 1:500 individuals. The underlying molecular defect is a mutation in the low-density lipoprotein (LDL) receptor gene encoding for liver cell-surface receptors, and as a result, LDL cholesterol (LDL-C) uptake by the liver out of the circulation is dramatically reduced.<sup>1,2</sup> Hence, patients with FH are clinically characterised by severely elevated LDL-C levels from birth onwards which strongly predispose to premature atherosclerosis and subsequent cardiovascular disease (CVD). If left untreated, the age- and sex-standardised cardiovascular mortality is even four to five times higher than that in the general population. In general, FH patients are advised to adhere to a healthy lifestyle including strict diet, frequent physical activity and no smoking. Since these lifestyle modifications do not sufficiently reduce LDL-C levels, drug therapy is considered in almost all FH patients. Primarily, aggressive cholesterol lowering is achieved by statin therapy; the effectiveness of these compounds in reducing mortality and morbidity is well established today.

In the Netherlands, the total number of patients with FH is estimated at 40,000 individuals. Even though FH is a common disorder, many patients are not diagnosed or only after a premature cardiac event has already occurred. Therefore, in 1994 a nationwide screening programme started (since 2003 supported by the government) to identify these patients in order to make early cholesterol-lowering treatment possible.<sup>3</sup> At present, 20,000 FH patients have been identified, among whom approximately 2260 women in the age ranging from 20 to 40 years. For this growing number of female FH patients who are usually intensively treated with lipid-lowering medication, a pregnancy wish or an established pregnancy could lead to concerns for both mother and child. Physicians are also faced with a

genuine dilemma, as no evidence-based guidelines are available for the management of these patients. Current practice is to advise to discontinue all systemically absorbed lipid-lowering medications with an option to replace them by bile acid binding resins, in order to avoid potential teratogenic effects in the unborn child. The National Institute for Health and Clinical Excellence (NICE) has published guidelines for the identification and management of individuals with FH including recommendations on treatment in the childbearing age, which are summarised in *table 1*.<sup>4</sup> In short, these recommendations consist of cessation of lipid-lowering drugs and accept markedly increased cholesterol levels that are acknowledged to give rise to more atherogenicity for the mother (and possibly even the newborn). In this contribution, we will present two representative cases with all the clinical considerations.

**Table 1.** Summary of NICE guidance on the management of fertility in women with familial hypercholesterolaemia<sup>4</sup>

<p><b>Period: prior to attempting to conceive</b></p> <ul style="list-style-type: none"> <li>• When lipid-lowering medication is first considered for girls and women of childbearing age, risks to the pregnancy and the foetus while taking lipid-lowering medication should be discussed.</li> <li>• Combined oral contraceptives are not generally contraindicated for women being treated with lipid-lowering therapy.</li> </ul> <p><b>Period: attempting to conceive and during gestation</b></p> <ul style="list-style-type: none"> <li>• There is no contraindication to pregnancy for the majority of women with FH.</li> <li>• Women wishing to become pregnant should be advised to stop use of statins three months prior to attempting to conceive.</li> <li>• Women with FH who are considering pregnancy or who are pregnant should be provided with shared care including expertise in both cardiology and obstetrics.</li> <li>• In the unusual situation where a woman has symptoms of CHD or homozygous FH and is intending to become pregnant, she should discuss her intentions with her cardiologist.</li> <li>• Women with FH who conceive while taking statins or other systemically absorbed lipid-lowering medications should be advised to stop treatment immediately and be referred to an obstetrician for foetal assessment.</li> <li>• It is not useful to regularly measure cholesterol concentrations during pregnancy.</li> </ul> <p><b>Period: lactation</b></p> <ul style="list-style-type: none"> <li>• Women with FH should be encouraged to initiate breastfeeding. Only resins should be considered as lipid-lowering therapy during lactation.</li> </ul>
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FH = familial hypercholesterolaemia; CHD = coronary heart disease.

## CASES

Patient A is a 35-year-old female diagnosed with FH five years previously. She was treated with atorvastatin 40 mg once daily (OD) and a cholesterol absorption inhibitor (10 mg OD). Her lipid profile was within target levels according to clinical guidelines with a LDL-C of 1.9 mmol/l. Because of a pregnancy wish, she discontinued lipid-lowering therapy and started folic acid substitution three months before stopping her oral contraceptives. She conceived three months later and the subsequent gestation period was uncomplicated.

During pregnancy, LDL-C was elevated up to 7.7 mmol/l. After delivery of a healthy son (AD 40 0/7, birth weight 3420 gram), she continued breastfeeding for three months. As a consequence she did not receive lipid-lowering medication for 15 months. IMT measurement was done before and after pregnancy and showed a mean increase of 0.0625 mm. The expected IMT increase for a FH women is approximately 0.01 mm each year, so 0.0125 mm in 15 months.<sup>5</sup>

Patient B is a 36-year-old female. She was diagnosed with FH when she was 18 years old and treated with simvastatin 40 mg OD; since then she had adopted a healthy lifestyle. At the age of 29 years she discontinued statin therapy as she aimed to conceive. During her first pregnancy the LDL-C increased to 9.3 mmol/l. Colestyramine was prescribed; however she did not start with colestyramine in view of the expected side effects. She gave birth to a healthy daughter (AD 39 1/7, birth weight 3540 gram) and she restarted statin therapy three months after delivery. At the age of 32, she was considering pregnancy again. After cessation of statin therapy (before conception) she decided to start with colestyramine therapy, bearing in mind her high cholesterol levels during the first pregnancy. Unfortunately, similarly high lipid levels were measured. She gave birth to a healthy daughter (AD 36 4/7, birth weight 3200 gram). Again, after three months of lactation, she restarted statin therapy. Due to two pregnancies, the patient did not receive adequate lipid-lowering medication for a total of 30 months.

These two cases describe different dilemmas for both these female FH patients who are on statin therapy and have a wish for pregnancy, and for their treating physicians. Next to the direct teratogenic effect of statins, the estimated risk of developing or progression of atherosclerosis with a reduced life expectancy is still not well delineated. This fact poses FH females and their treating physicians with serious conflicts that we will discuss hereafter.

## LIMITED EVIDENCE FOR CLINICALLY RELEVANT SIDE EFFECTS OF LIPID-LOWERING TREATMENT

There are no evidence-based guidelines with respect to the use of lipid-lowering compounds (e.g. statins) during pregnancy; observation and intervention studies are unfortunately lacking. Most information should therefore be considered as experience based instead of evidence based. Animal studies have shown conflicting evidence on potential teratogenicity of statins. Studies in both rat and rabbit models failed to show any teratogenic effect of simvastatin, however skeletal malformations have been noticed for lovastatin, cerivastatin and fluvastatin in the same animal models. Atorvastatin and mevinolin showed developmental toxicity and skeletal defects, respectively, but only at high supra-therapeutic doses that induced maternal toxicity, with a reduced maternal weight and reduced food

consumption. Due to results emerging from these animal studies, all statins are considered as contraindicated during pregnancy and therefore data on therapeutic statin doses during pregnancies in humans are scarce.

A case series of FDA reports in humans that was published in 2004, reported 178 gestational statin exposures from 1987 to 2001. After exclusion of cases involving first-trimester elective or spontaneous abortions, pregnancy loss due to maternal illness, foetal genetic disorders, transient neonatal disorders, or loss to follow-up, 52 cases could be analysed. Reported maximum doses were 40 mg OD for lovastatin, 10 mg OD for atorvastatin, 20 mg OD for simvastatin, and 0.25 mg OD for cerivastatin. Twenty cases reported structural birth defects (such as severe defects of the central nervous system and limb deficiencies), more often present after exposure to lipophilic statins as compared with hydrophilic statins. Lipophilic statins have been shown to enter foetal tissues after passage of placental circulation in animal studies.<sup>6</sup> In a post-marketing study, results of 134 women were analysed who had used lovastatin or simvastatin during pregnancy (of which 89% only during the first trimester). Because the percentage of spontaneous abortions, congenital anomalies, foetal deaths and stillbirths was not higher than the expected incidence of the general population, the authors concluded that there was no evidence for a correlation between exposure to statins during pregnancy and pregnancy outcome.<sup>7</sup> More recent reports lend further support to these conclusions, e.g. the Slone Epidemiology Center Birth Defects Study and the National Birth Defects Prevention Study.<sup>8,9</sup> Limitations of these analyses are incomplete datasets and the relatively rare occurrence of statin use during pregnancy. In addition, the patient group is rather heterogeneous, for example due to the inclusion of patients with diabetes or obesity. In a similar study, three groups of pregnant women with a history of dyslipidaemia treated with a statin, a fibrate and/or nicotinic acid, or no treatment in the first trimester were compared all having a similar prevalence of congenital abnormalities. The question remains, however, whether significant differences in stillbirths exist between the various groups.<sup>10</sup> These retrospective observations imply that negative effects as a result of statin use during the first trimester may be less severe than earlier reports have claimed. A cohort study on teratogenic effects of statin therapy in 64 pregnant women taking statins in the first trimester, and 64 pregnant women without exposure to statins, has shown no differences in prevalence of congenital anomalies, but gestational age at birth (38.4 vs 39.3 weeks) and birth weight (3.14 kg vs 3.45 kg) were significantly lower in the statin group compared with the non-statin group.<sup>11</sup>

Limited data on teratogenicity concerning other lipid-lowering agents is currently available (table 2). Ezetimibe, nicotinic acid and fibrates have all been associated with teratogenic effects in animal studies. Ezetimibe is known to pass from the maternal circulation into breast milk in rats. According to FDA and EMEA advice, it is preferable not to prescribe

**Table 2.** Lipid-lowering drugs around pregnancy

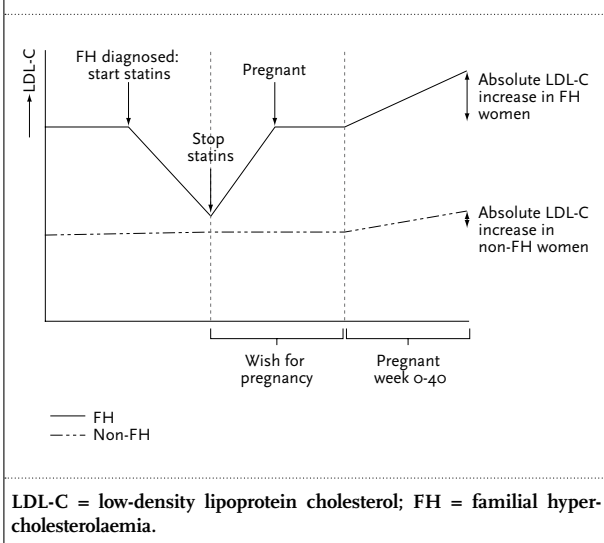
	Approved	Contraindicated
Preconceptional phase (<3 months before stopping contraceptives)	Colestyramine (12-16 g OD, max. 24 g daily) Vitamin supplementation MgO <sub>2</sub> in case of constipation Colesevelam?	Statins Fibrates Ezetimibe Nicotinic acid
During pregnancy	Idem	Idem
Lactation period	Idem	Idem

these agents during pregnancy and lactation.<sup>12</sup> The use of the bile acid binding resin colestyramine during pregnancy in humans has not shown an increased risk for congenital anomalies thus far. Clinical data on the use of the newer bile acid binding resin colesevelam during pregnancy are still lacking, but animal studies showed no adverse effects.

#### LIPID LEVELS DURING PREGNANCY

In the general population, maternal cholesterol levels (and subsequently LDL-C levels) increase by approximately 30 to 50% during pregnancy as a result of enhanced cholesterol synthesis in the liver, probably as a consequence of increased oestrogen levels. The increase in cholesterol levels starts from the first trimester onward and is the most pronounced in the third trimester. HDL-C also rise from the first trimester and will remain augmented during the rest of pregnancy. Triglyceride levels can rise even threefold compared with preconceptional levels.<sup>13,14</sup> The physiological explanation of this gestational hypercholesterolaemia and hypertriglyceridaemia may have a biological role in the need for increased sex steroids synthesis and maintenance of an adequate nutrient supply for both pregnant mother and foetus. Despite the increase in lipid levels, the lipid profile will not be considered as atherogenic in non-FH patients. Currently, only one study on pregnant FH women has been published.<sup>15</sup> This Scandinavian study analysed lipid profiles between week 17 and 36 of gestation in 22 FH patients in comparison to 149 normocholesterolaemic individuals. In both groups, a significant increase in total cholesterol, LDL-C and triglycerides was found. Although the relative increase in lipid levels was equal between the two groups, the absolute increase (LDL-C: 1.9 mmol/l vs 0.8 mmol/l, respectively) was more pronounced in the FH group due to elevated levels at baseline. The average LDL-C increased from 6.7 mmol/l to 8.6 mmol/l between week 17 and 36, as for the normocholesterolaemic females these levels were 3.1 mmol/l and 3.9 mmol/l, respectively. These effects are schematically illustrated in figure 1. HDL-C remained unchanged in both groups. No differences in birth weight, birth length and gestational age at delivery were observed.

**Figure 1. LDL-C levels during pregnancy in women with and without FH**



### CONSEQUENCES FOR THE MOTHER

Currently, it is unknown whether increased cholesterol levels during pregnancy will lead to enhanced atherosclerosis for the FH mother. Given the average birth rate of 1.8 children for a Dutch female and a lactation period of three months, the total 'unprotected' period consists of at least 27 months, plus the time it takes to conceive from the moment of discontinuing contraceptives. Considering the achieved cholesterol levels that exceed approximately threefold the physiological range, this growth of atheroma is not unlikely, even more so if treatment cessation spans a period much longer than the pregnancy itself. Subsequent pregnancies, long-term breastfeeding, or an unfulfilled pregnancy wish can prolong this period substantially. In normocholesterolaemic mothers, it has been shown that elevated lipid levels during pregnancy do not have adverse effects on endothelial function.<sup>16</sup> Results of the Framingham Heart Study (an extensive population cohort study) showed an elevated risk for CVD in (non-FH) women who had more than six pregnancies when compared with nulliparous women (relative risk 1.6; 95% confidence interval: 1.1 to 2.2).<sup>17</sup> However, another population-based study did not show a relationship between reproductive history and intima-media thickness, a surrogate marker for atherosclerotic disease, after correction for age.<sup>18</sup> The case of our first patient suggests that there is a considerable IMT increase during the period around pregnancy when therapy is discontinued, but currently no studies have investigated this hypothesis in FH women.

Finally, women with FH could have an increased risk for hypertensive disease during pregnancy since preeclampsia

is associated with hypercholesterolaemia.<sup>19</sup> The same holds true for recurrent miscarriage. Theoretically and from animal experiments statins might reduce the risk for these pregnancy complications.<sup>20</sup> However, further research is needed to investigate this hypothesis.

### FOETAL DEVELOPMENT

Besides maternal complications, it is also unknown whether high cholesterol levels in utero has a negative impact on foetal development. There are several recent indications that the foetus can acquire maternal cholesterol and use it for its own metabolic needs, such as cellular growth.<sup>12</sup> Currently there are no reports indicating lipotoxicity of high maternal cholesterol levels for the foetus, but it has been suggested that maternal hypercholesterolaemia during pregnancy could induce increased cardiovascular risk for offspring. An autopsy study in spontaneously aborted foetuses showed that offspring from (non-FH) hypercholesterolaemic mothers exhibit significantly more and larger preatherosclerotic aorta lesions than offspring from normocholesterolaemic mothers.<sup>21</sup> Another autopsy study showed that children from hypercholesterolaemic mothers show faster progression of preatherosclerotic lesions, compared with children from normocholesterolaemic mothers.<sup>22</sup> These results have been supported in animal studies.<sup>23-25</sup> Experiments in murine models showed that differences in arterial gene expression between offspring of normo- and hypercholesterolaemic mothers persist long after birth, supporting the assumption that foetal lesion formation is associated with genetic programming, which may in turn affect postnatal atherogenesis.<sup>26,27</sup> Maternal treatment of hypercholesterolaemia during pregnancy may reduce atherosclerosis in offspring.<sup>28</sup> However, this observation has not been repeated in human studies.

### CONCLUSION AND CLINICAL IMPLICATIONS

Thus far, prospective studies on statin use in FH patients around pregnancy are lacking<sup>12</sup> and this fact will raise some concern for FH patients with a pregnancy wish. Results from animal studies indicate that statins are associated with adverse foetal outcomes predominantly at supra-therapeutic levels. From available reports in humans, there is no clearly adverse safety signal, but the 'primum non nocere' principle does argue against the use of statins during pregnancy and lactation presenting absence of 'proven' safety. Women who experience an unplanned pregnancy can be reassured that the chance of an adverse pregnancy outcome is minor. When FH patients are using contraceptives, they should stop taking statins approximately three months

before discontinuation of contraceptives. Concerning lipid-lowering therapy, treatment with bile acid binding resins is the only option. However, since this drug reduces lipid levels by only 15% at the expense of significant side effects, the majority of women will not reach target levels for LDL-C even if they continue to use the drug throughout pregnancy. Using these drugs, supplementation of fat soluble vitamins needs to be considered. However, tolerance to colestyramine is poor, mainly because of constipation. An alternative may be the recently introduced compound colesevelam, a bile acid binding resin with significantly less side effects.<sup>29</sup> Currently, colesevelam is registered for patients with primary hypercholesterolaemia with LDL-C above target levels despite optimal therapy, and for patients who do not tolerate statin therapy.

