Magnesium wasting associated with epidermal-growth-factor receptor-targeting antibodies in colorectal cancer: a prospective study

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Summary

Background Preliminary evidence suggests that magnesium wasting occurs in patients who are treated with epidermal-growth-factor receptor (EGFR)-targeting antibodies for colorectal cancer. The mechanism of this side-effect is unknown, and if all or a subset of patients are affected is also unclear. We aimed to assess the incidence, characteristics, and predictive factors of magnesium wasting during treatment with EGFR-targeting antibodies, and to study the pathophysiology of this phenomenon.

Methods We measured prospectively magnesium concentrations in a cohort of 98 patients with colorectal cancer treated with EGFR-targeting antibodies with or without combined chemotherapy. The primary outcome measure was the slope of the serum magnesium concentrations over time. In 35 patients, 24-h urinary magnesium excretion was measured. In a subset of patients (n=5), an intravenous magnesium load test was done. 16 patients who had chemotherapy alone acted as controls. A clinical protocol was written before initiation of the study, but because this was a non-interventional study, the protocol was not formally registered.

Findings 95 (97%) patients had decreasing serum magnesium concentrations during EGFR-targeting treatment compared with baseline measurements. The mean serum magnesium slope during EGFR-targeting treatment (with or without combined chemotherapy) was significantly lower compared with chemotherapy alone (–0·00157 mmol/L/day, SD 0·00162 [95% CI –0·00191 to –0·00123] vs 0·00014 mmol/L/day, SD 0·00076 [–0·00026 to 0·00055]; t test, p<0·0001). 24-h urine analysis and intravenous magnesium load tests showed a defect in renal magnesium reabsorption.

Interpretation EGFR-inhibiting antibodies compromised the renal magnesium retention capacity, leading to hypomagnesaemia in most patients. Future studies should address the effects of exposure and target affinity. Our study suggests a pivotal role of the EGFR-signalling pathway in regulating magnesium homeostasis.

Introduction Inhibition of the epidermal-growth-factor receptor (EGFR) has become an integral part in the treatment of various cancer types, either as monotherapy or in combination with chemotherapy.1 Cetuximab is a chimeric monoclonal antibody that targets specifically the EGFR with high affinity and competitively inhibits endogenous ligand binding. Matuzumab is a humanised antibody and panitumumab a fully human antibody against the same target. Cetuximab and panitumumab showed good efficacy in the treatment of late-stage colorectal cancer, and all are being tested in the first-line setting in various combinations.

A previous study2 has detailed the onset and clinical course of severe hypomagnesaemia in one patient treated with cetuximab in combination with irinotecan. The researchers identified retrospectively 34 of 154 patients who were treated with cetuximab in their institution and who had serum magnesium concentrations measured at least once. They noted an incidence of grade 3 to 4 hypomagnesaemia in 10 of the 154 patients. Additionally, hypomagnesaemia was reported recently in 38% of patients who were treated with panitumumab, with grade 3 and 4 hypomagnesaemia occurring in 3% of those treated with panitumumab.3 Another retrospective study4 on 48 evaluable patients showed an incidence of grade 3 or 4 hypomagnesaemia in 13 of 48 (27%) patients treated with cetuximab; the researchers suggested an increasing incidence of severe hypomagnesaemia (ie, grade 0: within normal limits; grade 1: from lower limit of normal to 0·5 mmol/L; grade 2: <0·5 mmol/L to 0·4 mmol/L; grade 3: <0·4 mmol/L to 0·3 mmol/L; grade 4: <0·3 mmol/L; severe being grade 3 or 4) with treatment duration.

We aimed to assess prospectively the occurrence of magnesium wasting in a consecutive series of patients treated with EGFR-targeting antibodies, and study the characteristics and potential mechanisms of this phenomenon.

