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Screening for malnutrition in patients with neuroendocrine tumours

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ABSTRACT

Objectives: To investigate whether screening for malnutrition using the validated malnutrition universal screening tool (MUST) identifies specific characteristics of patients at risk, in patients with neuroendocrine tumours (NET)

Design: Cross-sectional study

Setting: University Hospitals Coventry & Warwickshire NHS Trust; European Neuroendocrine Tumour Society (ENETS) Centre of Excellence

Participants: Patients with confirmed NET (n = 178) of varying primary tumour sites, functioning status, grading, staging and treatment modalities.

Main outcome measure: To identify disease and treatment-related characteristics of patients with NET who score using MUST and should be directed to detailed nutritional assessment.

Results: MUST score was positive (≥1) in 14% of outpatients with NET. MUST-positive patients had lower faecal elastase concentrations compared to MUST negative patients (244 ± 37 vs 389 ± 19 µg/g stool; p = 0.011). MUST positive patients were more likely to be on treatment with long acting somatostatin analogues, as compared with MUST negative NET patients (65 vs 41%, p = 0.028); and showed higher plasma somatostatin concentrations (751 ± 681 vs 51 ± 3 pmol/l, p = 0.017). MUST positive patients were also more likely to have rectal or unknown primary NET, whereas frequencies of pancreatic, midgut and gastric NET, and goblet cell tumours of the appendix were comparable between MUST positive and MUST negative patients (all p > 0.2).

Conclusions: Given the high frequency of positive MUST scores in our large and diverse NET cohort and the clinical implications of detecting malnutrition early, we recommend routine use of malnutrition screening in all patients with NET and particularly in patients who are treated with long acting somatostatin analogues.

Strenghts and limitations of this study

- This study investigates the important clinical problem of malnutrition screening in patients with neuroendocrine tumours (NET).
- This is the first study to systematically investigate the tumour-specific characteristics and treatment modalities associated with malnutrition risk in patients with NET.
- Strengths of the study include the relatively large size of a well characterised diverse cohort of patients with NET, including information about tumour grading, staging, functional status, biomarkers and treatment modalities.
- Limitations include the observational, real-world nature of this study, with attendant limitations on availability of data subsets and power in regression analyses.

INTRODUCTION

Malnutrition is caused by insufficient delivery of nutrients or increased catabolism and is linked to major negative outcomes including excess morbidity, mortality and higher treatment costs [1-4]. The prevalence of malnutrition in cancer patients has been reported to range between 30 and 70%, depending on tumour type, stage and treatment modalities [5], but might be different in patients with neuroendocrine tumours (NET).

NET comprise a complex group of often slow growing neoplasms that are derived from primitive endocrine and nervous cells. The annual incidence of NET has recently tripled to 40-50 cases per million, which is thought to be at least in part related to increased awareness and improved diagnostic modalities [6]. Malnutrition in patients with NET might be frequent for various reasons which include functioning tumours producing hormones that affect gut transit[7-9], pancreatic masses, tumour infiltration of the mesentery in midgut NET[10 11], prior abdominal surgery, or treatment with somatostatin analogues [12-14].

The National Institute for Health and Care Excellence (NICE) recommends malnutrition screening in all adult inpatients and outpatients in at risk groups [2]. Several screening tools have been developed of varying complexity [5]. The malabsorption universal screening tool (MUST) is one of the more commonly used screening methods in NHS Trusts, due to its simplicity and previous validation in multiple settings which includes cancer patients [2 15 16] (figure 1). However, the potential utility of malnutrition screening in patients with NET has not been reported to date.

Here, we investigated in a large cohort of 178 patients with confirmed NET of varying primary tumour sites, grading, staging, functioning status and treatment modalities, to assess we could identify NET patients at risk of malnutrition using MUST, and whether MUST positive patients showed specific disease or treatment related characteristics.

MATERIALS AND METHODS

Participants and sample collection

The University Hospitals Coventry and Warwickshire NHS Trust (UHCW) audit department approved the study (audit number 1133/2015; July 2015). Data was obtained from the local database at the ARDEN NET centre, European Neuroendocrine Tumour Society (ENETS) Centre of Excellence (CoE) in UHCW. All patients with gastro-entero-pancreatic NET and available information regarding MUST were eligible for inclusion.