In conclusion, teratogenicity of statins and other lipid-lowering medications should be further investigated. In addition, large follow-up studies are needed to determine the effect of hypercholesterolaemia during pregnancy on CVD risk for FH women, as well as for their offspring.

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# Novel therapies for hypereosinophilic syndromes

S.A. Antoniu

Division of Pulmonary Disease, University of Medicine and Pharmacy 'Gr.T.Popa', Iasi, Romania,  
corresponding author: tel.: +40 232 239 408, fax: +40 232 270 918,  
e-mail: sabina.antonela.antoniu@pneum.umfiasi.ro

## ABSTRACT

**Background:** Conventional therapies (corticosteroids, cytotoxic agents or interferon- $\alpha$ ) or newer compounds such as imatinib are used specifically in subsets of hypereosinophilic syndromes (HES). However other therapies are still needed in this condition.

**Objective:** To review the novel therapies for HES discussing their advantages and shortcomings.

**Methods and Results:** Preclinical and clinical data on novel tyrosine kinase inhibitors, anti-IL-5 antibodies or anti-CD52 antibodies (alemtuzumab) are analysed. The former might represent appropriate options in case of imatinib resistance; the efficacy of anti-IL-5 monoclonal antibodies therapy is limited by the occurrence of rebound eosinophilia and alemtuzumab might be a promising anti-eosinophil therapy for all HES subsets.

**Conclusion:** Some of the novel therapies might become appropriate therapeutic options for HES.

## KEYWORDS

Hypereosinophilic syndromes, tyrosine kinase inhibitors, anti-IL-5 monoclonal antibodies, alemtuzumab

## INTRODUCTION

Hypereosinophilic syndromes (HES), along with systemic mastocytosis and chronic myelomonocytic leukaemia, belong to the group of atypical chronic myeloproliferative disorders.<sup>1</sup> HES were initially defined with Chusid's criteria represented by blood eosinophilia  $>1500/\text{mm}^3$  persisting for at least six consecutive months, exclusion of causes of secondary eosinophilia (i.e. allergy, parasitic infections,

etc) and presence of end-organ impairment or dysfunction related to hypereosinophilia.<sup>2</sup>

In HES skin, heart, neurological and lung involvement were commonly reported in addition to cardiac manifestations, which have the highest lethal potential.<sup>3</sup> Subsequently, however, based on identification of various genetic abnormalities several subtypes of HES were characterised: myeloproliferative, lymphoproliferative, autoimmune and familial.<sup>4</sup>

However, based on the current World Health Organisation (WHO) classification, three major subsets of previously termed HES are defined: patients with clonal eosinophilia (chronic eosinophilic leukaemia) caused by FIP1L1-PDGFR $\alpha$  fusion gene, patients with clonal IL-5/Th2 lymphocytes mediated hypereosinophilia defined as 'lymphoproliferative' HES and finally patients with no evidence of clonal hypereosinophilia labelled as having 'idiopathic/myeloproliferative' HES.<sup>5</sup>

Such reclassification is important in order to reshape, in a more targeted fashion, the therapeutic approach according to the presence or absence of clonal proliferation and to pathogenic mechanisms of eosinophil clonality: the subset with chronic eosinophilic leukaemia would best benefit from tyrosine kinase inhibitors, lymphoproliferative subsets of anti-IL-5 antibodies and corticosteroids, whereas for the 'idiopathic' subset conventional therapy including corticosteroids, cytotoxic agents or bone marrow transplantation may still be appropriate as first-line therapies.

This paper reviews the existing therapeutic methods for all HES subsets focusing on the novel therapies represented by tyrosine kinases, anti-IL-5 or anti-CD52 antibodies and discusses the rationale for their use based on available preclinical and clinical data.



## CURRENT THERAPIES FOR HES

The existing therapies which can be used in various forms of HES are represented by the conventional therapies and by the tyrosine kinase inhibitor imatinib mesylate (IM). The identification of various HES subsets accordingly led to a revision of the therapeutic recommendations and novel specific therapies such as IM replaced, for example, corticosteroids in HES/CEL subsets.

### Conventional therapies for HES

Conventional therapies include corticosteroids, cytotoxic agents, interferon- $\alpha$  or bone marrow transplantation: some of these options, such as corticosteroids and cytotoxic agents, were previously more widely used in all HES subsets in general but based on current knowledge of underlying pathogenic mechanisms they are not recommended as first-line therapy in all HES subsets.

Corticosteroids were initially the mainstay of HES treatment and are currently recommended as first-line therapy in FIP1L1-PDGFR $\alpha$ -negative HES subsets.<sup>6</sup> However, in such patients disease relapse has been reported when attempting to taper the corticosteroid dose and on the other hand the side effects associated with their long-term use, such as cardiovascular abnormalities, gastrointestinal disorders, myopathy or diabetes, may challenge their current therapeutic position and have prompted evaluation of other potential treatments.

Cytotoxic agents have been used in HES therapy based on extrapolation of their efficacy in other myeloproliferative disorders: hydroxyurea was most commonly reported to be used previously as HES therapy but the latency of its therapeutic effect on eosinophil count reduction and the potential haematological or gastrointestinal adverse events which would aggravate the symptoms of HES, are limiting factors for its wider use.<sup>4</sup> Currently they are indicted in HES subsets with corticosteroid resistance or when steroid tapering is necessary.<sup>6</sup> Other cytotoxic agents for which there are isolated reports of improvement in HES disease outcome are vincristine, cytarabine 2-chlorodeoxyadenosine and etoposide.<sup>7</sup>

Interferon-alpha is an immunomodulator which was found to reduce Th2-mediated IL-5 production, synthesis of GM-CSF and release of eosinophil-specific neurotoxin and eosinophil cationic protein and indirectly inhibited eosinophil differentiation.<sup>8-11</sup>

It is recommended for use in HES patients with organ damage and corticosteroids/cytotoxic treatment failure.<sup>9,12-14</sup> The interferon-alpha/hydroxyurea combination has been shown to increase the clinical efficacy.<sup>15</sup>

Allogeneic bone marrow transplantation has also been reported to be a potentially curative therapy. It is recommended to be used as an ultimate therapeutic measure in case of therapeutic refractoriness or intolerance

to available therapies or in case of multiple severe organ impairment, but is associated with major morbidity and even mortality.<sup>16,17</sup>

### Tyrosine kinase inhibitors: imatinib mesylate

FIP1L1-PDGFR $\alpha$  oncogene is generated by a deletion in chromosome 4q12 and results in the development of fusion protein (FIP1L1-PDGFR $\alpha$ ) with tyrosine kinase activities and was detected in the HES subset currently defined as CEL emerging rapidly as a therapeutic target for imatinib.<sup>18</sup> FIP1L1-PDGFR $\alpha$  oncogene occurs with a variable frequency predominantly in males, the endomyocardial fibrosis and mucosal ulceration were reported to occur more frequently in this subset, tryptase and B12 levels were found to be elevated, IgE levels are normal and thrombocytopenia, anaemia or myelofibrosis are other features of this disease phenotype.<sup>6,7</sup>

Imatinib mesylate (IM) is an aminopyrimidine which was previously found to block the Bcr-Abl tyrosine kinase, in the Philadelphia chromosome of chronic myeloid leukaemia. IM was demonstrated to exert inhibit tyrosine kinases such as platelet-derived growth factor receptor (PDGFR) or KIT, blocking their signalling pathways in Bcr-Abl negative disorders such as HES, systemic mastocytosis or gastrointestinal stromal tumours.<sup>1,19,20</sup>

Initial reports on clinical efficacy of IM came from isolated cases and then from case series: IM therapy was tentatively used for the first time in a HES patient previously treated with high doses of corticosteroids, hydroxyurea and INF- $\alpha$  and with a large eosinophil count in an attempt to reduce the doses of these medications: imatinib 100 mg/day resulted in a complete clearance of peripheral eosinophils after 35 days of therapy.<sup>21</sup> In a patient with multiple organ involvement and corticosteroid and cytotoxic agents refractoriness 14 days of IM therapy resulted in a complete haematological response (0% eosinophils in peripheral blood) and symptoms significant improvement.<sup>22</sup>

Subsequent case series reported complete haematological remissions in HES patients mostly with failure to respond to prior conventional therapies: such patients were reported to receive IM dosages ranging from 100 to 400 mg/day and both complete haematological response and duration of the therapeutic effects were found to vary.<sup>23,24</sup> In a particular study assessing the efficacy of IM in 11 HES patients, the complete remission was achieved within a median period of four weeks in ten out of 11 patients with IM doses within the same range as mentioned before, and the therapeutic effect was reported to persist for at least three months in nine out of 11 patients.<sup>18</sup> The most important findings of this study, however, were the identification of fusion gene FIP1L1-PDGFR $\alpha$  as the therapeutic target of imatinib therapy in five of the nine patients with long-standing

complete haematological response and the detection of a mutation in the fusion gene responsible for development of imatinib resistance.<sup>18</sup>

Overall in FIP1L1-PDGFRα positive patients with HES/CEL IM induced complete haematological remission (normalised white blood cell count and differential mainly eosinophil count), decreased bone marrow clonal cellularity and myelofibrosis and resulted in molecular remission as well.<sup>1,18,25-31</sup>

In FIP1L1/PDGFRα negative patients, however, IM therapy was not reported to have such spectacular effects as in the positive phenotype: in fact the efficacy was variable and both and besides reports of complete responses partial and non-response have been reported.<sup>1,18,28,30</sup>

Poor or absent therapeutic response to IM in FIP1L1/PDGFRα positive HES patients was a surprising although sporadic finding and was identified to be due to 'acquired' imatinib resistance which had also been detected previously in chronic myeloid leukaemia patients. Several mutations in the fusion gene are hypothesised to be responsible for imatinib resistance in HES and FIP1L1-PDGFRα T674I mutant is the only one currently documented clinically and used in preclinical studies to test alternative tyrosine kinase inhibitors such as nilotinib or sorafenib.<sup>7</sup>

## NOVEL THERAPIES IN HES

Several therapies are currently under investigation as potential HES therapies: compounds such as newer tyrosine kinases are evaluated as alternative options in case of imatinib resistance in the HES/CEL subset, anti-IL-5 antibodies are being investigated for lymphoproliferative HES phenotype whereas anti-CD52 antibodies, given their eosinophil inhibitory potential, are being assessed for all HES subsets with therapeutic refractoriness.

### Nilotinib

Nilotinib (AMN107) is another aminopyrimidine derivative sharing with imatinib many structural features as well as the potential of blocking kinases such as PDGFR, KIT or Bcr-Abl (breakpoint cluster region-abelson).<sup>32</sup> Unlike imatinib which blocks PDGFR activity to a greater extent, nilotinib exhibits a more potent inhibition on Bcr-Abl, and is able to act effectively, also on PDGFRα T674I mutated kinase which was identified to be associated with imatinib resistance in HES patients.<sup>18,33</sup> When tested *in vitro* on an EOL-1 cell line exhibiting FIP1L1-PDGFRα fusion kinase, nilotinib was found to exert a similar inhibitory effect to imatinib.<sup>34</sup> Nilotinib was also demonstrated to inhibit FIP1L1-PDGFRα activity in a Ba/F3 cell line and was also found to interfere with

the development of myeloproliferative disorder induced by FIP1L1-PDGFRα in a mice model of bone marrow transplantation.<sup>35</sup> When tested clinically in a phase II study performed in 11 HES patients receiving nilotinib 400 mg twice daily, the complete remission was achieved in only one patient whereas in five others it stabilised the disease and three patients exhibited disease progression.<sup>36</sup>

### Sorafenib

Sorafenib (BAY 43-9006) is a potent inhibitor of various tyrosine kinases such as vascular endothelial growth factor receptor (VEGFR), KIT, or PDGFR which is currently approved for advanced renal carcinoma and under evaluation for other malignancies.<sup>37</sup>

Sorafenib was found to inhibit imatinib-resistant proliferative activities of FIP1L1-PDGFRα T674I mutated tyrosine kinase in Ba/F3 cells and was found to induce apoptosis of the EOL-1 cell line.<sup>38</sup> When given in a patient with chronic eosinophilic leukaemia and FIP1L1-PDGFRα T674I mutation in blast crisis, a clinical response was promptly achieved. Unfortunately, this was short-lived as a sorafenib-resistant FIP1L1-PDGFRα D842V mutant developed rapidly that responded to sorafenib (Nexavar).<sup>39</sup> Another mutation S601P conferring dual imatinib/orafenib resistance was subsequently identified in a FIP1L1-PDGFRα positive HES patient.<sup>40</sup>

### PKC412

PKC412 is a staurosporine derivative currently under evaluation as an antitumour therapy for various malignancies, which was initially demonstrated to inhibit protein kinase C and several tyrosine kinases including PDGFR, FLT3 or VEGF.<sup>41</sup>

When tested in FIP1L1-PDGFRα positive or FIP1L1-PDGFRα T674I Ba/F3 cells lines PKC412 demonstrated its suppressing effects whereas in a murine model of bone marrow transplantation of FIP1L1-PDGFRα T674I induced myeloproliferative disease, it prolonged survival and reduced white cell counts and spleen weight significantly when compared with placebo or imatinib-treated animals.<sup>42,43</sup>

### Dasatinib

Dasatinib (BMS-354825) is another pluripotent kinase inhibitor for Bcr-Abl, KIT, or PDGFRβ which was found to also be active against imatinib-resistant Bcr-Abl isoforms and to inhibit T-cell activation and proliferation.<sup>44,45</sup> Dasatinib was found to be effective in patients with chronic myeloid leukaemia or with Bcr-Abl-positive acute lymphoblastic leukaemia after imatinib therapy failure and the fact that it targets kinases involved in HES pathogenesis might also qualify it to also be assessed in this condition.<sup>46</sup>

## ANTI-INTERLEUKIN-5 THERAPIES: MEPOLIZUMAB, RESLIZUMAB

In the lymphoproliferative HES subset the pathogenic mechanism is represented by a deregulation of T-cell homeostasis, with generation of abnormal Th<sub>2</sub> clones upregulated to produce increased levels of IL-5, IL-4 and other cytokines/chemokines involved in eosinophil proliferation and activation and in B-cell differentiation with increased IgE production.<sup>47</sup> The most frequently described clone was a CD4<sup>+</sup>(Th<sub>2</sub>) lymphocyte population lacking CD3 membrane expression (CD3<sup>-</sup>CD4<sup>+</sup>).<sup>48</sup> The fact that IL-5 is produced by eosinophils might also raise the issue of differentiating HES subsets and the clue for achieving this would be demonstration of a Th<sub>2</sub>-driven 'cytokine profile': IL-5, IL-4, IL-13, IL-3, and GM-CSF in lymphoproliferative HES forms.<sup>6</sup>

Interleukin-5 has also evolved as another therapeutic target in HES. As mentioned above, it is not only produced by Th<sub>2</sub> lymphocytes but also by eosinophils or basophils/mast cells. It upregulates eosinophil counts and activities via an IL-5 receptor, which is a heterodimer with an IL-5 specific binding chain  $\alpha$  (IL-5R $\alpha$ ) expressed only on eosinophils and basophils and a non-ligand chain  $\beta$ , which is common to GM-CSF and IL-3 receptors as well.<sup>49</sup> IL-5R $\alpha$  selectivity for eosinophils explains IL-5 complex involvement in proliferation, differentiation, activation and survival of these cells and qualified it to be tackled with various specific therapies in different allergic diseases including asthma.<sup>50</sup> Hypereosinophilic syndromes were also included in investigational plans for two anti-IL-5 therapies: SCH55700 and mepolizumab.