Methods

Patients and procedures Between Feb 26, 2003, and Dec 15, 2005, we enrolled consecutive patients with metastatic colorectal cancer for whom treatment with EGFR-targeting antibodies was initiated at the University Hospital Gasthuisberg, Leuven, Belgium. A clinical protocol was written before initiation of the study, but because this was a non-interventional

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study the protocol was not formally registered. Most patients were enrolled in phase I, II, or III trials: EVEREST (irinotecan and cetuximab); CRYSTAL (irinotecan, fluorouracil, and folinic acid [FU/FA], with or without cetuximab); BABEL (irinotecan and cetuximab); MAURICE (irinotecan and matuzumab); SALVAGE (cetuximab monotherapy); ACROBAT (oxaliplatin, FU/FA and cetuximab); and ABX-408 (panitumumab monotherapy). All patients included in these clinical trials gave written informed consent for repetitive blood monitoring.

Serum magnesium concentrations were assessed at baseline (ie, within 1 week before start of treatment with EGFR-targeting antibodies) and every 2 weeks. Patients who did not have at least two serum magnesium measurements due to short treatment duration or missing data were excluded from the analysis. In a subset of patients, concentrations of serum parathyroid hormone (PTH) were measured at baseline and during hypocalcaemia. Clinical data, such as use of diuretics and response to treatment, were recorded by ST, PP, CV, and EVC. Adverse events, including hypomagnesaemia, hypocalcaemia, diarrhoea, asthenia, and skin toxicity, were graded according to common toxicity criteria (CTC) version 2.5

24-h urine collections were not preplanned, but were obtained from 15 patients at baseline and in 35 patients on treatment. Urinary magnesium excretion was measured and the fractional excretion of magnesium (FEMg) was calculated as follows: FEMg=[U[Mg]×P[Cr]×100]/(0.7×S[Mg]×U[Cr]), where U, P, and S refer to the urine, plasma, and serum concentrations of magnesium (Mg) and creatinine (Cr).6

In five patients who were treated with cetuximab and in one control, an intravenous magnesium load test was done during treatment according to Walder and colleagues:7 patient 1 (control) at 30 weeks after start of chemotherapy (without cetuximab); patient 2 after 25 weeks of cetuximab; patient 3 after 14 weeks of cetuximab; patient 4 after 67 weeks of cetuximab; patient 5 after 28 weeks of cetuximab; and patient 6 after 26 weeks of cetuximab. Patients provided written informed consent for this additional test to be done. We infused 73·89 mg/kg magnesium sulphate over 2 h, and collected blood and urine samples 30 min before, every 30 min during, and 30 min after the intravenous infusion. We measured plasma and urine magnesium, calcium, and creatinine concentrations. We calculated the ultrafiltrable filtration fraction of plasma magnesium as 0·7×plasma [Mg] and creatinine (Cr).8

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All electrolytes were assessed according to standard operating procedures at our institution. More specifically, serum and urinary total magnesium and serum total cal-
Cation concentrations were measured by use of a colorimetric assay in the Modular P analyzer (Roche, Basel, Switzerland). Serum calcium concentrations were corrected for hypoalbuminemia.

Statistical analysis
For the assessment of the changes in serum magnesium, a two-stage analysis was used. In the first stage, a curve-fitting analysis for linear, polynomial, and logarithmic models was done. A linear regression analysis was fitted to the data of each participant separately. The dependent variable was the change in serum magnesium concentration from baseline. The independent variable was the time since start of treatment. In the second stage, the slopes obtained in the first stage were compared between groups with Student’s t tests, with correction for differences in variances, where appropriate. All models were checked for validity of the underlying normality assumptions. A similar approach was used with only three serum magnesium values: at baseline, week 4, and week 8. A Kaplan-Meier analysis was used to study the distribution of the time until development of hypomagnesaemia. Multiple regression analysis with backward elimination procedure was done with the slope as the dependent variable with age, sex, use of diuretics, maximum grade of diarrhoea, maximum grade of asthenia, maximum grade of skin rash, maintenance dosage (for cetuximab), total treatment duration, and baseline serum magnesium and creatinine concentrations as covariates. Given the sample size, this number of covariates is not prone to over-fitting. Tests for collinearity and interactions were done. A Cox regression model with backward Wald stepwise elimination procedure for time to development of grade 1 hypomagnesaemia was built by use of the same clinical covariates as in the multiple regression analysis. The proportionality assumption was tested. Statistical analysis was done by use of SPSS for Windows 13.0. All tests were done at the 5% level of significance.