Patients with NET were characterised according to age, gender, anthropometric data, the location of the primary tumour, staging, histological grading (surgical sample or diagnostic biopsy), presence or absence of functional symptoms (i.e. flushing or diarrhoea), treatment modalities received, for example treatment with somatostatin analogues, information about prior abdominal surgery for any reason, and NET related biomarkers (most recent overnight fasted gut hormone profile from within the previous 6 months including Chromogranin A, Chromogranin B, gastrin, vasoactive intestinal polypeptide (VIP), somatostatin, glucagon and pancreatic polypeptide; and 24-hour urine 5-hydroxyindoleacetic acid (5-HIAA) concentrations sampled with the usual dietary precautions). Further characteristics such as biomarkers for screening for exocrine pancreatic insufficiency or heart failure were available if measured for clinical reasons.

Measurement of biomarkers

Routine biochemical markers were performed in the Biochemistry laboratory at UHCW. Plasma gut hormone profiles were sampled after at least 10 hours of overnight fast. Analyses were performed by radioimmunoassay at Hammersmith Hospital. Samples for 24-hour urine were obtained following restriction of known factors that can cause false high measurements of urinary 5-HIAA. Analyses were performed using HPLC at Heartlands Hospital,

Birmingham. Faecal elastase-1 concentration in stools was determined using an enzyme-linked immunosorbent assay (ScheBo® Pancreatic Elastase-1 Stool Test), measured at City Hosptial, Birmingham, UK. The human faecal elastase-1 antibody used here is immunologically specific and is not affected by enzyme replacement therapies.

Statistical analyses

Anthropometric data were presented as mean ± SD, all other data as mean ± SE. Normal distribution was assessed using Kolmogorov-Smirnov test. Ordinal data were correlated using Spearman's analyses to assess the associations between variables. Due to the relatively small number of patients in the respective sub-groups, all MUST positive patients were pooled for comparison with MUST negative patients. Backward stepwise binary logistic regression analyses were used to assess the influence of variables on MUST score (positive vs negative) as the dependent variable, with models tested using NET related characteristics such as location of the primary tumour, tumour grading and staging, functioning status, treatment with somatostatin analogues, NET related biomarkers (normal or pathological), and prior abdominal surgery as independent variables; and simultaneous adjustment for age, gender and serum creatinine. A p value < 0.05 was considered statistically significant. Data analyses were performed using IBM SPSS Statistics version 22 (Chicago, Illinois).

RESULTS

Baseline characteristics

MUST data were available in n = 178 patients of the NET cohort in the ARDEN NET centre. The cohort comprised 79 males and 99 females. Age was 63.6 ± 15.6 years, body weight 70 ± 18.7 kg, body height 167 ± 11 cm, BMI 26.9 ± 6.5 kg/m^2, and serum creatinine 84.3 ± 3.2 µmol/l. Previous abdominal surgery for any reason (NET related and not NET related) was performed in n = 103 (58%) of the patients. Histological grading was available in n = 139 (78.1%) of the patients, if performed for clinical reasons. Out of the n = 139 patients with histological grading, n = 88 (49.4%) had a G1 NET, n = 34 (19.1%) had a G2 NET, and n = 17 (9.6%) had a poorly differentiated (G3) neuroendocrine carcinoma. Out of the 178 NET patients in this cohort, n = 76 (42.7%) were on treatment with somatostatin analogues (Sandostatin LAR 30 mg once monthly; or Somatuline Autogel 120 mg once monthly); of those, n = 42 (55.3%) had a well differentiated midgut NET and n = 12 (15.8%) had a well differentiated pancreatic NET. Further tumour characteristics of the cohort are shown in (figure 2).

Patients scoring positive using MUST

Fourteen percent of the NET patients (n = 25/178) scored ≥1 using MUST, which classifies the patient as “medium risk for malnutrition” and should trigger a recommendation of “further observation” of nutrition status according to BAPEN/NICE guidelines [2 15]; and 5.6% of the NET patients scored ≥ 2 (n = 10/178), which classifies the patient as “high risk for malnutrition” and should trigger treatment of malnutrition and ideally referral to the dieticians or multidisciplinary nutrition team [15].