SCH55700 is a humanised murine monoclonal anti-IL-5 neutralising antibody of the IgG<sub>4</sub>/ $\kappa$  subtype.<sup>51</sup> A single 1 mg/kg dose of SCH55700 was tested in four patients with HES refractory/intolerant to conventional therapies and was found to produce a normalisation of the peripheral eosinophil count and to improve organ symptoms and signs. Therapeutic response was not found to be predicted by serum interleukin-5 (IL-5) levels or by presence of the FIP1L1/PDGFR $\alpha$  mutation. An eosinophil-lowering effect was found to last up to 12 weeks but a rebound of eosinophil count and symptoms were reported when levels of the compound decreased. SCH55700 retreatment given on a monthly basis reduced eosinophilia and symptoms, but the amplitude of therapeutic effect was lower when compared with original treatment.<sup>52</sup>

Rebound eosinophilia, which is reported to occur after SCH55700 treatment, was found to be due to an upregulating factor hypothesised to be IL-5 itself. High levels of IL-5 were found in sera retrieved one month after SCH55700 treatment and *in vitro* SCH55700 post-treatment sera retrieved from patients with HES and eosinophilic gastroenteritis with peripheral eosinophilia were found

to prolong survival of normal eosinophils, unlike pretreatment sera. This effect was reversed by the murine monoclonal anti-IL-5 antibody TRFK5.<sup>53</sup>

A single dose of SCH55700 was also tested clinically in a pilot study in a small sample of asthma subjects with severe persistent asthma despite oral or high doses of inhaled corticosteroids, and was found to reduce, in a dose-dependent manner, blood eosinophilia and to improve lung function, without demonstrating a significant effect on clinical outcome measures of disease activity.<sup>54</sup>

SB-240563 (mepolizumab) is the other humanised mouse monoclonal anti-IL-5 antibody of the IgG<sub>1</sub>/ $\kappa$  subtype which was evaluated for HES, asthma or other eosinophilic disorders: the first report of therapeutic efficacy of mepolizumab in a HES patient showed that three subsequent weekly doses of 750 mg mepolizumab significantly reduced peripheral eosinophilia, IL-5 levels and organ-related symptoms; however rebound eosinophilia was reported to occur rapidly, within days after the last dosage, and was also reported in other subsequent studies performed in other eosinophilic conditions.<sup>55</sup>

In a subsequent case series including three patients with FIP1L1-PDGFR $\alpha$  negative corticosteroid-resistant HES and related severe dermatitis, mepolizumab 750 mg of mepolizumab doses were given at a two weekly interval for variable durations ranging from five to eight months: after the initial two mepolizumab infusions which were associated with significant clinical and haematological improvement, disease rebound was reported in two of the three patients.<sup>56</sup>

In an open-label study mepolizumab was given intravenously every four weeks for 12 weeks in four FIP1L1-PDGFR $\alpha$  negative HES patients, most of them having multiple organ involvement: blood eosinophilia was found to decrease rapidly and significantly in three of these four patients, with the effect lasting at least eight weeks after the last dosage. Health-related quality of life was found to be improved as well as lung function and no rebound eosinophilia was reported.<sup>57</sup>

Mepolizumab therapy given as a 750 mg infusion every four weeks for a 36-week period was subsequently tested in a randomised, double-blind, placebo-controlled trial in 85 FIP1L1-PDGFR $\alpha$  negative HES patients receiving prednisone monotherapy, 20 to 60 mg per day. The primary endpoint of efficacy (reduction of the prednisone dose to  $\leq 10$  mg/day for  $\geq 8$  consecutive weeks) was reached in 84% of the patients treated with monoclonal antibody compared with 43% of patients in the placebo group (HR, 2.90;  $p < 0.001$ ). A peripheral blood eosinophilia count of  $< 600/\mu\text{l}$  for  $\geq 8$  consecutive weeks was achieved in 95% of patients receiving mepolizumab, as compared with 45% of patients receiving placebo (HR 3.53;  $p < 0.001$ ). Serious adverse events occurred in seven patients receiving mepolizumab and in five patients receiving placebo.<sup>58</sup>

When the therapeutic effects of three-monthly infusions of mepolizumab on peripheral blood eosinophil counts were assessed in 25 patients with various eosinophilic diseases; 23 were found to respond to anti-IL-5 therapy with a significant reduction in peripheral eosinophil counts. Haematological responsiveness was not related to the levels of baseline plasma IL-5 or the presence of FIP1L1-PDGFR $\alpha$  fusion gene. The effect persisted three months after final infusion in 76% of subjects. However, mepolizumab therapy was associated with an increase in IL-5 serum levels due to generation of an IL-5-mepolizumab precipitating complex.<sup>59</sup> Mepolizumab was planned to be launched on the market with the commercial name of Bosatria but the application was withdrawn based on inconclusive risk-benefit data.<sup>60</sup>

#### ALEMTUZUMAB: ANTI-CD 52 MONOCLONAL ANTIBODY

CD52 is a surface protein expressed by human eosinophils. An *in vitro* study evaluating expression and functional activity of CD52 on human eosinophils and neutrophils of healthy donors and hypereosinophilia patients showed that only eosinophils expressed this protein. Preincubation of eosinophils with mouse anti-CD52 monoclonal antibody alone, and preincubation of eosinophils with mouse anti-CD52 and cross-linking by goat antimouse antibody resulted in a significant inhibition of reactive oxygen species production after stimulation with C5a. A similar effect was found after incubation with humanised anti-CD52 monoclonal antibody and cross-linked by goat antihuman antibody and was reported to occur on eosinophils retrieved from patients with hypereosinophilia.<sup>61</sup>

Alemtuzumab (CAMPATH) is a humanised monoclonal anti-CD52 which was evaluated initially in three cases with HES refractory to conventional therapy and to imatinib, one of them exhibiting CD3-CD4+ abnormal phenotype: alemtuzumab resulted in sustained haematological and clinical responses.<sup>62,63</sup> More recently, alemtuzumab was given in weekly cycles at a dose of 30 mg or 10 mg three times per week either intravenously or subcutaneously in a pilot study involving nine patients with FIP1L1-PDGFR $\alpha$ -negative HES who had previously received conventional therapies and various tyrosine kinase inhibitors. Complete haematological remission was detected in eight patients within the first four weeks of therapy; alemtuzumab withdrawal in five patients resulted in a disease flare after a median period of 3.5 weeks.<sup>64</sup>

In another study 11 patients with advanced HES/CEL received alemtuzumab therapy, first in escalating doses (5, 10, 30 mg intravenously on days 1-3) followed by the titrated tolerated dose three times a week for a total of 12 doses.

In patients with a complete haematological response (i.e. normalisation of the peripheral blood eosinophil count) once-weekly maintenance therapy was given. Complete haematological response was achieved in ten patients (91%) after a median of two weeks therapy and lasted three months (median duration). Bone marrow abnormal eosinophilia disappeared in four of seven evaluable patients. Subsequently seven of the ten patients relapsed, five after therapy cessation. Alemtuzumab retreatment resulted in a second complete haematological remission in two patients. Cytomegalovirus reactivation was reported in two patients and orbital lymphoma successfully treated in one patient.<sup>65</sup>

#### CONCLUSIONS

Hypereosinophilic syndromes include various phenotypes defined based on the immunogenetic abnormality, which results in abnormal bone marrow and organ eosinophil proliferation. Currently three main disease subsets are considered: HES/CEL or FIP1L1-PDGFR $\alpha$  positive subset, lymphoproliferative HES and 'idiopathic' HES.

In the HES/CEL subset, IM is currently recommended to be the first-line therapy given its demonstrated inhibitory activities on FIP1L1-PDGFR $\alpha$  fusion protein. Fortunately in HES, reports of IM resistance are not common and this still preserves its therapeutic effectiveness. However, when this occurs, this may pose a significant therapeutic challenge. There might be several mutations rendering the abnormal tyrosine kinase more abnormal, this time from a therapeutic point of view but T674I is currently reported to occur most frequently in HES.

Therefore newer tyrosine kinase inhibitors have to face this 'genetic provocation' and to demonstrate a better efficacy and safety as compared with IM.

The long-term efficacy of the anti-IL-5 monoclonal antibodies in the lymphoproliferative form of HES is unclear and questionable due for example in the case of mepolizumab to the rapid development of rebound eosinophilia. Studies discussed in this review focused on this aspect too, but further data are needed in order to gain a better picture on this phenomenon. One (with luck the only) major reason for such behaviour might be represented by the immunogenicity of such compounds: the existing anti-IL-5 monoclonal antibodies are *humanised* rat-derived compounds and this structural particular might at least in theory increase the risk of immunogenicity. The fact that rebound eosinophilia was reported to occur quite rapidly after initial anti-IL-5 antibodies and if immunogenicity only is to be considered, this might reflect a certain 'immune aggressivity' of such compounds in the specific HES setting which indeed undermines their efficacy. Given these facts,

feasible options would be to test existing investigational anti-IL-5 therapies combined to corticosteroids or other immunosuppressive therapies, to discover newer fully human monoclonal antibodies or other therapies targeting Th2-related IL-5 pathway.

Alemtuzumab is also a promising anti-eosinophil therapy. It has the disadvantage of treating the end-effect which, however, can be inversely seen as a major advantage as the end pathogenic effect in HES is the same irrespective of the genetic and/or immune pathogenic defects. However, more clinical data on this compound are needed including findings on long-term safety.

Overall many of the novel therapies for HES discussed above seem to be promising if further evaluated and appropriately tested and, given that newer therapeutic approaches are desirable especially in subsets such as idiopathic HES, supportive data are essential.

Recent reclassification of HES subsets led to a certain 'individualisation' of its therapies according to disease subset: the major benefit is represented by a more appropriate therapeutic targeting which consequently results in a better disease outcome. However, this therapeutic targeting seems to be unevenly distributed among the subsets and this yields an inadequate therapeutic coverage of some disease subsets. In this respect there is a need to discover other therapies for HES subsets with unmet therapeutic.

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# Self-monitoring of blood glucose in tablet-treated type 2 diabetic patients (ZODIAC)

N. Kleefstra<sup>1,2\*</sup>, J. Hortensius<sup>1</sup>, S.J.J. Logtenberg<sup>1</sup>, R.J. Slingerland<sup>3</sup>, K.H. Groenier<sup>4</sup>, S.T. Houweling<sup>2,5</sup>, R.O.B. Gans<sup>6</sup>, E. van Ballegoie<sup>2†</sup>, H.J.G. Bilo<sup>1,6</sup>

<sup>1</sup>Diabetes Centre, Isala Clinics, Zwolle, the Netherlands, <sup>2</sup>Medical Research Group Langerhans, <sup>3</sup>Department of Clinical Chemistry, Isala Clinics, Zwolle, the Netherlands, <sup>4</sup>Department of General Practice, University of Groningen, Groningen, the Netherlands, <sup>5</sup>General Practice Sleeuwijk, Sleeuwijk, the Netherlands, <sup>6</sup>Department of Internal Medicine, University Medical Center Groningen, Groningen, the Netherlands, \*corresponding author: tel.: +31 (0)38-424 77 63, fax: +31 (0)38-424 33 67, e-mail: kleefstra@langerhans.com

## ABSTRACT

**Background:** Whether self-monitoring of blood glucose (SMBG) improves glycaemic control in patients with type 2 diabetes mellitus (T2DM) not using insulin is questionable. Our aim was to investigate the effects of SMBG in patients with T2DM who were in persistent moderate glycaemic control whilst not using insulin.

**Methods:** Patients were eligible when between 18 and 70 years of age, with an HbA<sub>1c</sub> between 7 and 8.5%, using one or two oral blood glucose lowering agents.

Forty-one of the anticipated 52 patients were randomly assigned to receive either SMBG added to usual care, or to continue with usual care for one year. A fasting glucose value and three postprandial glucose values were measured twice weekly (including a Saturday or a Sunday). The primary efficacy parameter was HbA<sub>1c</sub>. Furthermore, health-related quality of life and treatment satisfaction were assessed using the Short-form 36 Health Survey Questionnaire (SF-36), the Type 2 Diabetes Symptom Checklist (DSC-r), the Diabetes Treatment Satisfaction Questionnaire (DTSQ) and the WHO-Wellbeing Index (WHO-5).

**Results:** Change in HbA<sub>1c</sub> between groups was -0.05% (95% CI: -0.51, 0.41; p=0.507). Also, there were no significant changes between groups on the DTSQ, DSC type 2, WHO-5 or SF-36, except for the SF-36 dimension 'health change' which was lower in the SBMG group (mean difference: -12 (95% CI: -20.9, -3.1).

**Conclusion:** On top of the absence of a clinical benefit, tablet-treated T2DM patients experienced some worsening of their health perception. We therefore argue that the

use of SMBG in this patient group is questionable, and its unlimited use and promotion should be reconsidered.

## KEYWORDS

Blood glucose self-monitoring, diabetes mellitus type 2, haemoglobin A<sub>1c</sub>, glycosylated, quality of life

## INTRODUCTION

Self-monitoring of blood glucose (SMBG) is an important tool in the management of diabetes mellitus in patients using insulin. For patients with type 1 diabetes mellitus, it is almost impossible to achieve good glycaemic control without SMBG.<sup>1</sup> In patients with type 2 diabetes mellitus (T2DM) using insulin SMBG can also help to improve glycaemic control.<sup>2,3</sup>

However, there still is much debate about the use and effectiveness of SMBG in non-insulin-treated T2DM.<sup>4</sup> A Cochrane review published in 2005 concluded that SMBG might be effective in improving glycaemic control in patients with T2DM who are not using insulin, translating into a possible benefit in haemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) of approximately 0.39%.<sup>4,5</sup> However, only two of the six studies included in this systematic review were rated as being of good methodological quality.<sup>6,7</sup> These two studies did not show a beneficial effect of SMBG on glycaemic control.

Our aim was to investigate the effects of SMBG on glycaemic control, quality of life and treatment satisfaction in patients with T2DM not using insulin, who are in persistent moderate glycaemic control. To answer our research question we designed a randomised controlled trial to compare SMBG use with usual care.

## MATERIALS AND METHODS

### Participants

In 1998, the Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC) Study was initiated in the Zwolle region (the Netherlands), as part of a shared care diabetes project.<sup>8</sup> Patients included in this shared care project were eligible for the present study if they met the following criteria: T2DM, 18 to 70 years of age, HbA1c 7 to 8.5% at previous annual check-up, use of one or two different oral blood glucose-lowering agents (moreover, when two oral blood glucose-lowering drugs were taken, they should not both be used at maximum dosage), oral blood glucose-lowering agents had not been changed during the past three months, no use of insulin, no use of devices for SMBG at the start of the study or in the preceding six months, and sufficient knowledge of the Dutch language to understand the requirements for the study. Patients meeting the eligibility criteria were asked to participate and were included in the study after written informed consent, whenever the HbA1c value during the current annual check-up was between 7 to 8.5% as well.

### Intervention

Patients in the intervention group (SMBG group) were instructed to measure their blood glucose values four times a day (one fasting glucose concentration and three post-meal glucose concentrations (1.5 hours after the meal), twice weekly, on one weekday and one day in the weekend for a period of one year. Patients were requested to record these glucose values in a study diary. Patients in the SMBG group were all provided with a single glucose monitor (Accu-check Aviva, Roche Diagnostics Corp., Indianapolis, IN). No further education except how to handle the device was given, in order to ensure that besides the intervention, there were no education differences with the control group. Patients were taught, and could also see in their diary, which glucose values were considered normal or acceptable (fasting 4 to 8 mmol/l and postprandial 4 to 10 mmol/l), and which were abnormal. In case of blood glucose values below 3.5 mmol/l or above 20 mmol/l, patients were instructed to evaluate their self-monitoring and to perform an extra measurement. If this subsequent value was again above 20 mmol/l, the patient was requested to contact the study nurse (during office hours) or the general practitioner (outside office hours). When the value was again below 3.5 mmol/l, the patient would follow the instructions in case of hypoglycaemia.

Patients in the control group continued with usual care from their own healthcare provider. No other instructions were given, except for the explicit request not to use any form of SMBG during the study.

All patients continued to receive care from their own healthcare provider every three months during the study. Healthcare providers were asked not to make changes in glucose-lowering agents during the study period. Every three months the HbA1c was measured. If it exceeded 8.5%, glucose-lowering therapy was intensified, according to the Dutch guidelines at the time of the study. First, when possible, metformin was started or increased to the maximum (tolerated) dose. Second, when possible, a sulphonylurea derivate was started or increased to the maximum (tolerated) dose. When a patient was already being treated with a thiazolidinedione, the dose was increased to the maximum (tolerated) dose. If two maximally dosed oral blood glucose-lowering agents were not sufficient to lower HbA1c below 8.5%, insulin therapy was initiated.

### Measurements

HbA1c levels were measured every three months. Furthermore, data collected at baseline and after 12 months included: diabetes duration, smoking with number of cigarettes, alcohol with number of units of alcohol, macrovascular complications (yes or no and date), medication, length (no shoes), weight (no shoes or coat), blood pressure, serum creatinine, lipid profile (non-fasting) with total cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LDL), triglycerides, total cholesterol/HDL and urinary albumin/creatinine ratio. All laboratory tests were performed in local hospital laboratories, where staff was unaware of treatment allocation.

In addition, at baseline, and after six and 12 months, patients were asked to fill in a questionnaire containing the Dutch versions of the Short-Form 36 Health Survey Questionnaire (SF-36),<sup>9-11</sup> the WHO five-item Wellbeing Index (WHO-5),<sup>12,13</sup> the Diabetes Treatment Satisfaction Questionnaire (DTSQ) and the Diabetes Symptoms Checklist.<sup>14</sup> The SF-36 and WHO test scores range from 0 to 100, with 100 representing the best possible well-being.<sup>14</sup> The DTSQ score can range from 0 (very dissatisfied) to 36 (very satisfied). The two additional items measuring perceived frequency of hypoglycaemia and hyperglycaemia are scored from 0 (none of the time) to 6 (most of the time). To measure the presence and the perceived burden of diabetes-related symptoms, the revised version of the type 2 Diabetes Symptom Checklist (DSC-r) was used.<sup>15</sup> Scores on the eight scales can range from 0 to 5, with higher scores indicating more troublesome symptoms.

### Outcome

Our pre-specified primary endpoint was HbA1c difference between groups. Our secondary endpoints were differences



between groups in HRQoL measures, diabetes-related complaints, treatment satisfaction, cumulative incidence of (necessity to start) insulin therapy, bodyweight and body mass index (BMI). For the primary endpoint, separate analyses were performed for patients who were compliant to the intervention (at least 80% of requested glucose measurements).

### Randomisation

Randomisation was done using an independent third party. After inclusion and informed consent at the first visit, the study nurse or the investigator made a telephone call to a third party, who had numbers ranging from 1 to 60 in non-transparent envelopes, and was asked to draw an envelope. When an uneven number was drawn, the patient was allocated to the intervention group who had to perform SMBG (SMBG group). With an even number, the patient was allocated to continued usual care (no monitoring; control group).