Role of the funding source
The sponsor of the study had no role in the study design, conduct of the study, data collection, data management, data analysis, data interpretation, preparation of the report, review of the report, or approval of the report. ST, HP, and EVC had full access to all of the raw data and had final responsibility to submit for publication.

Results
Table 1 shows the characteristics of the 98 patients who had colorectal cancer and who were treated with EGFR-targeting antibodies.
targeted treatment. The median interval between serum magnesium sampling was 1–8 weeks (range 0–6–11–7).

To study the concentrations of serum magnesium over time, we fitted the data to linear and logarithmic models. For each patient, a linear-fit analysis of the serum magnesium concentrations during anti-EGFR treatment showed good correlation coefficients. The mean adjusted $R^2$ was 0.773 (SD 0.241). Only four participants had $R^2$ values below 0.3; in these participants, outlying aberrant data points were responsible for the poor correlation coefficient. A curve-estimation regression for polynomial or logarithmic models did not yield better results than the linear model. The slopes of the change from baseline of serum magnesium concentrations had a normal distribution with large interindividual variability (mean $-0.00157$ mmol/L/day, SD $0.00162$, range $0.00225$ to $-0.00757$). This variability is depicted in the wide array of linear models in the patient group (figure 1). Importantly, for 95 of 98 (97% of the patients), a progressive magnesium loss was noted.

We tested the effectiveness of three early time points (weeks 0, 4, and 8) in characterising the magnesium slope for a given patient for two reasons: first, to provide a simplified approach; and second, to see if early characterisation of the rate of magnesium wasting was possible. The linearity of the slopes calculated on the whole dataset predicts that early characterisation would be feasible. This is indeed what we found; we noted a good correlation between the slope that was calculated with the entire dataset and the slope calculated with three early time points. This might be useful in clinical practice. The comparison between the two methods is illustrated in the webfigure.

The median time to onset of hypomagnesaemia (ie, magnesium concentration below the lower limit of normal) was 99 days (SD 126, range 12–639). According to the Kaplan-Meier analysis, the estimated proportion of patients with normal serum magnesium concentrations (ie, $>0.65$ mmol/L) was 46% (95% CI 34–58) after 6 months of treatment and 26% (11–41) after 1 year (figure 2). The incidence of hypomagnesaemia according to treatment duration is given in table 2.

In nine evaluable patients who continued to be followed in our institution, rapid recovery of serum magnesium concentrations occurred in the absence of magnesium substitution after treatment discontinuation. Resolution of hypomagnesaemia to normal values was seen at the first post-treatment assessment (30 days after the end of treatment) for five patients with grade 1 hypomagnesaemia, whereas resolution to normal values in three patients with grade 4 hypomagnesaemia took between 60 days and 90 days after the end of treatment.

By assessment of patients in the control group of the CRYSTAL trial (5-FU/FA/irinotecan, with or without cetuximab; n=6) and other patients treated with 5-FU/FA/irinotecan or oxaliplatin (n=10), we were able to study the effect of cetuximab on serum magnesium concentration slopes, controlling for the effect of chemotherapy. The mean concentration of serum magnesium slope during EGFR-targeting antibody treatment (with or without combined chemotherapy) was significantly lower compared with those seen in patients treated with chemotherapy alone ($-0.00157$ mmol/L/day, SD $0.00162$, 95% CI $-0.00191$ to $-0.00123$) vs $0.00014$ mmol/L/day, SD $0.00076$; $t=4.1, p<0.0001$; figure 3).