Correlation analyses

Spearman analyses showed a weak but statistically significant negative correlation of total MUST score with serum creatinine concentrations ($r = -0.1$, $p = 0.04$); and a positive correlation with age ($r = 0.17$, $p < 0.001$). There was a statistically significant negative correlation of total MUST score with faecal elastase levels ($r = -0.28$, $p = 0.009$). Treatment with long acting somatostatin analogues ($r = 0.14$, $p = 0.056$), a combined measure of pathological NET tumours markers in urine and plasma (either raised 5-HIAA or raised markers in the fasted gut hormone profile; $r = 0.15$, $p = 0.06$) and serum BNP concentrations ($r = 0.16$, $p = 0.07$) as a marker for the presence of cardiac involvement tended to show weak correlations with total MUST score. Total MUST score was not correlated with other NET related markers, including functioning status, tumour grade, or tumour stage; and presence of prior abdominal surgery (all $p > 0.20$).

Regression analyses

For the prediction of MUST scores, backward stepwise binary logistic regression analyses with MUST score as the dependent variable (positive vs negative) and age, location of the primary tumour, functioning status, tumour stage, tumour grade, faecal elastase concentrations, and treatment with somatostatin analogues as the independent variables was tested. In step 7 of this stepwise model, only the use of somatostatin analogues remained a statistically significant predictor ($p < 0.001$; chi-square test 9.204, $p = 0.002$), with 100% correct prediction of MUST negative patients, but poor prediction of MUST positive patients. When additionally including the location of the primary tumour and faecal elastase concentrations, again use of somatostatin analogues was a significant predictor of MUST ($p = 0.006$), but not the location of the primary tumour ($p = 0.22$), with faecal elastase concentrations showing a trend ($p = 0.12$). Chi-square test again showed a significant

improvement of the model (20.59, p = 0.024), with 98% correct prediction of MUST negative patients, but correct prediction of MUST positive patients remained low at 25%. The best model included age, location of the primary tumour, tumour stage, functioning status, faecal elastase concentrations and treatment with somatostatin analogues (chi-square 24.014, p = 0.046), with use of somatostatin analogues (p = 0.001) and location of the primary tumour (p < 0.001) as the only significant predictors, 97% correct prediction of MUST negatives and 37.5% correct prediction of MUST positives.

Use of somatostatin analogues (yes vs no) with location of the primary tumour, tumour grade, tumour stage, and functioning status as the independent variables was predicted by location of the primary tumour (p = 0.013), tumour grade (p = 0.003), presence of distant metastases (M1, p = 0.002) and functioning status (p = 0.032), with reasonable correct prediction of both treated (81%) and untreated (83.6%) patients (qui-square 69.154, p < 0.001).

Characteristics of MUST positive compared with MUST negative patients

ANOVA analyses demonstrated specific characteristics of NET patients with a positive as compared with a negative MUST score (table 1). NET patients who scored ≥ 1 using MUST were significantly more often treated with somatostatin analogues as compared to patients who did not score using MUST (64 vs 39%; p = 0.02) and in agreement with this, showed significantly higher plasma somatostatin concentrations (table 1). When stratifying the entire cohort according to treatment with somatostatin analogues, 21.1% (n = 16 of 76 patients) who were treated with somatostatin analogues scored ≥ 1 using MUST, as compared to 8.8% (n = 9 of 102 patients) who were not on treatment with somatostatin analogues (p = 0.02). MUST positive patients also showed significantly lower faecal elastase levels (table 1). Again, in the entire cohort of NET patients, faecal elastase concentrations were also significantly lower in the 76 patients who were on treatment with somatostatin analogues, as

compared with the 102 patients who were not on treatment with somatostatin analogues (329 ± 26 vs 421 ± 23 µg/g stool; p = 0.011). Finally, patients who scored using MUST had significantly more often NET of the rectum or of unknown origin, as compared with NET patients who did not score (figure 3). Frequencies of midgut NET, pancreatic NET, goblet cell tumours of the appendix and gastric NET were not significantly different between MUST positive and negative patients (all p > 0.2; figure 3).

DISCUSSION

Malnutrition is an adjustable risk factor [17], but associated with severe adverse clinical outcomes if not addressed [1 2]. Patients with NET do not typically present with major weight loss or acute illness before reaching the very final stages with extensive metastatic disease or carcinoid heart disease. This is related to the fact that NET are often slower growing and less aggressive tumours at least when well differentiated NET, as compared with other types of cancer [18]. Nevertheless, malnutrition in patients with NET could be present for other reasons, including chronic loose stools, osmotic diarrhoea [18], and excess secretion of serotonin precursors stimulating small bowel motility [7-9]. Pancreatic mass effects, tumour infiltration of the mesentery [10 11] and NET related treatment [12-14] are further potential risk factors.