### Statistical analysis

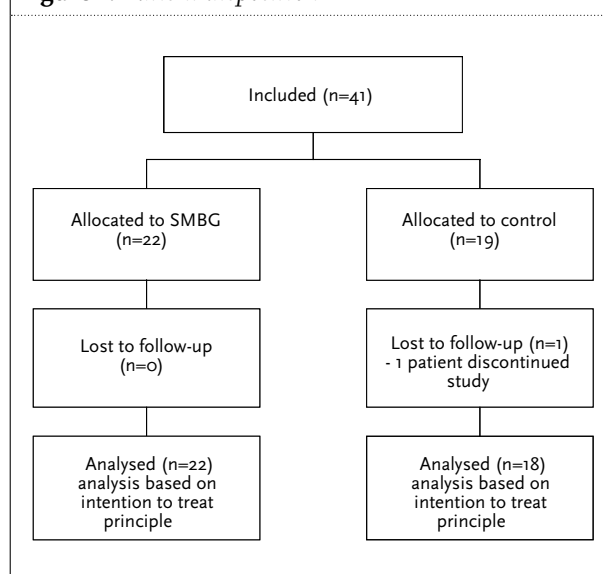
Mean HbA<sub>1c</sub> of patients with HbA<sub>1c</sub> 7 to 8.5% in our shared care diabetes project not using SMBG was 7.45 (standard deviation (SD) 0.38). Powered to detect a 0.39% absolute reduction in HbA<sub>1c</sub> in a one-year follow-up of patients performing SMBG as compared with control patients, with a power 95%, alpha 0.05 two-tailed, the total sample size of the study should be 52. To take dropout into account, the aim was to include 60 patients.

To evaluate differences in target variables over time and between the groups, we used the repeated measures of the general linear model (GLM) with the Greenhouse-Geiser test to compensate for lack of sphericity. Concerning HbA<sub>1c</sub>, in case of missing values, these values were imputed by the Expectation Maximisation (EM) algorithm using the available HbA<sub>1c</sub> values. The baseline value was set as covariate. SPSS software, version 14.0, was used for all the analyses.

## RESULTS

Patients were recruited from March 2006 until October 2007. A total of 41 patients were included in the study and randomised (figure 1) of which one patient in the control group refused to continue the study and withdrew consent. Of the 22 patients in the SMBG group, 17 (77%) performed at least 80% of the requested glucose registrations. Two patients performed half of the expected registrations, and from three patients no SMBG results at all were available; one of these patients did not perform SMBG at all, and gave as a reason that he could not find the time to do it, the second patient did not perform SMBG because he judged SMBG too difficult to perform. The third patient

Figure 1. Patient disposition



did not return his diary during his last visit and despite phone calls, letters and house visits, no contact could be established afterwards.

Patient baseline characteristics are presented in table 1. Median HbA<sub>1c</sub> levels were 7.5 and 7.6% in the SMBG and control group, respectively. BMI and diabetes duration were different between groups. HbA<sub>1c</sub> levels at different time points in the study in both groups are presented in table 2. After 12 months, HbA<sub>1c</sub> dropped 0.1% in both groups with no significant difference between the SMBG and control group (-0.05% (95% CI: -0.51, 0.41; p=0.51)). When performing this analysis in the subgroup of compliant

Table 1. Baseline characteristics

	SMBG (n=22)	Control (n=18)
Gender (male)	12 (55)	13 (72)
Age (years)	59.5±8.0	58.7±7.8
Diabetes duration (year) *	5.0 (4.0, 7.0)	8.0 (3.8, 11.3)
Body mass index (kg/m <sup>2</sup> )	32.7±5.8	29.0±4.6
Systolic blood pressure (mm Hg)	151±21	147±18
Serum creatinine (µmol/l)	93±20	94±22
Cockcroft (ml/min) * †	91 (78, 121)	96 (72, 110)
Albumin creatinine ratio *	1.50 (0.58, 3.75)	1.0 (0.63, 3.20)
HbA <sub>1c</sub> (%)*	7.5 (7.1, 7.9)	7.6 (7.3, 8.1)
HDL (mmol/l)	1.32±0.34	1.17±0.27
LDL (mmol/l)	2.35±0.71	2.48±1.05
Use of 2 oral blood glucose-lowering agents	12 (55)	12 (67)
Macrovascular complication	6 (27)	2 (11)

Data are mean ± SD or n (% of known data) unless otherwise indicated. \* Data are median (P<sub>25</sub>, P<sub>75</sub>); † estimated creatinine clearance; SMBG = self-monitoring of blood glucose; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

**Table 2.** HbA1c per treatment group

	Baseline	3 months	6 months	9 months	12 months	Change within groups *	Change between groups (95%CI)†
SMBG	7.6±0.5	7.5±0.6	7.4±0.7	7.5±0.8	7.5±0.8	-0.1±0.9	-0.05 (-0.51, 0.41)
Control	7.7±0.4	7.6±0.6	7.7±0.6	7.6±0.6	7.5±0.5	-0.1±0.8	‡

Data are mean ± SD within groups unless otherwise indicated. \*from baseline to 12 months; † mean difference between groups with 95% CI; ‡ p=0.51; SMBG = self-monitoring of blood glucose.

patients, the between-group difference was -0.04% (95% CI: -0.52, 0.45; p=0.70). In a post-hoc analysis, adding BMI and diabetes duration as covariates (intention-to-treat analysis) did not change the results (-0.07% (95%CI: -0.56, 0.43; p=0.67)). Three patients in the intervention group progressed to insulin therapy vs none in the control group (p=0.10). No effects on BMI and weight were seen (data not shown).

Data concerning HRQoL outcome are presented in table 3. Scores on the subscales of the SF-36 mostly show a small and non-significant worsening in the SMBG group compared with the control group, except for the dimension 'health change'. After 12 months the score on this subscale was 12.0 (95% CI: -20.9, -3.1) points lower in the SMBG group compared with control (p<0.01). The dimension 'health change' consists of one item (with five possible answers) in the questionnaire: 'Compared with one year ago, how would you rate your health in general now?'. Concerning the WHO-5 questionnaire, the DTSQ and the DSC-r, no significant differences were found. Also, no significant differences were found for the separate eight scales of the DSC-r (data not shown).

## DISCUSSION

SMBG did not improve glycaemic control in patients with moderately controlled type 2 diabetes treated with oral

glucose-lowering agents in this study. Furthermore, SMBG did not have any positive effect on HRQoL, well-being, treatment satisfaction or diabetic symptoms. On the contrary, patients performing SMBG reported a decline in their health in general during the one-year study, compared with the control group.

After the two studies of high methodological quality, which were included in the Cochrane review from 2005 and did not find an effect of SMBG on glycaemic control, three other large randomised controlled trials of high methodological quality have been published (table 4).<sup>6,7,16-19</sup> In general, the results of our study are in line with these trials. One publication reported a positive effect of SMBG on HbA1c of 0.24% (95% CI: 0.03, 0.45).<sup>18</sup> This concerned a 27-week study in 610 patients, in which patients in the SMBG group were requested to perform SMBG five times a day (before each meal, two hours after the main meal and before bedtime), two days a week (one working and one non-working day); on top of that once a month postprandial measurements were taken after each meal. Unfortunately, this study did not measure HRQoL or treatment satisfaction. The two other studies did not find an effect of SMBG on HbA1c.<sup>16,17</sup> Farmer *et al.* compared a control group with less intensive and more intensive SMBG.<sup>16</sup> Differences in HbA1c compared with the control group were -0.14% (95% CI: -0.35, 0.07) and -0.17% (95% CI: -0.37, 0.03), for the less intensive and more intensive

**Table 3.** Health-related quality of life scores (SF-36), wellbeing (WHO-5), diabetes treatment satisfaction (DTSQ) and diabetes symptoms (DSC-r) per treatment group

Questionnaire	SMBG		Control		Change between groups (95%CI)
	Baseline mean ± SD	1 year mean ±SD	Baseline mean ±SD	1 year mean ±SD	
SF-36 physical component score	42.2±10.4	44.3±9.8	48.5±10.6	47.9±7.9	-0.0 (-5.2, 5.1)
SF-36 mental component score	55.5±7.4	53.1±9.5	50.6±10.6	51.6±7.7	-1.4 (-6.6, 3.7)
WHO-5 total score	68.0±20.7	74.4±14.5	71.0±17.9	76.3±11.4	-0.6 (-8.2, 7.0)
DTSQ total score	29.3±4.8	32.1±3.8	30.7±4.2	30.7±4.0	1.2 (-1.6, 4.1)
DTSQ hypo*	1.0 (0.0, 2.5)	1.0 (0.0, 2.0)	0.0 (0.0, 1.0)	0.0 (0.0, 2.0)	0.3 (-0.5, 1.1)
DTSQ hyper	2.2±1.6	2.3±1.9	2.6±1.7	1.9±1.9	0.5 (-0.8, 1.8)
DSC-r total score*	0.5 (0.2, 1.0)	0.4 (0.3, 1.1)	0.7 (0.4, 1.0)	0.9 (0.3, 1.4)	-0.1 (-0.5, 0.3)

Data are mean ± SD or mean change (95% CI) unless otherwise indicated. Data are median (P<sub>25</sub>, P<sub>75</sub>); SMBG = self-monitoring of blood glucose.

**Table 4.** Randomised controlled trials of SMBG of high methodological quality in patients with type 2 diabetes not using insulin: effects on HbA1c

Study	Treatment arm		Intervention vs. control
	Intervention	Control	
Allen	12.4→10.4	11.7→9.7	~0.0 (p>0.95)
Davidson	8.5→7.7	8.4→7.8	-0.2 (95% CI: -1.1, 0.6)
Farmer*	1) 7.41→7.28 2) 7.53→7.36	7.49→7.49	1) -0.14 (95% CI: -0.35, 0.07) 2) -0.17 (95% CI: -0.37, 0.03)
O'Kane	8.8→6.9	8.6→6.9	-0.07 (95% CI: -0.38, 0.25)
Barnett	8.12→6.95	8.12→7.20	-0.24 (95% CI: -0.45, -0.03)

\*Group 1 received less intensive SMBG and group 2 more intensive self-monitoring of blood glucose (SMBG).

SMBG groups, respectively.<sup>16</sup> Furthermore, the health utility score as measured with the EuroQoL (EQ-5D) questionnaire was lower in the more intensive intervention group compared with the control group.<sup>16</sup> In the study by O'Kane *et al.* the effect on HbA1c of SMBG compared with control was -0.07% (95% CI: -0.38, 0.25), and they reported a significantly worse outcome on the depression scale of the well-being questionnaire in the SMBG group compared to the control group.<sup>17</sup>

The one-year follow-up study of Farmer *et al.* had two different intervention groups (n=453).<sup>16</sup> Patients in the less intensive intervention group (performing SMBG three times a day (one fasting and two pre- or postprandial values), two days a week) were instructed to strive for preprandial glucose concentrations of 4 to 6 mmol/l and postprandial concentrations of 6 to 8 mmol/l. No further information about how to interpret glucose values was given to subjects. In addition to the care as given in the 'less intensive group', the more intensive group received training and support in timing, interpretation and using results, also to enhance motivation and maintain adherence to diet, physical activity and drug regimens. The more intensive group was also encouraged to experiment with SMBG to explore the effects of specific activities. The study by O'Kane *et al.* also had a one-year duration, and included 184 patients with new onset diabetes.<sup>17</sup> Patients in the SMBG group were requested to measure four fasting and four postprandial values per week and received advice on interpretation and appropriate (lifestyle) responses to high and low readings.

An important limitation of our study is the sample size. We needed 52 and aimed at 60 patients, but were only able to include 41 patients due to a variety of reasons. In 2007, out of the 10,403 patients between 18 and 70 years of age in the ZODIAC project, 74% had an HbA1c below

7% during their annual check-up and were therefore not eligible for inclusion.<sup>8</sup> Furthermore, many of the patients with higher HbA1c levels were not persistently in the HbA1c range of 7 to 8.5%, or were on a maximum dosage of oral blood glucose-lowering agents, or already performed SMBG. Regarding our results, the 95% confidence interval is wider than the relevant difference of 0.39% our study was powered on, i.e. -0.51 to 0.41, which means that this magnitude of benefit cannot be excluded in the patients performing SMBG, but also not in the control group. SMBG can be performed in different frequencies and at many different moments during the day. SMBG can be performed with or without exact knowledge about the interpretation and use of glucose values. We instructed patients to perform one fasting glucose measurement and three glucose measurements 90 minutes post-meal twice a week. We gave information about which values were acceptable or unacceptable, but not about how to reach good control. Patients were not assisted more often by a healthcare provider with knowledge and advice about how to achieve glucose values in the target range. So, the SMBG performed in our study is more a structured form of self-measurement than self-regulation, which is more often done and easier to do in cooperation with patients on insulin.

What can be regarded as a strong point of our study is that we used a form of SMBG which, in our opinion, reflects what happens in daily practice. Furthermore, by using this study design, we were able to rule out the effects of education on HbA1c. The difference in intervention between the groups in our study is the performance of SMBG itself and not some other form of education, which in itself is reported to improve HbA1c by 0.32%.<sup>21</sup>

In conclusion, tablet-treated T2DM patients, rating their health over a one-year period, experienced a worsening on the dimension 'health change' of the SF-36 when performing SMBG. Failing to find a clinical benefit, we conclude that there appears to be no evidence for a positive impact of SMBG on HRQoL or treatment satisfaction in T2DM patients treated with oral glucose-lowering agents, although we cannot completely rule this out based on this study. We therefore argue that the use of SMBG in this patient group is questionable, and its use should be reconsidered.

#### ACKNOWLEDGEMENTS

The authors would like to especially thank Reinou Friso for all the help with the study. Furthermore, we would like to thank Ina Brink, Anja Blok, Gerda de Vries, Helen van der Zee, Els Zoon, Rita Ebbink en Heloise van der Vegt for their help with conducting the study.

The authors acknowledge the Medical Research Foundation and Roche Diagnostics for their financial

support. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

This study was presented at the 45th Annual Meeting of the European Association for the Study of Diabetes, Vienna, Austria, 29 September to 2 October 2009 and at the 14th Annual Conference of the Federation of European Nurses in Diabetes, Vienna, Austria, 25 and 26 September 2009.

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# Rhabdomyolysis following pandemic influenza A (H1N1) infection

Shiuan-Chih Chen<sup>1,4,5</sup>, Keh-Sen Liu<sup>2,4</sup>, Horng-Rong Chang<sup>3,5,6</sup>, Yuan-Ti Lee<sup>2,4,5</sup>, Chun-Chieh Chen<sup>1,4</sup>, Meng-Chih Lee<sup>1,5</sup>

<sup>1</sup>Department of Family and Community Medicine, Chung Shan Medical University Hospital, Taichung, Taiwan, Divisions of <sup>2</sup>Infectious Diseases and <sup>3</sup>Nephrology, Internal Medicine, Chung Shan Medical University Hospital, Taichung, Taiwan, <sup>4</sup>School of Medicine and <sup>5</sup>Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan, \*corresponding author: tel.: +886-4-24739595 ext. 34970, fax.: +886-4-23248137, e-mail: sp954455@seed.net.tw.

## ABSTRACT

Rhabdomyolysis is uncommon but potentially life-threatening. We present a 17-year-old patient who developed rhabdomyolysis following pandemic influenza A (H1N1/09) infection. With aggressive hydration her renal function remained normal throughout the entire disease course, and she steadily improved clinically. Although pneumonia and acute respiratory distress syndrome are the most common severe complications of H1N1/09 infection, clinicians should be aware that H1N1/09 infection may be complicated by rhabdomyolysis.

## KEYWORDS

Rhabdomyolysis, pandemic, influenza A (H1N1)

## INTRODUCTION

Rhabdomyolysis, most frequently caused by crush injury, a comatose or postictal state, postoperative surgical trauma, or excessive physical exertion, is a syndrome characterised by muscle necrosis and the release of intracellular muscle constituents into the circulation.<sup>1,4</sup> The severity of disease can range from asymptomatic elevations in serum muscle enzymes to life-threatening conditions. Pandemic influenza A (H1N1/09) infection is a rare cause of rhabdomyolysis. We describe a pandemic influenza A (H1N1/09)-infected patient with complicating rhabdomyolysis.

## CASE REPORT

A previously healthy 17-year-old girl presented to us with a cough of three days duration. Two days before presentation, she had myalgia and weakness which resolved that day after bed rest. One day before presentation, the patient treated herself with over-the-counter cold medications for a presumed common cold, without significant improvement. She noted recent contact with a classmate suffering fever and cough of unknown cause. She denied other symptoms and did not have any medical or family history, regular or illicit drug use, recent immunisation, travel, trauma, extraordinary exercise or similar episodes of myalgia. On examination she was slender, afebrile, normotensive and not lymphadenopathic. Heart, lung, motor function, sensory response and skin examinations were normal. A rapid antigen test for influenza A was positive. Urinalysis was positive for trace blood with a pH of 6.5 but no red or white blood cells on microscopy. Other laboratory data showed a markedly raised creatine kinase (CK) at 192.372 U/l (normal 0.533 to 3.001 U/l), and elevated aspartate aminotransferase (AST) at 4.318 U/l (normal 0.217 to 0.633 U/l), lactic dehydrogenase (LDH) at 5.084 U/l (normal 1.667 to 3.167 U/l), alanine aminotransferase (ALT) at 1234 U/l (normal 0.050 to 0.617 U/l) and CK-MB at 1.134 U/l (normal 0 to 0.167 U/l). C-reactive protein and erythrocyte sedimentation rate, initially slightly elevated, normalised one day later. Chest X-ray, electrocardiogram, complete blood counts, routine clinical chemistries, thyroid function, coagulation tests, troponin I, and antinuclear antibody test were normal. Blood/throat swab cultures for bacteria, urine myoglobin and serology for cytomegalovirus and Epstein-Barr virus were all negative.