In univariate analysis, the serum magnesium concentration slope was inversely correlated with age (Pearson’s correlation; $P=-0.276; p=0.0088$) and baseline serum magnesium concentrations (Pearson’s correlation;
we found a very weak correlation between serum magnesium concentration slope and serum calcium concentration slope during the treatment ($R^2=0.052, p=0.047$). Appearance of any grade of hypocalcaemia was restricted to hypomagnesaemia of grade 2 or higher. Although not planned, for six patients, PTH concentrations were noted at baseline and during hypocalcaemia. All baseline measurements were within normal ranges (ie, PTH 3–40 ng/L). During hypomagnesaemia, PTH values remained in the normal range, but in the setting of accompanying hypocalcaemia, these values were inappropriately low (patient 1: baseline PTH 14.4 ng/L, during grade 4 hypomagnesaemia and grade 3 hypocalcaemia PTH 14.4 ng/L; patient 2: baseline 11.3 ng/L, during grade 3 hypomagnesaemia and grade 2 hypocalcaemia 31.9 ng/L; patient 3: baseline 23.1 ng/L, during grade 4 hypomagnesaemia and grade 3 hypocalcaemia 23.6 ng/L; patient 4: baseline 23.1 ng/L, during grade 3 hypomagnesaemia and grade 2 hypocalcaemia 37.9 ng/L; patient 5: baseline PTH 12.1 ng/L, during grade 2 hypomagnesaemia and grade 1 hypocalcaemia 2.7 ng/L; patient 6: baseline 3.2 ng/L, during grade 2 hypomagnesaemia and grade 1 hypocalcaemia 3.4 ng/L).

Patients with grade 3 and 4 hypomagnesaemia experienced severe fatigue, cramps, and somnolence. Mental alteration was reported in one patient. No seizures were recorded. However, symptoms related to hypomagnesaemia were not assessed with a validated questionnaire in our study. Oral magnesium substitution with once daily 162 mg magnesium was initiated in 30 patients with grade 1 or 2 hypomagnesaemia, but was generally badly tolerated and resulted in poor therapeutic compliance. Nine more severely affected patients with grade 2 (n=3) and grade 3 or 4 (n=6) hypomagnesaemia were treated by weekly intravenous infusion of 3 g magnesium sulphate for the remaining duration of treatment. Seven patients with at least four consecutive serum magnesium concentration data points before and on substitution with intravenous magnesium sulphate were used to assess the effect of intravenous supplementation on magnesium wasting. We did not find a significant difference between the serum magnesium concentration slopes before and on substitution ($-0.00158$ mmol/L/day, $SD 0.00138$ vs $-0.00180$ mmol/L/day, $SD 0.00211$, respectively).

In one patient who had been hospitalised for grade 4 hypomagnesaemia, serum magnesium concentrations were measured shortly after five daily intravenous magnesium infusions. In this patient, we documented that the benefit of substitution with 3 g intravenous magnesium sulphate lasted only 48 h because of ongoing renal loss.
antibodies. A progressive decrease in serum magnesium concentrations was seen in 97% of patients after initiation of treatment. Both the rate of magnesium loss and the duration of treatment will decide the time of occurrence of hypomagnesaemia during treatment with EGFR-targeting antibodies. This finding explains why current reports underestimate the incidence of magnesium wasting, because they concentrate only on patients who are overtly hypomagnesaemic, i.e., the more severely affected patients who had time to develop hypomagnesaemia during the relatively short treatment intervals.2,4 The decrease of serum magnesium concentrations in patients who were treated with EGFR-inhibiting antibodies, with or without chemotherapy, was significantly different from those in patients who had received chemotherapy alone. Chemotherapy has been associated with shifts in erythrocyte cellular-to-plasma-magnesium ratios.9 However, in our series, we can conclude that magnesium wasting is specifically due to EGFR inhibition. This finding is confirmed by the rapid normalisation of serum magnesium concentrations on discontinuation of EGFR inhibition, while in most cases, patients went on to receive more chemotherapy. Magnesium loss was reported with all tested monoclonal antibodies against EGFR, suggesting a class effect. However, the incidence and severity might vary between products. As our patient series was heterogeneous, we cannot characterise possible pharmacokinetic differences between various EGFR-inhibitory compounds nor provide a comprehensive assessment of the expected incidence of hypomagnesaemia for all of these compounds. Future studies should address the effects of dose, exposure, and affinity for the target. Whether small molecule inhibitors of the EGFR kinase induce magnesium wasting, and to what extent, is unknown.