In our outpatient cohort of patients with various types of NET, 14% had a MUST score of > 1, which should trigger referral to the dietitians and the nutrition support team [2 15]. When comparing the cohort of patients who scored using MUST with the patients who did not score, we identified distinct characteristics of MUST positive patients with NET. MUST positive patients were more likely to have unknown primary or rectal NET, which might be related to delayed diagnosis and widespread disease in these patients. Furthermore, MUST positive patients showed significantly lower faecal elastase concentrations, although the frequency of pancreatic NET was not significantly different between groups, arguing against possible pancreatic mass effects as the main driving factor. Most importantly, MUST positive patients were 1.5-fold more likely to be on treatment with long acting somatostatin analogues and consequently showed significantly higher plasma somatostatin concentrations. This relationship could be causal, related to rapid onset suppression of pancreatic exocrine secretion by somatostatin [19 20] and consequent steatorrhea [21], and reflected by significantly

lower faecal elastase concentrations in the entire cohort of NET patients who were treated with somatostatin.

Further known mechanisms are in agreement with a possible role of somatostatin in conveying malnutrition in patients with NET. Impairment of hepatic bile acid physiology by somatostatin has been reported [22]. Furthermore, intravenous somatostatin inhibits glucose, triglyceride, amino acid, and calcium absorption by direct effects on the intestinal mucosa [12 13 23]; and decreases gastric acid secretion by 90% in healthy volunteers [23]. In addition, suppression of various gut hormones such as cholecystokinin and glucagon like peptide-1 by somatostatin are well described [21 24 25], and diarrhoea, steatorrhea and weight loss are key features of excess hormone producing somatostatinomas [26]. It might be argued that patients who were treated with long acting somatostatin analogues were more prone to score using MUST related to functioning status and advanced disease progression. However, in our analyses only treatment with somatostatin analogues remained a statistically significant predictor of MUST in all tested models, further supporting a potentially causal involvement. The observational nature of this study needs to be mentioned as a limitation, as well as reduced sample sizes when including not routinely measured biomarkers in the regression models. Confirmation of our findings in multicentre settings with access to large and diverse NET patient cohorts will be useful.

In summary, somatostatin analogues are key treatment modalities in patients with well differentiated NET but may cause transient or permanent gastrointestinal side effects such as bloating and cramping in up to 30% of the patients [27-29]; and, based on our findings, appear to predispose to some degree of malnutrition in patients with NET. Without systematically screening NET patients for malnutrition, mild impairment of digestive processes might be missed or attributed to functioning aspects of the NET, rather than recognised and treated as a

possible side effect of the treatment. Referring these patients for early nutritional intervention could lead to improvement of the nutritional status and quality of life [30].

Acknowledgements

Contributors: MOW, NB and SQ were involved in the study concept and design. SK, KG, JHLW, LD, AN, SF, WS and SS were involved in the acquisition of clinical data. CD was involved in the analyses of biochemical markers. SQ, NB, LD, GKD and MOW were involved in the collection of MUST data. MOW and JH did the statistical analyses. MOW and MD supervised the MSc project of SQ. MD provided important intellectual content and critical review of the manuscript. SQ provided input to the first draft of the manuscript. MOW drafted the manuscript, and all authors critically revised it for important intellectual content. MOW supervised the study and is the guarantor.

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Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no competing interests

Ethical approval: The UHCW audit department had approved this study (audit number 1133/2015; July 2015). All study data were accessed using techniques compliant with the Health Insurance Portability and Accountability Act of 1996 (HIPAA), and, because this study involved analysis of pre-existing, de-identified data, it was exempt from institutional review board approval.

Transparency: The lead author (the manuscript's guarantor) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Data sharing: No additional data available.

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Tables

Table 1 Characteristics of MUST positive compared with MUST negative patients with neuroendocrine tumours (NET) of varying primaries, tumour grading, staging and functioning status. Data are given as mean ± SE. SSA, long acting somatostatin analogues; 5-HIAA, 5-hydroxyindoleacid

	MUST positive n = 25	MUST negative n = 153	p value
Treatment with SSA (%)	64 ± 10	39 ± 4	**0.020**
Plasma somatostatin (pmol/l)	751 ± 681	51 ± 3	**0.017**
Faecal elastase (µg/g stool)	244 ± 37	389 ± 19	**0.011**

Figure legends

Figure 1: Simplified scheme of use of the MUST score (adapted from [31]). MUST was positive in 14% of the screened patients (25/178 patients with NET). The majority of the patients with positive MUST scored 1 (n = 15) or 2 (n = 8), mostly related to BMI < 20 kg/m^2 (n = 18) and/or, less frequently, recent weight loss (n = 9). Only n = 2 of the patients had a MUST score of ≥ 3.