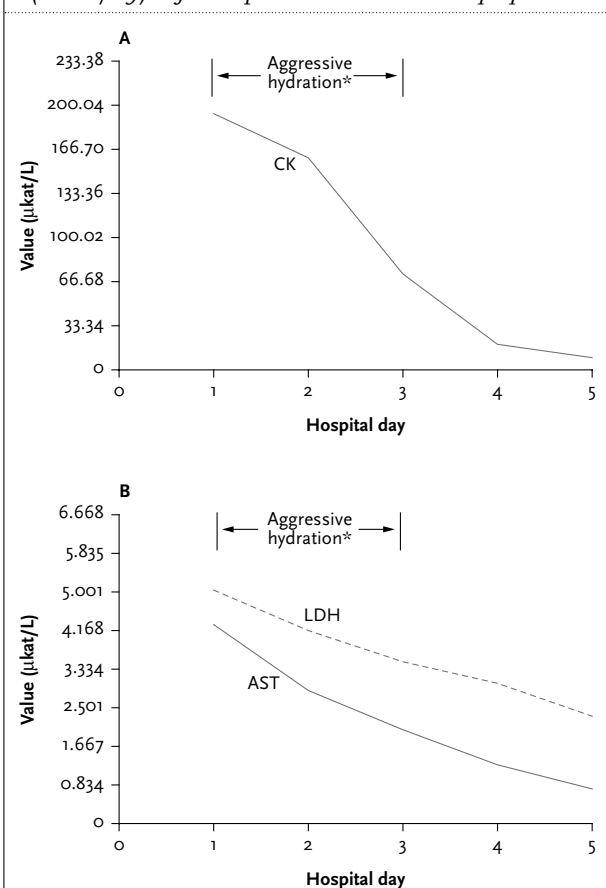
The girl was admitted with a diagnosis of rhabdomyolysis presumably from influenza A infection. Three hours after admission, she had fever which resolved with acetaminophen and oseltamivir (75 mg twice daily for five days) one day later. Pandemic influenza A (H1N1/09) virus was identified by real-time reverse-transcriptase polymerase chain reaction of nasopharyngeal smear at the National Laboratory of Taiwan Centers for Disease Control. With aggressive hydration for rhabdomyolysis, her renal function and electrolytes remained normal and the muscle enzyme values gradually declined as the patient improved clinically. On hospital day 5, the ALT and LDH had normalised, AST was just above normal, and CK decreased to 10.119 U/l (figure 1); the patient was subsequently discharged. One week later, she remained asymptomatic, and her AST and CK had normalised.

## DISCUSSION

Rhabdomyolysis has multiple aetiologies, among others, trauma, intense exercise, infection, drugs or toxins, genetic defects, and metabolic or neuromuscular diseases have been described.<sup>1,4</sup> Viral aetiology, mostly influenza, has been reported to be the predominant cause of infection-induced rhabdomyolysis.<sup>2,4,5</sup> The mechanisms of rhabdomyolysis caused by influenza virus remain unclear. Certain hypothetical mechanisms, including muscle damage due to direct viral invasion or induction by an immune-mediated action, have been proposed.<sup>2,4,6</sup> Recent reports<sup>7,8</sup> showed that >40% of pandemic influenza A (H1N1/09) admissions had abnormal muscle enzyme values, implying that pandemic influenza A (H1N1/09) virus might cause muscle damage or inflammation. The classic triad of rhabdomyolysis includes myalgia, red-to-brown or dark urine and muscle weakness. However, <10% of patients with rhabdomyolysis show all three classic features and 3.6% have dark urine,<sup>2,3</sup> implicating a potentially insidious onset. The diagnosis of rhabdomyolysis is confirmed by laboratory studies. Myoglobinuria can produce pigmenturia thus aiding in the diagnosis of rhabdomyolysis. Although myoglobinuria is usually detected in cases of rhabdomyolysis, rhabdomyolysis does not necessarily result in visible myoglobinuria. Myoglobin has a short half-life (2-3 hours) and could be rapidly and unpredictably eliminated by hepatic metabolism and renal excretion.<sup>9</sup> The test for myoglobin in plasma or urine would be negative before any medical attention is sought; thus, the diagnosis of rhabdomyolysis cannot be completely ruled out.<sup>1,4,9</sup> The classic laboratory finding as the diagnostic criteria for rhabdomyolysis is an elevated serum CK of  $\geq 5$  times the normal value, in which the CK is almost entirely of skeletal muscle fraction.<sup>1,10</sup> The complications of rhabdomyolysis include hyperkalaemia, hypocalcaemia, cardiac dysrhythmias, cardiac arrest, acute renal failure, disseminated intravascular coagulation and compartment syndrome. Acute renal failure is the most common among them and has been reported in 13 to 50% of patients with rhabdomyolysis.<sup>3</sup> The mechanisms of renal damage include tubular obstruction, the toxic effect of free chelatable iron on tubules, and vasoconstriction.<sup>1,4</sup> Hypovolaemia or dehydration and aciduria (urine pH <6.5) have been suggested as crucial factors in the development of renal failure from rhabdomyolysis;<sup>1,2</sup> therefore, early and aggressive fluid repletion and bicarbonate therapy if necessary are the standard treatment to prevent acute renal failure.<sup>1,4,9</sup>

To date, there have been only three case reports of pandemic influenza A (H1N1/09)-associated rhabdomyolysis in the literature;<sup>11-13</sup> two cases appeared to have respiratory distress syndrome or pneumonia with complicating rhabdomyolysis and one case with

**Figure 1.** Serum creatine kinase (panel A), aspartate aminotransferase, and lactic dehydrogenase (panel B) levels during hospitalization in pandemic influenza A (H1N1/09)-infected patient with rhabdomyolysis



AST = aspartate aminotransferase; CK = creatine kinase; LDH = lactic dehydrogenase. Normal range: AST = 0.217-0.633 µkat/l; CK = 0.533-3.001 µkat/l; LDH = 1.667-3.167 µkat/l. \*Isotonic saline was initially administered intravenously at 1-2 liters/hour and then was adjusted to maintain a urine output of >1 ml/kg/hour.

mild asthma had triadic features of rhabdomyolysis. In the present report, the previously healthy patient did not present with severe respiratory illness caused by pandemic influenza A (H1N1/09) infection, and there were no apparent symptoms suggestive of rhabdomyolysis, implying the capacity of this novel virus for causing this clinical effect with obscure presentation.

## CONCLUSION

Clinicians should consider a pandemic influenza A (H1N1/09) infection in any person with cold-like symptoms and suspected contact history in the worldwide pandemic surroundings. Although pneumonia and acute respiratory distress syndrome are the most common severe complications of the pandemic influenza A (H1N1/09) infection,<sup>7,8,14</sup> physicians should also be aware that pandemic influenza A (H1N1/09) infection may be complicated by rhabdomyolysis.

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# Falsely elevated lactate in severe ethylene glycol intoxication

Y. Sandberg<sup>1</sup>, P.P.M. Rood<sup>2</sup>, H. Russcher<sup>3</sup>, J.J.M. Zwaans<sup>2</sup>, J.D. Weigel<sup>4</sup>, P.L.A. van Daele<sup>1\*</sup>

Department of <sup>1</sup>Internal Medicine, <sup>2</sup>Emergency Medicine, <sup>3</sup>Clinical Chemistry, <sup>4</sup>Critical Care Medicine, Erasmus MC, University Medical Center, Rotterdam, the Netherlands, \*corresponding author: e-mail: p.l.a.vandaele@erasmusmc.nl

## ABSTRACT

A 29-year-old male presented at the emergency department of our hospital in a confused state. He had a history of psychoses and substance abuse. Physical examination revealed hyperventilation and abdominal tenderness. Blood gas analysis in the emergency department using an ABL 725 Radiometer analyser showed a severe metabolic acidosis with massive lactate elevation. Lactate acidosis due to mesenteric ischaemia was suspected. However, toxicology screening demonstrated ethylene glycol intoxication. Treatment with ethanol infusion and acute haemodialysis was started. Repeated laboratory measurements using a clinical chemistry analyser showed minimal plasma lactate elevation. Falsely elevated lactate measurement is a little known phenomenon that can occur in ethylene glycol intoxication and can cause serious delay in diagnosis. Therefore, elevated lactate concentrations measured on intensive care unit and emergency department blood gas analysers should be confirmed by a clinical chemistry analyser in the main laboratory in case of suspected ethylene glycol intoxication.

## KEYWORDS

Ethylene glycol intoxication, lactate, blood gas analyser

## INTRODUCTION

Ethylene glycol is a colourless and odourless fluid that has a sweet taste. It is a component of antifreeze fluid, which is the major source of exposure in poisonings. Poisoning with ethylene glycol can occur through attempted inebriation, intentional self-harm, or unintentional ingestion. The signs and symptoms of ethylene glycol intoxication generally develop in three distinct

### What was known on this topic?

Ethylene glycol metabolites, especially glycolic acid and glyoxylic acid, can cause falsely elevated lactate levels and was described for the first time in 1999.

### What does this add?

This case together with the additional experiments show that analytical interference of glycolic acid occurs on different instruments in many Dutch hospitals. The majority of blood gas analysis machines demonstrate falsely elevated lactate levels and all Radiometer blood gas analysis instruments are affected.

stages. Stage 1 (30 min to 12 hours after ingestion): gastrointestinal and nervous system involvement; stage 2 (12 to 24 hours after ingestion): cardiopulmonary dysfunction with profound metabolic acidosis; stage 3 (24 to 72 hours after ingestion): acute renal failure which can be oliguric or anuric. The mortality of ethylene glycol intoxication is variable, ranging from 1 to 22%.<sup>1</sup>

Ethylene glycol metabolites are structurally similar to lactate and can cause artificial elevation of lactate concentration. This is especially the case when using blood gas machines in the emergency department and intensive care unit.<sup>2-4</sup> We present a case of ethylene glycol intoxication and demonstrate the substantial potential for misdiagnosis.

## CASE REPORT

A 29-year-old man with a history of psychoses and substance abuse presented at the emergency department of our hospital in a confused state. His medication was



penfluridol with unknown dose. On examination his temperature was 36.1°C, the pulse 110 beats/min, the blood pressure 180/100 mmHg, and a score of 11 on the Glasgow Coma Scale (possible range, 3 to 15, with higher scores indicating better status). The respiratory rate was 50 breaths/min, and the oxygen saturation 100%. His pupils were equal, round, and reactive to light. Further physical examination revealed a diffusely tender abdomen and hypoactive bowel sounds. Testing of arterial blood in the emergency department using the ABL 725 blood gas analyser (Radiometer Medical, Denmark) indicated severe metabolic acidosis with a lactate concentration of 24 mmol/l. Full laboratory investigations showed an elevated creatinine, an anion gap of 25 mmol/l and osmolal gap of 6 mOsmol/kg (table 1). Both a chest radiograph and computed tomography (CT) scan of the head were normal and because of physical exhaustion mechanical ventilation was started. A normal CT scan of the abdomen ruled out that the lactate elevation was caused by mesenteric ischaemia. Urine toxicological screening indicated cannabinoid use. The urine sediment showed calcium oxalate crystals. Toxicological screening of serum at the time of admission showed an ethylene glycol level of 640 mg/l (10.3 mmol/l) and was negative for ethanol and methanol. Remarkably, a second plasma lactate level measured on an Dx-C-800 automated chemistry analyser (Beckman Coulter) in the hospital's main laboratory was

only 4.7 mmol/l. Ethanol infusion, bicarbonate infusion and haemodialysis were initiated immediately and the patient was admitted to the intensive care unit. Shortly afterwards, he could be extubated. The renal failure has completely recovered.

### PATHOPHYSIOLOGY AND CLINICAL MANIFESTATIONS

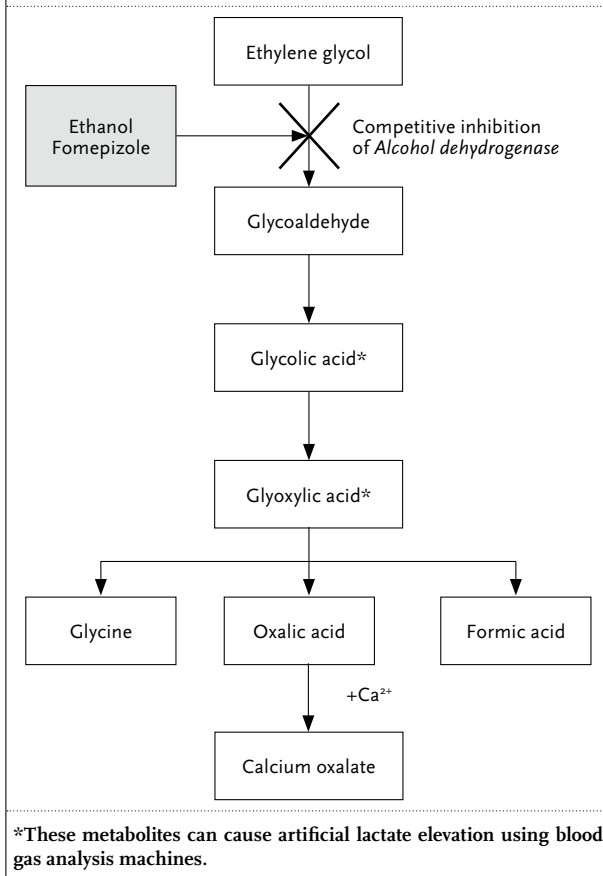
Ethylene glycol itself is relatively nontoxic, but it is metabolised by successive oxidations to toxic metabolites such as glycolic acid, glyoxylic acid and oxalic acid (figure 1).<sup>5</sup> The prominent metabolic acidosis and organ failure are caused by circulating glycolic acid. Oxalic acid may combine with ionised calcium in the plasma to form calcium oxalate crystals. Calcium oxalate precipitates in the renal tubules and is thought to cause renal failure.<sup>6,7</sup> Detection of typical calcium oxalate crystals in the urine supports the diagnosis of ethylene glycol intoxication but is a late and non-specific finding. The increased anion gap is attributable to ethylene glycol and its metabolites, the osmolal gap is only increased shortly after ethylene glycol ingestion.<sup>8</sup> The time span between ingestion and presentation in our case was more than 12 hours and explains the normal osmolal gap at presentation.

**Table 1. Results of laboratory tests**

Measurement		Time			
		13.00	16.00	17.00	20.00
Sodium	mmol/l	145	151	149	147
Potassium	mmol/l	5.1	4.7	4.0	4.1
Urea	mmol/l	6.1	7.1	-	8.4
Creatinine	µmol/l	135	160	-	187
Chloride	mmol/l	118	112	115	113
Calcium	mmol/l	2.37	2.23	-	-
Glucose	mmol/l	11.9	10.6	10.0	-
pH		7.11	7.09	7.11	7.45
pCO <sub>2</sub>	kPa	0.8	7.2	4.7	2.7
pO <sub>2</sub>	kPa	19.2	56.7	59.1	22.7
Bicarbonate	mmol/l	1.9	15.7	10.8	18.8
Oxygen saturation		98	99	100	99
Lactate dehydrogenase	U/l	983	-	-	547
Creatinine kinase	U/l	1515	-	-	763
Anion gap <sup>1</sup>	mmol/l	25	23	23	15
Osmolal gap <sup>2</sup>	mOsmol/kg	6	-	-	-
Lactate (POCT) <sup>3</sup>	mmol/l	24	23	23	
Lactate (Chemistry analyser)	mmol/l	4.7	4.3	2.6	3.2
Ethylene glycol	mg/l	640	-	-	234
Ethanol	g/l	0	-	-	0.9

<sup>1</sup>Anion gap = [Na<sup>+</sup>] - ([Cl<sup>-</sup>] + [HCO<sub>3</sub><sup>-</sup>]); <sup>2</sup>Osmolal gap = measured osmolality - (2x [Na<sup>+</sup>] + [glucose] + [urea]); <sup>3</sup>POCT = point-of-care test; - = not done.

**Figure 1.** Metabolic transformation of ethylene glycol



## TREATMENT

The metabolism of ethylene glycol occurs primarily through alcohol dehydrogenase. Ethanol is a competitive substrate for alcohol dehydrogenase, which has greater affinity for ethanol than for ethylene glycol. Therefore ethanol is effective and inhibits the metabolism of ethylene glycol (figure 1). Although it is difficult to dose and has sedative and behavioural effects, ethanol is inexpensive and easily obtained. An alternative is fomepizole (4-methylpyrazole). It is also a competitive inhibitor of alcohol dehydrogenase and prevents the formation of toxic acid metabolites. It is easy to dose, easy to administer, and side effects are rare.<sup>5</sup> However it is expensive and not available in all hospitals. Haemodialysis is used to clear both ethylene glycol and its toxic metabolites more quickly.