The linearity of the serum magnesium concentration slope suggests that the rate of magnesium loss for an individual patient is constant. However, a high interindividual variability exists in the rate of magnesium wasting. We did a multivariate analysis to assess potential variables affecting the slope of magnesium loss. Increasing age was a variable associated with more severe magnesium wasting. Ageing is associated with a progressive decline in renal function and the development of glomerulosclerosis and interstitial fibrosis, which might enhance magnesium wasting.10 Also, higher baseline serum magnesium concentrations were associated with steeper slopes. This might indicate baseline differences in magnesium regulation, which affect the propensity to magnesium wasting during EGFR-targeted treatment. Total treatment duration, which is decided by disease control and any toxic effects, was found to be inversely correlated to the severity of magnesium wasting. The high interindividual variability in the rate of magnesium wasting is strikingly similar to that in the heterogeneous development of skin toxicity reported in patients during EGFR inhibition. Both these phenomena could be explained by germline variations in genes encoding for proteins involved in the EGFR signalling pathway.

In healthy individuals, serum magnesium concentrations are tightly controlled and vary between 0.70 mmol/L and 1.10 mmol/L.11 Magnesium is ultrafiltered in the glomeruli. Subsequently, 70% of magnesium is reabsorbed passively in the thick ascending loop through paracellin 1. In the presence of hypomagnesaemia, the kidney aims at preserving magnesium reserves by lowering fractional excretions below 0.5–1%, with the normomagnesaemic range being 3–5% as shown by our data and those of Schlingmann and colleagues.12 This additional active reabsorption of up to 10% can take place in the distal convoluted tubule through the activity of the transient receptor potential cation channel TRPM6.13 In our patients, decreased gastrointestinal uptake was not studied, however, defective TRPM6 intestinal activity might have a role in triggering magnesium depletion. TRPM6 is expressed predominantly in mouse kidney, colon, caecum, and lung. Dietary magnesium concentrations affect TRPM6 mRNA levels, suggesting regulatory feedback loops.14 We found clear evidence of defective renal magnesium handling. The fractional excretion of magnesium in the urine was inappropriately high in the setting of serum hypomagnesaemia. To further elucidate this loss of adaptive magnesium reabsorption, we used the intravenous magnesium load test, which allows quantification of renal magnesium leak thresholds and differentiation between causative defects located in the thick ascending loop (paracellin 1) or distal convoluted tubule (TRPM6). In our patients, the magnesium load test revealed strongly lowered leak thresholds, comparable with those seen in patients with hereditary loss of function mutations in the TRPM6 gene (hypomagnesaemia with secondary hypocalcaemia [HSH]).15 Calciuria increased normally, implying intact paracellin 1 function at the level of the thick ascending loop. Patients with lower serum magnesium concentrations at the time of the test had a significantly lower threshold. This suggests a tight causal relation between the altered renal leak threshold and serum magnesium steady-state levels. Unexplained, however, is the continuous decrease of the serum magnesium concentrations in our patients, suggesting a progressive downward resetting of the leak threshold. This could suggest a progressive effect of EGFR inhibition on the proteins involved in renal magnesium retention at the level of the distal convoluted tubule, such as TRPM6. TRPM6 is expressed along the entire gastrointestinal tract and in the kidney, mainly in the distal convoluted tubule.16–18 EGFR is expressed in the same regions of the kidney and EGFR-kinase activity has been shown to be necessary for correct membranous localisation and activity of related transient receptor potential
How loss of EGFR activity could progressively impair correct TRPM6 localisation or function is unclear. Alternatively the inhibition of TRPM6 is immediate and complete, but compensatory mechanisms fail progressively. Interestingly, EGFR is known to have a role in kidney cell viability and repair after injury. A decreased rate in cell turnover or increase in apoptosis could be involved in the progressive worsening. However, after discontinuation of EGFR inhibition a rapid recovery is seen, suggesting a dynamic inhibition and excluding extensive cell injury. Researchers have reported that vascular endothelial growth factor (VEGF) can regulate intracellular magnesium concentrations through phosphatidylinositol-3 kinase (PI3K)-dependent signalling. Additionally, magnesium-induced chemotaxis of human umbilical vein endothelial cells (HUVEC) was also shown to be dependent on protein kinase C activity and protein tyrosine phosphorylation. These findings suggest tyrosine kinase activity might be involved in regulating magnesium homeostasis.