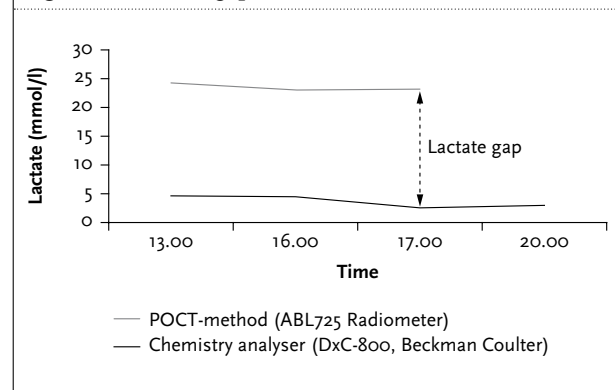
## DISCUSSION

A remarkable finding in our case was the discrepancy between the lactate level measured on a blood gas analyser in the emergency department and the plasma lactate level measured on a clinical chemistry analyser (figure 2). Slightly elevated lactate concentrations can be found in ethylene

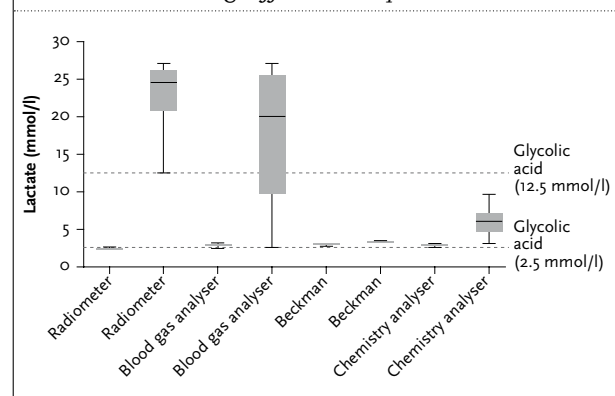
glycol intoxication,<sup>9</sup> but ethylene glycol does not cause excess lactate production.<sup>4</sup> Glycolic acid and glyoxylic acid can both cause artificial elevation of lactate.<sup>2,10-13</sup> Certain types of L-lactate oxidase allow cross reaction with these ethylene glycol metabolites. Especially blood gas analysers (using L-lactate oxidase) are affected,<sup>2</sup> while analysers using lactate dehydrogenase are free of interference. Measuring a 'lactate gap' using two different technologies (figure 2), only one of which is sensitive to glycolic acid, is suggested to be helpful in diagnosing advanced ethylene glycol poisoning.<sup>2,12,14</sup> However, when ethylene glycol intoxication is in the differential diagnosis, the ethylene glycol concentration should be directly measured.

We wished to gain insight into how widespread the problem of false lactate elevation due to glycolic acid interference is. Therefore samples were spiked with various concentrations of glycolic acid ([2.5 mmol/l] and [12.5 mmol/l]). The lactate values were determined in 30 Dutch hospitals using different clinical chemistry analysers and blood gas machines, including Radiometer ABL analysers (figure 3). The majority of measurements (81%) on blood gas analysers showed falsely elevated lactate levels. Radiometer blood gas analysers were available in 12 hospitals and were all affected. The chemistry

**Figure 2.** Lactate gap



**Figure 3.** The effect of glycolic acid upon blood lactate measurements using different analysers



analysers demonstrated no or only minimally elevated lactate concentrations.

## CONCLUSION

This case demonstrates the potential to misdiagnose ethylene glycol intoxication as a lactate acidosis due to falsely elevated lactate measurement. Although serum lactate elevations can be detected in patients with ethylene glycol intoxication, such elevations are usually minor. The falsely elevated lactate levels likely occur because of the incomplete specificity of L-lactate oxidase. Knowledge of this analytical interference is essential in every patient presenting with severe metabolic acidosis and massive lactate elevation. Elevated lactate concentrations on blood gas analysers should be confirmed by a chemistry analyser in case of suspected ethylene glycol poisoning. On the other hand, the lactate gap between measurements with different analysers can help in diagnosing a possible ethylene glycol poisoning.

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# An adult with vague abdominal complaints and atypical colonoscopic findings

G. van der Horn<sup>1\*</sup>, E.R. Ranschaert<sup>1</sup>, I.J.M. Dubelaar<sup>1</sup>, I.P. van Munster<sup>2</sup>

Departments of <sup>1</sup>Radiology and <sup>2</sup>Gastro-enterology, Jeroen Bosch Hospital, 's- Hertogenbosch, the Netherlands, \*corresponding author: tel.: +31(0)73-699 20 00, fax: +31(0)73-6992601, e-mail: g.horn@jzbz.nl

## CASE REPORT

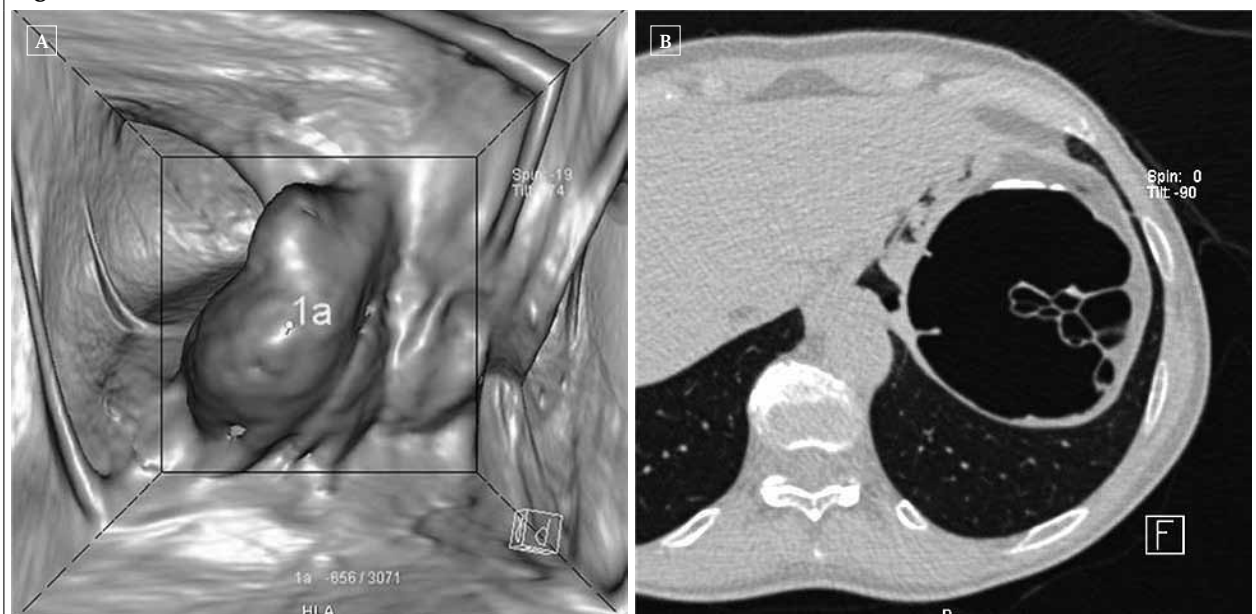
A 58-year-old male presented with a history of vague abdominal pain for several months. Physical examination and laboratory tests were negative. Routine ultrasound examination showed no relevant abnormalities. The patient was not reassured and optical colonoscopy was performed to exclude colonic pathology. A polyp-like lesion with intact overlying mucosa was seen and interpreted as an external lesion; therefore no biopsy was taken. Instead, a computed tomography colonography (CTC) was carried out and a

multilobulated broad-based polypoid lesion was found at the splenic flexure (*figure 1A*). On the two-dimensional (2D) images (*figure 1B*) the lesion demonstrated a rather 'foamy' aspect caused by multiple air-containing lobules.

## WHAT IS YOUR DIAGNOSIS?

See page pagina 327 for the answer to this photo quiz.

Figure 1.



# A tropical surprise?

B. Tomlow\*, K. Pogány

Department of Internal Medicine, Maastad Hospital, Rotterdam, the Netherlands,  
\*corresponding author: tel.: +31 (0) 20-753 94 76, e-mail: B.Tomlow@MCA.nl

## CASE REPORT

A 56-year-old Surinamese woman with a medical history notable for Crohn's disease presented to our emergency department with unexplained fatigue, fever and severe pain in her right leg for a few weeks. In addition she explained that she had had a small spot on the same leg for the last five years, which she noticed for the first time after a visit to Suriname. It was a reddish skin plaque, which has been slowly growing ever since. In the last few months it had grown explosively and caused severe pain. The vital signs revealed a body temperature of 38.7 °C, a blood pressure of 100/65 mmHg, heart rate of 76 beats/min and a respiratory rate of 18 breaths/min. On physical examination, the right leg revealed an ulcerated lesion with a size of 20 cm long and 10 cm wide, with hard, raised edges, on the lateral side of the lower leg (figures 1 and 2). Chest examination revealed clear and

symmetrical breathing sounds and normal heart sounds. Laboratory examination showed a raised C-reactive protein level of 175 mg/l (normal value 0 to 10 mg/l), leucocyte count of  $16.0 \times 10^9/l$  (normal value 3.5 to  $10.0 \times 10^9/l$ ) and a haemoglobin level of 4.4 mmol/l (normal value 7.5 to 9.5 mmol/l) with a mean corpuscular volume of 72 fl (normal value 80 to 100 fl). All the other findings were within normal limits. The microcytic anaemia was successfully treated using a single erythrocyte transfusion and prolonged iron supplementation. The lesion on the lower right leg, however, was still of unknown origin.

## WHAT IS YOUR DIAGNOSIS?

See page pagina 328 for the answer to this photo quiz.

Figure 1.



Figure 2.



# An unusual cause of constipation by a rectal mass

H.F. Dresselaars\*, J.J.M. van Meyel

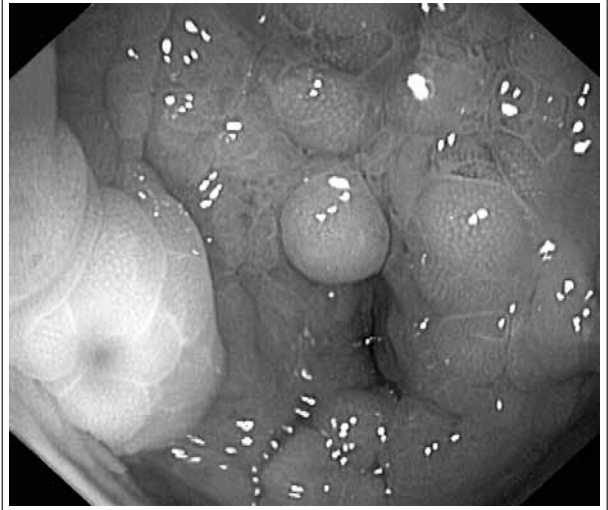
Sint Lucas Andreas Hospital, Amsterdam, the Netherlands, \*corresponding author: tel.: 06-47677373, fax: +31 (0)20-683 87 71, e-mail: lenadresselaars@hotmail.com

## CASE REPORT

A 65-year-old female patient presented at the emergency department with a four-week history of total constipation, 4 kg weight loss and mild nausea. There was no medical history of note and blood tests were normal besides a microcytic anaemia (haemoglobin 6.3 mmol/l). Rectal examination revealed a rigid mass filling the whole lumen. Further physical examination was unremarkable. Colonoscopy was performed showing a vitreous, circular growing, non-ulcerative mass of 10 centimeters in length (*figure 1*). Biopsy of the lesion showed hyperplastic tissue and some atypical cells; no dysplasia was found. The diagnosis of primary colorectal adenocarcinoma was rejected since atypical cells were only found beneath the muscularis mucosa – leaving the mucosa relatively spared – and immunostains did not meet the criteria (CDX-2 negative, cytokeratine (CK) 20 negative and CK7 positive).

Computed tomography of thorax and abdomen showed profound mediastinal, intra- and retroperitoneal lymphadenopathy and lesions in the lungs suspicious for metastatic disease. Besides the rectal mass no abnormalities of other abdominal organs were seen. X-ray of the colon with contrast showed no additional obstruction elsewhere in the bowel.

Figure 1.



**WHAT KIND OF INVESTIGATION  
NEEDS TO BE DONE NEXT TO MAKE  
THE DIAGNOSIS?**

See page pagina 329 for the answer to this photo quiz.

AN ADULT WITH VAGUE ABDOMINAL COMPLAINTS AND ATYPICAL  
COLONOSCOPIC FINDINGS

## DIAGNOSIS

Pneumatosis cystoides coli (PCC) is a rare condition defined as an abnormal location of gas within the colonic wall.<sup>1,2</sup> It typically presents with multiple gas-filled cysts in the submucosa and/or subserosa of the colon. The size of the cysts may range from a few millimetres to several centimetres. PCC is regarded as the colonic variant of pneumatosis cystoides intestinalis (PCI).<sup>1</sup> Distinction is made between primary idiopathic and secondary forms of PCI.<sup>1</sup> The primary form (PCC) has no known associated predisposing factors.<sup>1,2</sup> The secondary form, however can be caused by a wide variety of underlying pathologies such as gastrointestinal disorders, pulmonary and infectious disease and prior chemotherapy.<sup>1</sup> Numerous hypotheses have been proposed to explain the pathogenesis, including mechanical, biochemical and bacterial causes.

Clinically PCI may present with a variety of symptoms.<sup>3</sup> PCC is a benign entity and the clinical course is usually positive. When PCC or PCI is an incidental finding, conservative management should be the treatment of choice. Antibiotics and elementary diet have been described for patients with mild symptoms, with ambiguous results.<sup>1,2</sup> High-pressure oxygen treatment seems effective in patients with more severe symptoms.<sup>1,2</sup> Our patient was treated conservatively because the PCC was interpreted as an incidental finding. The symptoms diminished and even disappeared after nine months.

PCC is very difficult to detect on plain film because of the coexistence of normal gas between mucosal folds or a mixture of gas and faecal material. With a barium enema and even with conventional colonoscopy, the intraluminal bulging of the submucosal air-containing cysts can be mistaken for a polypoid lesion or even a malignancy. With CTC one can precisely delineate the extent of the lesions and differentiate PCC from an adenomatous polyp or malignancy. From an endoluminal three-dimensional

perspective, PCC can have a polypoid appearance similar to the findings with conventional colonoscopy, although the mucosal surface cannot be reliably discerned. On the 2D images, however, the air composition of the cysts is readily apparent, especially in a lung-window setting.<sup>1-5</sup> Since CT scan also provides a survey of the entire abdominal cavity, it is possible to exclude other conditions such as pneumoperitoneum or gas in the portal venous system,<sup>2</sup> although this is limited because of the lower signal-to-noise ratio in the currently used low-dose setting and the absence of IV-contrast administration.

## CONCLUSION

Our patient was diagnosed with a pneumatosis cystoides coli in the descending colon. CT colonography is the modality of choice in diagnosing PCC, allowing the exact location and configuration of the abnormalities to be described.

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## DIAGNOSIS

To differentiate between framboesia (*Treponema pallidum pertenue*), leprosy (*Mycobacterium leprae*), *Rickettsia* and other causes, radiological imaging was performed and both incision biopsies and serology were taken. Conventional radiological imaging of the lower right leg revealed an abnormal soft tissue contour and lateral proximal tibia of the anterior side. The skeleton showed a normal structure and mineralisation without periosteal response. An MRI of the leg showed staining of the superficial ulcer around the raised edges with slight oedema in the adjacent subcutis. There did not seem to be any growth into the surrounding muscles, tendons or bone. The serology was negative. The biopsies showed a hyperplasia and hyperpigmentation on the basal side advancing in the epithelium showing an abnormal desmoplastic stroma. This eventually confirmed the diagnosis of a well-differentiated squamous cell carcinoma. Squamous cell carcinoma (SCC) is a malignant form of cancer that may occur in many organs, including the skin, lips, mouth, oesophagus, urinary bladder, prostate, lungs, vagina, and cervix and is the second most common cancer of the skin.<sup>1,2</sup> Sunlight exposure and immunosuppression are

risk factors for SCC of the skin with chronic sun exposure being the strongest environmental risk factor.<sup>3</sup> Squamous cell carcinoma can generally be treated by excision or surgery while nonsurgical options for the treatment of cutaneous SCC include topical chemotherapy, topical immune response modifiers, photodynamic therapy, radiotherapy, and systemic chemotherapy. Radiation therapy is a primary treatment option for patients in whom surgery is not feasible and is an adjuvant therapy for those with metastatic or high-risk cutaneous SCC.<sup>3,4</sup>

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ANSWER TO PHOTO QUIZ (PAGE PAGINA 326)  
AN UNUSUAL CAUSE OF CONSTIPATION BY A RECTAL MASS

Gastroscopy demonstrated a poorly distensible stomach with an ulcerative mass involving the majority of the viscus. Biopsy showed a poorly differentiated adenocarcinoma and chronic inflammation. Signet ring cells were present (figure 2). No *Helicobacter pylori* was found. Morphological appearance resembled the rectal mass. Linitis plastica of the stomach with metastatic disease in the rectum was diagnosed.

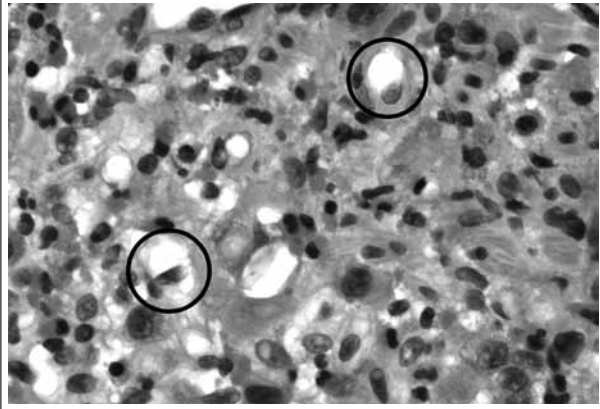
The term linitis plastica refers to the desmoplastic inflammatory-like reaction that tumour cells or their products elicit in the stroma, often more striking than the infiltrative cells themselves. This reaction makes hollow

organs rigid with thickened walls, as can be seen on CT and during endoscopy in progressive disease.<sup>1</sup>

Linitis plastica-type gastric carcinoma is a poorly differentiated adenocarcinoma accounting for a maximum of 14% of the advanced gastric cancers.<sup>2</sup> Histological examinations reveal independent signet ring cells infiltrating in deeper layers of gastric tissue, leaving the mucosa relatively intact. The latter makes endoscopic diagnosis often difficult. Deeper biopsies might be necessary.<sup>3</sup>

The stomach is the most common primary site of metastatic linitis plastica; however, other sites include the breast, gallbladder, bladder and prostate gland. Metastases, when present, can virtually always be found in the peritoneum and often in the gastrointestinal tract.<sup>1,3</sup> The prognosis is very poor with a five-year survival of less than 10%.<sup>2,3</sup>

Figure 2.



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# The influence of a soiled finger in capillary blood glucose monitoring

J. Hortensius<sup>\*</sup>, N. Kleefstra<sup>1,2</sup>, R.J. Slingerland<sup>3</sup>, M.J. Fokkert<sup>3</sup>, K.H. Groenier<sup>4</sup>, S.T. Houweling<sup>2,5</sup>, H.J.G. Bilo<sup>1,6</sup>

<sup>1</sup>Diabetes Centre, Isala Clinics, Zwolle, the Netherlands, <sup>2</sup>Langerhans Medical Research Group, <sup>3</sup>Department of Clinical Chemistry, Isala Clinics, Zwolle, the Netherlands, <sup>4</sup>Department of General Practice, University of Groningen, Groningen, the Netherlands, <sup>5</sup>General Practice Sleenwijk, Sleenwijk, the Netherlands, <sup>6</sup>Department of Internal Medicine, University Medical Center, Groningen, the Netherlands, <sup>\*</sup>corresponding author: tel.: +31 (0)38-424 25 18, fax: +31 (0)38-424 76 94, e-mail: h.hortensius@isala.nl

Dear Editor,

There are several aspects concerning self-monitoring of blood glucose (SMBG) which can influence the outcome of the measurement.<sup>1</sup> For example, there is no general agreement regarding the use of the first or second drop of blood for glucose monitoring. Our aim was to investigate the influence of wiping a drop of blood on glucose concentrations measured by SMBG when having a finger soiled with different sugar-containing products, and the effects of cleaning a soiled finger with chlorhexidine. Twenty-five non-diabetic adults participated. After they had washed their hands, their fingers were soiled with various sugar-containing products. The products used were dextrose, fruit, jam, honey, and chocolate paste. Glucose concentrations were determined in the first, second and third drop of capillary blood (intervention 1). Furthermore, measurements were done in one drop before and in two drops of blood after cleaning a soiled finger with chlorhexidine (intervention 2). All results were compared with a control measurement from a clean and dry finger.

Glucose levels were determined with the Accu-Check Inform II (Roche, Almere, the Netherlands). The general linear model (GLM) with repeated measures (multivariate testing) was used to test for overall changes in glucose concentrations. Furthermore, using the 'simple' contrast, each concentration was compared with the control.

Table 1 shows glucose concentrations in sequential drops of blood in different circumstances. In both interventions the highest glucose concentrations were found in the first drop of blood, with a significant decrease in sequential blood glucose concentrations from the first drop of blood to control ( $p=0.013$  and  $p<0.0005$  respectively). In 53% of the cases in intervention 1 and in 34% of the cases in intervention 2, the blood glucose concentration in the third drop of blood was still more than 10% higher than the control measurement. In the literature only one study describes the difference between glucose concentrations in the first and the second drop of blood.<sup>2</sup> This study showed no differences between the readings. The study was conducted in volunteers

**Table 1.** Glucose concentrations in different sequential drops of blood

	Intervention 1			Measurement before cleaning	Intervention 2		Control
	1st drop	2nd drop	3rd drop		1st drop	2nd drop	
Median, ranges	10.7 (4.8-33.3)	6.2 (4.5-24.1)	5.8 (4.3-15.3)	8.4 (5.4-33.3)	5.9 (4.3-33.3)	5.4 (4.4-6.9)	5.1 (4.3-7.3)
Absolute difference vs control; median	5.2 (0.0-28.6)	0.9 (-1.0-19.0)	0.6 (-0.9-10.0)	3.2 (-0.5-28.6)	0.8 (-0.3-28.2)	0.2 (-1.1-1.6)	--
> 10% higher vs control	89%	63%	53%	87%	63%	34 %	--

n=25; glucose in mmol/l; median (interquartile ranges).

without soiled fingers, however. Soiled fingers can have a great effect on blood glucose readings. Our study shows that it is difficult to clean the fingers properly. A new study has to be performed to investigate aspects concerning SMBG in people with diabetes. In the meantime, the results of our study emphasise the potential inaccuracy of SMBG, even when measuring in the second or third drop of blood, and even after cleaning the finger with chlorhexidine.

#### ACKNOWLEDGEMENT

The authors would like to thank Geke Douw-van Til and Elma Mensink and all the volunteers for their support in this study.

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Bijsluiter

Bijsluiter

# Five-year incidence of type 2 diabetes mellitus in patients with familial combined hyperlipidaemia

Dear Editor,

With great interest we read the article by Brouwers *et al.* in which they described the five-year incidence of type 2 diabetes mellitus (T2DM) in patients with familial combined hyperlipidaemia (FCHL).<sup>1</sup> After a mean follow-up period of 4.8±0.5 years, logistic regression analyses were used to compare the age- and sex-adjusted incidence and prevalence of T2DM between FCHL patients (n=56) and their spouses (n=54). The incidence was significantly higher in the FCHL group compared with spouses (14 vs 2%, odds ratio 9.1; 95% CI 1.0 to 81.4). Based on these results, the authors concluded that FCHL patients, as compared with healthy controls, are predisposed to the development of T2DM. However, the mean baseline body mass index (BMI) of the spouses and FCHL patients was 25.5±3.7 and 28.1±3.9 (kg/m<sup>2</sup>), respectively (p<0.05). The authors did not adjust for this baseline difference in their analyses. Instead, they adjusted

for the change in BMI during the follow-up period, which was not relevantly different between the two groups. Since bodyweight is an extremely important factor in the development of T2DM, we are very interested if the higher incidence of T2DM in FCHL patients still remains after adjustment for the baseline difference in BMI.

**K.J.J. van Hateren, N. Kleefstra**

*Diabetes Center, Isala Clinics and Langerhans Medical Research Group, Zwolle, the Netherlands, e-mail: k.j.j.van.hateren@isala.nl*

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## Response to letter to the editor

We agree that the degree of obesity is an important determinant of incident T2DM. However, baseline BMI was not a significant predictor of new-onset T2DM in our cohort (p=0.12), which is probably accounted for by the small sample size, as discussed in our article.<sup>1</sup> Odds ratio (OR) and confidence intervals (CI) for incident T2DM did not change substantially when we additionally adjusted for baseline BMI, although it was no longer significant (OR: 9.1; 95% CI: 1.0 to 81.4; p=0.04, age- and sex-adjusted, versus: OR: 7.5; 95% CI: 0.8 to 70.4; p=0.07, age-, sex- and BMI-adjusted), which again might be attributed to the small sample size. These outcomes in combination with an increased susceptibility to develop hepatic fat accumulation and insulin resistance<sup>2,3</sup> – both involved in the pathogenesis of T2DM<sup>4</sup> – suggest that FCHL patients have an increased risk to develop T2DM, independent of the degree of obesity. Nevertheless, these data do require confirmation in an independent FCHL cohort.

**M.C. Brouwers, C.J. van der Kallen, N.C. Schaper, M.M. van Greevenbroek, C.D. Stehouwer,**  
*Maastricht University Medical Centre, Maastricht, the Netherlands*

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# Ascertainment and verification of diabetes in the EPIC-NL study

I. Sluijs<sup>\*</sup>, D.L. van der A<sup>2</sup>, J.W.J. Beulens<sup>1</sup>, A.M.W. Spijkerman<sup>3</sup>, M.M. Ros<sup>2</sup>, D.E. Grobbee<sup>1</sup>, Y.T. van der Schouw<sup>1</sup>

<sup>1</sup>University Medical Center Utrecht, Julius Center for Health Sciences and Primary Care, Utrecht, the Netherlands, <sup>2</sup>National Institute for Public Health and the Environment, Center for Nutrition and Health, Bilthoven, the Netherlands, <sup>3</sup>National Institute for Public Health and the Environment, Center for Prevention and Health Services Research, Bilthoven, the Netherlands, <sup>\*</sup>corresponding author: tel.: +31 (0)88-756 81 95, fax: +31(0)88-756 80 99, e-mail: i.sluijs-2@umcutrecht.nl

## ABSTRACT

**Background:** The objectives of this study were to describe in detail the ascertainment and verification of prevalent and incident diabetes in the Dutch contributor to the European Prospective Investigation into Cancer and Nutrition (EPIC-NL cohort) and to examine to what extent ascertained diabetes agreed with general practitioner (GP) and pharmacy records. **Methods:** In total, 40,011 adults, aged 21 to 70 years at baseline, were included. Diabetes was ascertained via self-report, linkage to registers of hospital discharge diagnoses (HDD) and a urinary glucose strip test. Ascertained diabetes cases were verified against GP or pharmacist information using mailed questionnaires. **Results:** At baseline, 795 (2.0%) diabetes cases were ascertained, and 1494 (3.7%) during a mean follow-up of ten years. The majority was ascertained via self-report only (56.7%), or self-report in combination with HDD (18.0%). After verification of ascertained diabetes cases, 532 (66.9%) were defined as having diabetes, 495 (21.6%) as non-diabetic individuals, and 262 (11.5%) as uncertain. Of the 1538 cases ascertained by self-report, 1350 (positive predictive value: 87.8%) were confirmed by GP or pharmacist. Cases ascertained via self-report in combination with HDD were most often confirmed (334 (positive predictive value: 96.0%)). **Conclusions:** Two out of three ascertained diabetes cases were confirmed to have been diagnosed with diabetes by their GP or pharmacist. Diabetes cases ascertained via self-report in combination with HDD had the highest confirmation.

## KEYWORDS

Ascertainment, diabetes, hospital discharge diagnoses, self-report, verification

## INTRODUCTION

Diabetes is an important cause of morbidity and mortality and its incidence is increasing worldwide.<sup>1,4</sup> In 2030 the prevalence of diabetes is expected to have increased by 57% compared with that in 2000.<sup>4</sup> Type 2 diabetes accounts for 90% of these cases.<sup>5</sup>

Accurate identification of diabetes cases in epidemiological studies is of great importance to obtain valid estimates of diabetes risk. In population-based studies, self-reported presence of disease is often used as part of disease ascertainment. Several studies compared self-reported diagnosis of diabetes with diagnosis according to the medical records or medical claims.<sup>6-12</sup> All studies presented high levels of agreement, with 73 to 95% of self-reported diabetes cases being confirmed and kappa values of agreement ranging from 72 to 92%. Alternative sources, such as hospital discharge data, can be used for ascertaining diabetes cases as well. Combining self-report data with alternative ascertainment sources might contribute to a higher identification of diabetes cases. However, still little is known about the validity of diabetes diagnoses from alternative sources such as hospital discharge registries.<sup>13</sup> Moreover, the validity of diabetes ascertained via a combination of self-report data and alternative sources is so far unknown.

In this article we describe in detail the ascertainment and verification of prevalent and incident diabetes cases in the Dutch cohort contributing to the European Prospective Investigation into Cancer and Nutrition (EPIC-NL). In this cohort of 40,011 Dutch adults with a mean age of 50 years, ascertainment of diabetes cases was based on several sources, including self-report, hospital discharge data, and a self-administered urinary glucose strip test. We present to what extent these different and combined

ascertainment sources for the diagnosis of diabetes agree with general practitioner (GP) medical and/or pharmacy records. Moreover, we investigated whether agreement differed by age.

## MATERIALS AND METHODS

### Setting

EPIC-NL consists of the two Dutch contributions to the European Prospective Investigation into Cancer and Nutrition (EPIC),<sup>14</sup> i.e. Prospect-EPIC and MORGEN-EPIC. The individual cohorts of EPIC-NL were set up simultaneously in 1993-1997 and were merged according to standardised processes into one large Dutch EPIC cohort in 2007. Its design and baseline characteristics are described elsewhere.<sup>15</sup>

The Prospect-EPIC Study includes 17,357 women aged 49 to 70 years at baseline, participating in the national breast cancer screening program, and living in the city of Utrecht and its surroundings.<sup>16</sup> The MORGEN-EPIC cohort consists of 22,654 men and women aged 21 to 64 years selected from random samples of the Dutch population in three towns in the Netherlands (Amsterdam, Doetinchem, and Maastricht).<sup>17,18</sup> All participants signed informed consent before study inclusion. The study complies with the Declaration of Helsinki and was approved by the Institutional Board of the University Medical Center Utrecht (Prospect) and the Medical Ethical Committee of TNO Nutrition and Food Research (MORGEN).

### Ascertainment of diabetes

Three sources of ascertaining diabetes were used in our study: self-report, hospital discharge diagnoses (HDD) and urinary glucose strip test (in the Prospect part of the cohort only). Details of all three sources are given below.

#### *Self-report*

At baseline, all individuals who agreed to participate received a self-administered general questionnaire containing questions on demographic characteristics, presence of chronic diseases, and risk factors for chronic diseases.<sup>19</sup> Response rates for this questionnaire were 45% for MORGEN (Amsterdam 33%, Maastricht 45%, Doetinchem 68%) and 35% for Prospect. The baseline questionnaire contained three questions on diabetes. Participants were asked if they had been diagnosed with diabetes previously, and if yes, additional questions on the year of diagnosis and type of treatment were asked.

To detect changes in health status and exposure, two follow-up questionnaires were sent to all surviving participants within regular intervals of three to five years. Response rates for these questionnaires were 64% for the first and 57% for the second questionnaire for the

Amsterdam-Maastricht part of MORGEN and 75 and 78%, respectively, for the Doetinchem part of the cohort. Response rates for Prospect were 78% for the first and 73% for the second questionnaire. These questionnaires included a question about whether diabetes was diagnosed since the last questionnaire, and if so, which physician (GP or specialist in internal medicine) (MORGEN) or which hospital (Prospect) was involved in treatment. Furthermore, information on year of diagnosis and medication use was collected.

#### *Hospital discharge diagnoses*

Diagnoses of diabetes were also obtained from the Dutch Center for Health Care Information, which is responsible for a standardised computerised register of hospital discharge diagnoses. Admission files were filed continuously from all general and university hospitals in the Netherlands from 1990. Data on sex, date of birth, dates of admission and discharge were recorded whenever a patient was discharged from the hospital. One mandatory principal diagnosis and up to nine optional additional diagnoses were reported. All diagnoses were coded according to the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9). The database was linked to the EPIC-NL cohort on the basis of date of birth, sex, postal code, and GP with a validated probabilistic method.<sup>20</sup> Follow-up was complete until 1 January 2006. Participants who had a principal or additional diagnosis of diabetes at discharge (ICD codes 250) were ascertained as diabetes cases in our cohort.

#### *Urinary glucose strip test*

Among prospect participants only, a urinary glucose strip test (Clinistix, Bayer Diagnostics, Tarrytown, NY, USA) was sent out with the first follow-up questionnaire. The lower threshold for detection of glucose with this test was 5.5 mmol/l. Participants were asked to report in the questionnaire whether the strip had turned purple after waiting ten seconds, indicating glucosuria. Participants who reported having a positive test were ascertained as diabetes cases in our cohort and advised to contact their GP.

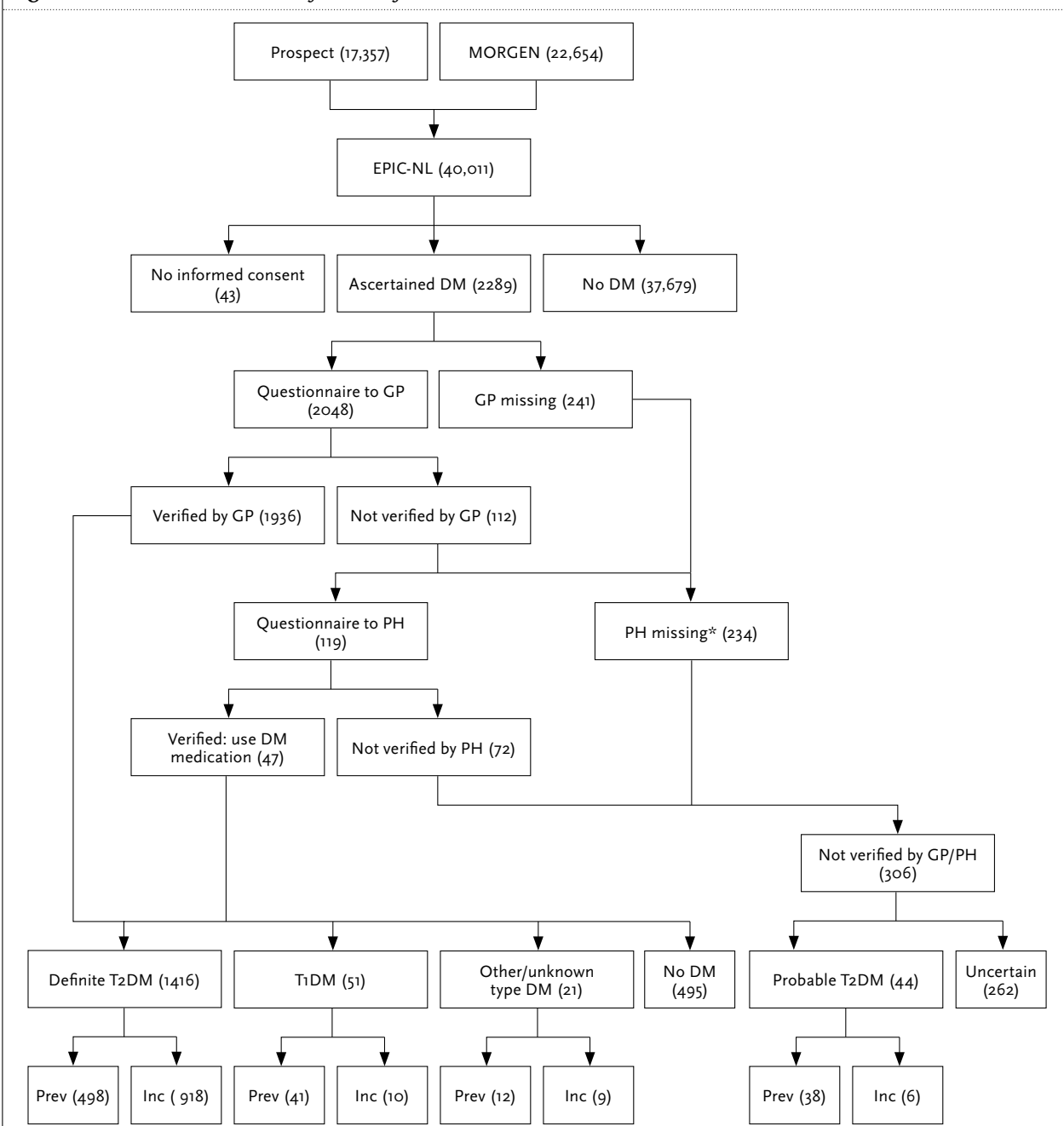
### Verification of diabetes

The verification process of ascertained diabetes cases is visualised in *figure 1*. Verification was carried out by means of GP or pharmacist information. We only verified diabetes in patients who gave signed informed consent for obtaining follow-up information.