We have clearly established magnesium wasting as a generalised side-effect of EGFR-targeting antibodies. The proportion of patients showing symptoms related to hypomagnesaemia in our cohort was low. We think that reporting the symptoms we noted in this heavily pretreated cancer population might not accurately reflect the proportion of hypomagnesaemia as the cause of these symptoms. Prospective collection of symptoms in patients with cancer who are untreated and treated with EGFR inhibitors by use of validated questionnaires is warranted. Additionally, patients with advanced cancer might sometimes have subclinical hypomagnesaemia.

In the setting of increasing treatment durations, early identification of those patients that are at increased risk is important. We show this might be achieved by assessing the serum magnesium concentration slope using values obtained at week 0, 4, and 8. Careful monitoring of serum magnesium is warranted during the duration of treatment because symptoms of hypomagnesaemia can evenly remain unrecognised. Cardiac abnormalities related to hypomagnesaemia can be present in patients without any clinical symptoms but with grade 2 hypomagnesaemia or worse.

The clinician should be aware of these issues and should weigh up the risks and benefits carefully when prescribing drugs known to prolong the QT interval. Also, patients receiving combination treatments that are nephrotoxic and potential magnesium-wasting agents, such as cisplatin, require special attention. In our series, we confirm that hypocalcaemia was associated with hypomagnesaemia concentrations of grade 2 ($<0.50$ mmol/L) or higher. Hypocalcaemia responded to correction of magnesium concentrations and resolved after discontinuation of EGFR-targeting antibodies. PTH was not assessed in all patients. The relation between hypomagnesaemia and PTH resistance or suppression has already been discussed in the published studies. Concentrations of immunoreactive PTH in most patients with hypomagnesaemia or hypocalcaemia have been either low or normal indicating inappropriately low PTH secretion. We found that a weekly regimen of intravenous magnesium substitution could not correct serum magnesium depletion during treatment, or even stop the depletion. Magnesium loss through the kidney precluded retention of the intravenous magnesium substitution for more than 48 h in severely affected patients. Daily intravenous supplementation, however, was effective in the acute setting in our patients, as previously suggested by Fakih and co-workers.

No dose decreases of EGFR-targeting antibodies were done on the basis of hypomagnesaemia. Oral substitution in patients with hypomagnesaemia is currently cumbersome and relatively inefficient. This might lead to treatment discontinuation in severely affected patients. Magnesium absorption in the gut is partially mediated by TRPM6. We are currently doing a prospective randomised trial comparing low-dose and high-dose oral magnesium substitution in patients treated with cetuximab. We expect diarrhea to remain a problem with all magnesium salt substitutions, and evidence exists in patients who have congenital-TRPM6 deficiencies that only high-dose oral substitution can stabilise concentrations of serum magnesium.

**Contributors**

ST had responsibility for the integrity of the data and the accuracy of the data analysis. ST, HP, and EVC were responsible for the study concept and design, and drafted the report. ST, PP, CV, and EVC were responsible for data collection. ST, HP, PP, KC, JGH, and EVC did the data analysis and interpretation. ST, HP, and EVC drafted the report. All authors revised the report. HP did the statistical analysis. ST, HP, KC, PP, CV, and EVC were involved with administrative, technical, or material support. ST and EVC were responsible for study supervision.

**Conflicts of interest**

The authors declared no conflicts of interest. ST and EVC are Senior Clinical Investigators of the Fund for Scientific Research—Flanders (Belgium; FWO); PP is a recipient of a Clinical Research Mandate of the Centre Hospitalier Universitaire de Liège. Merck and Amgen have provided research funding, not related to this study, to the Digestive Oncology Unit, University Hospital Gasthuisberg, Leuven.

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