#### *GP information*

In the Netherlands, general practice is the optimal source for providing information on the patients' health and illness as virtually all non-institutionalised Dutch citizens are registered with a GP practice. In the Dutch healthcare system the GP is the gatekeeper and controls access to

**Figure 1.** Ascertainment and verification of diabetes cases in the EPIC-NL cohort



Values are expressed as n. DM = diabetes mellitus; GP = general practitioner; Inc = incident; PH = pharmacist; Prev = prevalent; T1DM = type 1 diabetes mellitus; T2DM = type 2 diabetes mellitus.. \*Pharmacist was only known for Prospect part of the cohort.

specialised medical care. The GP has a complete overview of the medical status of the patient. All potential incident and prevalent diabetes cases, ascertained through the three previously described methods, were validated against GP information obtained via mailed questionnaires. Information on name and address of the participants' GPs was obtained from the baseline questionnaire. Extensive efforts were made to obtain accurate current GP contact details. For Prospect and the Doetinchem part of the MORGEN cohort, information on participants' GPs

was updated in the follow-up questionnaires. For the Amsterdam and Maastricht part of the MORGEN cohort, updates were inquired in the first follow-up questionnaire only. Addresses were checked and, if necessary, updates were made using various data sources, such as online medical address books and internet sites.

*GP questionnaire*

The GP questionnaire contained 12 questions on diabetes. GPs were asked if diabetes had been diagnosed,

and if so, in what year and which type (1, 2, other or unknown) of diabetes. Additional questions on how the diagnosis was established and on treatment during the first year after diagnosis and current treatment (diet, oral glucose lowering medication, insulin) were asked. Also, the GP was asked whether the patient suffered from long-term complications such as neuropathy and hypertension. GPs were requested to respond within four weeks upon receipt of the questionnaire. If no response was received within the set time limit, GPs were sent a reminder letter and were subsequently contacted by telephone if they did not respond to this second letter either. GPs received a financial compensation (€ 18, which is equal to a 20-minute consultation) for each returned questionnaire.

#### *Pharmacist questionnaire*

When the GP was unknown or the current GP was unwilling to participate, we used pharmacist information to verify the diagnosis of diabetes, via a mailed questionnaire. Pharmacists' data were only available for the Prospect cohort.

The pharmacist questionnaire contained eight questions concerning use of diabetes medication. The pharmacist was asked whether the participant had used any diabetes medication (i.e. oral glucose-lowering medication or insulin), currently and in the past. Also the year of initiation of diabetes medication was asked. The reminder procedure was the same as for the GPs. No financial compensation was given for returned questionnaires.

#### *Definitions verified cases*

All ascertained diabetes cases, confirmed to be diagnosed with diabetes by the GP, were classified as definite diabetes cases, and split by type of diabetes (1, 2, other or unknown). Ascertained patients for whom the GP did not confirm the diagnosis were defined as not having diabetes. Furthermore, since insulin and glucose-lowering medication are used exclusively for the treatment of diabetes and not for any other illness or disease, participants with confirmed use of diabetes medication by the pharmacist were verified as definite diabetes cases. However, participants who did not use any diabetes medication could not be classified as not having diabetes, because not all persons with type 2 diabetes require insulin or glucose-lowering medication.

If information from both the GP and the pharmacist was absent, we classified participants as probable diabetes cases when two or more ascertainment sources indicated the participant had been diagnosed with diabetes. All probable cases were defined as type 2 diabetes cases. In further analyses, those with probable and definite diagnoses of type 2 diabetes were grouped together as type 2 diabetes cases. Cases ascertained through one ascertainment source

without any verification from the GP or pharmacists were classified as uncertain.

#### **Data analysis**

Median age, mean BMI, and distribution of sex and highest education at baseline (n (%)) were computed. Age was categorised in ten-year intervals, and the youngest two age groups were taken together, because of the relatively low number of cases in these groups. BMI was calculated as measured weight divided by measured height squared ( $\text{kg}/\text{m}^2$ ). Education level was categorised into low (primary education or lower vocational education), middle (advanced elementary education or intermediate vocational education or higher general secondary education for three years or longer) and high (Bachelor or Master of Science degree). In addition, ascertainment and verification information on diabetes status were presented according to age. Finally, we determined the percentage of agreement between information on diabetes status from different ascertainment sources and verification via GP and pharmacist. As we only verified the diabetes cases, and not the non-cases, we only calculated the percentage of ascertained diabetes cases that were confirmed to have diabetes by their GP or pharmacist. This percentage can be interpreted as the positive predictive value (PV+) for having diabetes, with GP and pharmacist information being the reference standard. Kappa values of chance corrected agreement were calculated according to the following equation:  $[\text{kappa} = (\text{observed agreement} - \text{expected agreement}) / (1 - \text{expected agreement})]$ . SPSS (version 14.0) for windows was used for the data analysis.

## **RESULTS**

The study population had a median age of 51.4 years and a mean BMI of 25.7. One quarter were male, and 40% had a low education level (*table 1*).

In total, 2289 (5.7%) participants were ascertained as being diagnosed with diabetes, of which 795 (2.0%) were ascertained at baseline and 1494 (3.7%) during a mean follow-up of 10.1 (SD 1.9) years. Of all ascertained diabetes cases, more than half (56.7%) were ascertained via self-report only and 13.5% via linkage with HDD. One in ten was ascertained via the urinary glucose strip test. The remaining cases were ascertained through a combination of self-report and linkage with HDD (18.0%) and a minority via both self-report and the urinary glucose strip test (1.6%) or all three ascertainment sources (0.1%) (data not shown).

#### **Verification procedure**

For 2048 (89.5%) of ascertained diabetes cases, we were able to send questionnaires to the GP. For 190 (8.3%) ascertained diabetes cases in the Prospect cohort we



**Table 1.** Baseline characteristics of the EPIC-NL cohort\*

Age, median (range)	Years	51 (20-70)
Sex	Male	10,260 (25.6)
	Female	29,751 (74.4)
Education	High	8095 (20.4)
	Middle	15,761 (39.7)
	Low	15,844 (39.9)
BMI, mean (SD)	Kg/m <sup>2</sup>	25.7 (4.0)

\*n=40,011; Values are expressed as n (%) unless indicated otherwise.

were unable to obtain validation information via the GP. Of 119 (62.6%) of these 190 individuals, current contact details were available for the pharmacists, and therefore questionnaires were sent to their pharmacists (figure 1). Total response rate for the GP and pharmacy questionnaires was 91.5% (94.5% for GP and 71.4% for pharmacy questionnaire). For 306 (13.3%) ascertained diabetes cases it was not possible to verify their diabetes status via GP or pharmacist. Of these individuals, 44 had two or more ascertainment sources indicating presence of diabetes. Consequently, these individuals were defined as probable type 2 diabetes cases (figure 1). Of all verified cases, 95.5% were verified by GP information, 2.3% by pharmacist information and 2.2% by multiple ascertainment information.

**Verified diabetes status**

After verification, 1460 (63.8%) individuals were defined as having type 2 diabetes (definite and probable), 51 (2.2%) as having type 1 diabetes and 21 (0.9%) as having another or unknown type of diabetes. In total, 495 (21.6%) of ascertained diabetes cases were not confirmed to have been diagnosed with diabetes and the remaining 262 (11.5%) were defined as potential, but not verified diabetes cases (table 2, figure 1). Prevalence and incidence of type 2 diabetes increased with age. Compared with younger persons, diagnoses of diabetes were more often confirmed in older persons and presence of diabetes was less often uncertain in older persons (table 2).

Of all ascertained prevalent cases, 104 (13.1%) switched to incident cases after verification, as the GP or pharmacist

reported the diagnosis date of diabetes to be after the inclusion date in the study. Of all incident cases, 68 (4.6%) switched to prevalent cases as the GP or pharmacist reported the diagnosis date of diabetes to be before the inclusion date in the study (data not shown).

**Ascertainment sources**

Cases ascertained via self-report only were often confirmed to have been diagnosed with diabetes by GP or pharmacist (PV+ 85.4%), whereas for cases obtained either by linkage with HDD or urinary glucose strip test, this was a minority (PV+ 39.6 and 22.0% respectively). We found a PV+ of 82.9% for diabetes ascertained via self-report in combination with the urinary glucose strip test, and a PV+ of 96% for diabetes ascertained via both self-report and HDD. The PV+ for diabetes ascertained by self-report (total) and HDD (total) was 87.8 and 73.9% respectively, whereas this was 31.3% for diabetes ascertained through the urinary glucose strip test (total) (table 3). The PV+ was higher for ascertained prevalent diabetes via self-report when they also reported receiving treatment with tablets or insulin (95.3%), as compared with those who reported only following a diet (61.5%) or receiving no treatment (67.6%) for their diabetes.

**DISCUSSION**

In the EPIC-NL cohort, two out of three of 2289 diabetes cases, ascertained via self-report, linkage with HDD and/or a urinary glucose strip test, were confirmed to have been diagnosed with diabetes by their GP or pharmacist. Diabetes ascertained via self-report only or in combination with linkage with HDD was confirmed relatively often.

Several limitations need to be discussed. First, we verified ascertained diabetes against GP information, which cannot be considered the golden standard. However, GPs have a complete overview of the medical status of patients and were therefore considered the best possible option for verification. Second, we did not establish the accuracy of self-reported absence of diabetes, which is equally important for clinical studies. Others reported 0.3 to 5% of self-reported non-diabetic individuals were verified as diabetes cases.<sup>6,8,11,12</sup>

**Table 2.** Verified diabetes status, according to age at baseline among ascertained diabetes cases\*

Age (years)	Verified diabetes status					
	Prevalent type 2 diabetes <sup>#</sup>	Incident type 2 diabetes <sup>#</sup>	Type 1 diabetes	Other / unknown type diabetes	No diabetes	Uncertain
20-39	8 (8.3)	19 (19.8)	11 (11.5)	1 (1.0)	31 (32.3)	26 (27.1)
40-49	42 (13.8)	133 (43.8)	13 (4.2)	6 (2.0)	69 (22.7)	41 (13.5)
50-59	257 (22.6)	467 (40.9)	19 (1.7)	10 (0.9)	260 (22.8)	126 (11.1)
60-70	229 (30.5)	305 (40.7)	8 (1.1)	4 (0.5)	135 (18.0)	69 (9.2)
Total	536 (23.4)	924 (40.4)	51 (2.2)	21 (0.9)	495 (21.6)	262 (11.5)

\*n=2289. Values are expressed as n (%). <sup>#</sup>Definite and probable diabetes cases.

**Table 3.** Verified diabetes status according to ascertainment source, among ascertained diabetes cases, verified by GP or pharmacist\*

Ascertainment source	Verified diabetes status	
	Diabetes	No diabetes
Self-report only	984 (85.4)	168 (14.6)
HDD only	90 (39.6)	137 (60.4)
Glucosuria only	48 (22.0)	170 (78.0)
Self-report + HDD	334 (96.0)	14 (4.0)
Self-report + glucosuria	29 (82.9)	6 (17.1)
Self-report + HDD + glucosuria	3 (100)	0 (0)
Total, self-report <sup>#</sup>	1350 (87.8)	188 (12.2)
Total, HDD <sup>§</sup>	427 (73.9)	151 (26.1)
Total, glucosuria <sup>&amp;</sup>	80 (31.3)	176 (68.7)

HDD = hospital discharge diagnoses; \*n = 1983; values are expressed as n (%); <sup>#</sup>combination of self-report only and self-report + other ascertainment sources; <sup>§</sup>combination of HDD only and HDD + other ascertainment sources; <sup>&</sup>combination of glucosuria only and glucosuria + other ascertainment sources.

Furthermore, presence of diabetes may go undetected for up to 12 years.<sup>21</sup> Verifying non-diabetic individuals would thus include checking diagnoses with GPs and determining fasting glucose values for identification of undetected diabetes of 37,722 participants. This was not feasible in the framework of this study. As a consequence, we could not calculate sensitivities, specificities or kappa values. Yet, we estimated kappa values with percentages of non-diabetic individuals verified as diabetes cases found in literature (0.3 to 5%).<sup>6,8,11,12</sup> This resulted in kappa values of 53 to 83%. Third, we calculated PV+ from our study. As the PV+ is dependent on the diabetes prevalence in the population, we should be cautious in comparing with other studies and generalising our results. Fourth, participants for whom we were unable to verify their diabetes diagnoses were relatively young. These participants may have switched GPs more often because of a more flexible lifestyle. Other factors, such as cohort effects, may also be responsible. Unfortunately, the number of diabetes cases per separate cohort was too small to further examine this.

Another Dutch study that also verified self-reported diabetes against GP information found a PV+ of 73%, and a kappa value of 75%, among 899 hypertensive patients.<sup>6</sup> We observed a higher PV+ (87.7%). However, comparability is complicated by the hypertensive study population and small number of diabetes cases. Others also reported relatively high PV+ values (76 to 95%) when self-reported diabetes was verified against medical records, medical claims or an interview, in apparently healthy or disabled persons.<sup>7-12,22</sup> Kappa values ranged from 72 to 92%. One study reported lower PV+ values in younger persons,<sup>9</sup> which is in line with our study. This was, however, not confirmed elsewhere.<sup>8</sup>

PV+ for ascertainment of diabetes via HDD was relatively low (39.6% for HDD only, 73.8% for total HDD). Coding of HDD from discharge letters has been shown to be reliable,<sup>23</sup> and it is therefore unlikely that errors in coding largely explain this. Another possibility is that additional HDD of diabetes may be the result of temporarily elevated glucose levels, induced by stress caused by the principal disease. This was, however, not confirmed by our data as PV+ were apparently similar for additional and principal HDD of diabetes (data not shown). Another study found a PV+ of 72.3% for HDD-derived diabetes verified against drug treatment data,<sup>13</sup> in line with our findings.

It has often been reported that urinary glucose strip tests are of limited use for detection of diabetes.<sup>24-29</sup> We observed a rather low PV+ of 31.3% for diabetes ascertained via the urinary glucose strip test, which confirms these findings. The PV+ for diabetes ascertained via both urinary glucose strip test and self-report was comparable with the PV+ for self-report only. In contrast, the PV+ for diabetes ascertained via both linkage with HDD and self-report was 10% higher compared with self-report only. This implies the urinary glucose strip test had limited additional value above self-report, whereas linkage with HDD was of additional value for ascertainment of diabetes.

In conclusion, two-thirds of ascertained cases of diabetes in the EPIC-NL cohort were confirmed to have been diagnosed by their GP or pharmacist. Older participants were confirmed relatively often. Ascertainment of diabetes via self-report may give a valid indication of the presence of diabetes. This may be combined with ascertainment via linkage with HDD, to increase validity. However, single reliance on linkage with HDD or reliance on a self-administered urinary glucose strip test is not recommended for ascertainment of diabetes from this study.

#### ACKNOWLEDGEMENTS

The authors would like to thank J.J. Metselaar-van den Bos, E. Wilson, F.L. Büchner and A.G. Niehoff for their contributions to the verification of diabetes cases.

The EPIC-NL study was funded by 'European Commission: Public Health and Consumer Protection Directorate 1993-2004; Research Directorate-General 2005'; Dutch Ministry of Public Health, Welfare and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF) (the Netherlands).

This work was partly supported by the InterAct project, funded by the European Union (Integrated Project LSHM-CT-2006-037197 in the Framework Programme 6 of the European Community).

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The *Netherlands Journal of Medicine* publishes papers in all relevant fields of internal medicine. In addition to reports of original clinical and experimental studies, reviews on topics of interest or importance, case reports, book reviews and letters to the editor are welcomed.

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