Figure 2: Characteristics of the NET cohort. (A) location of the primary tumour, (B) tumour staging, (C) histological grading (well differentiated, grade 1 and 2; poorly differentiated, grade 3)

Figure 3: MUST positive compared with MUST negative patients with NET. Patients who scored using MUST were significantly more likely to have rectum NET ($p < 0.05$) or a NET with an unknown primary ($p < 0.01$). Other types of NET were not significantly different between MUST positive and MUST negative patients (all $p > 0.2$). Black bars: MUST positive patients; grey bars: MUST negative patients. pNET, pancreatic neuroendocrine tumour

Figure 1

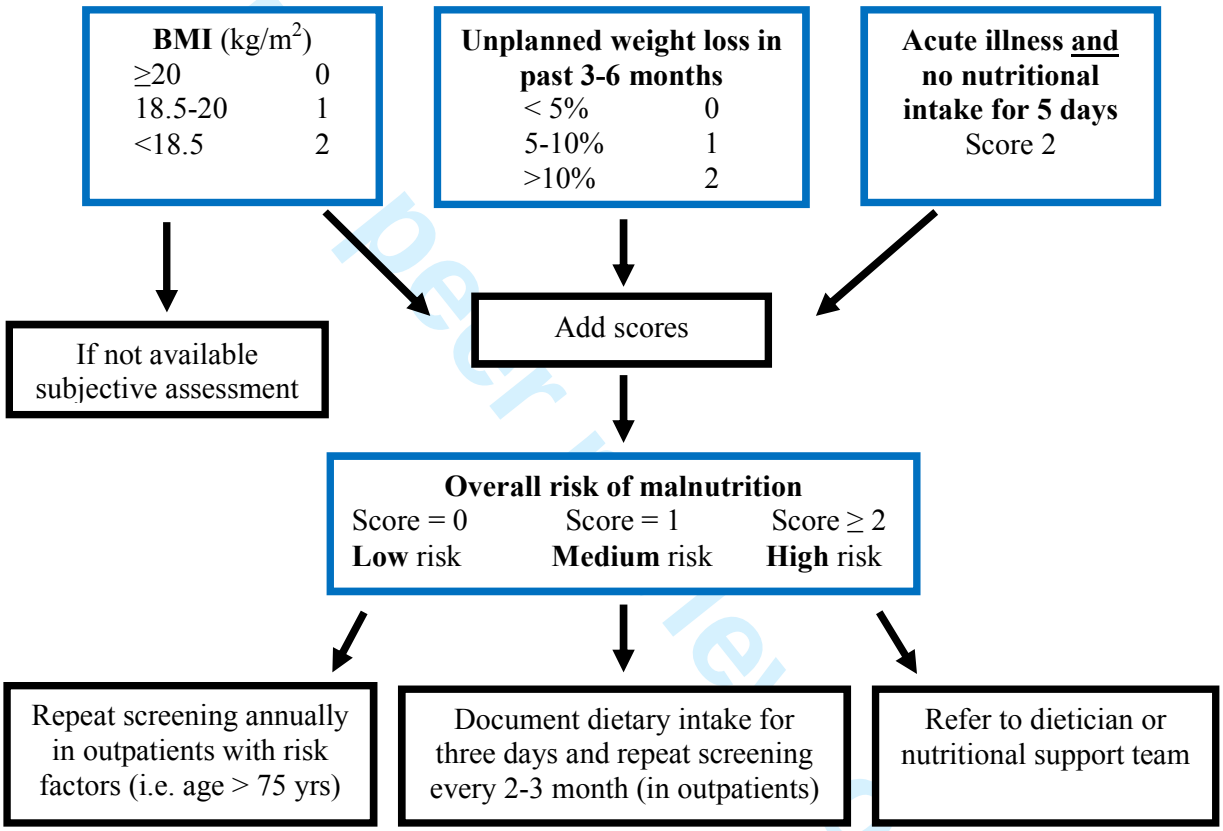


Figure 2

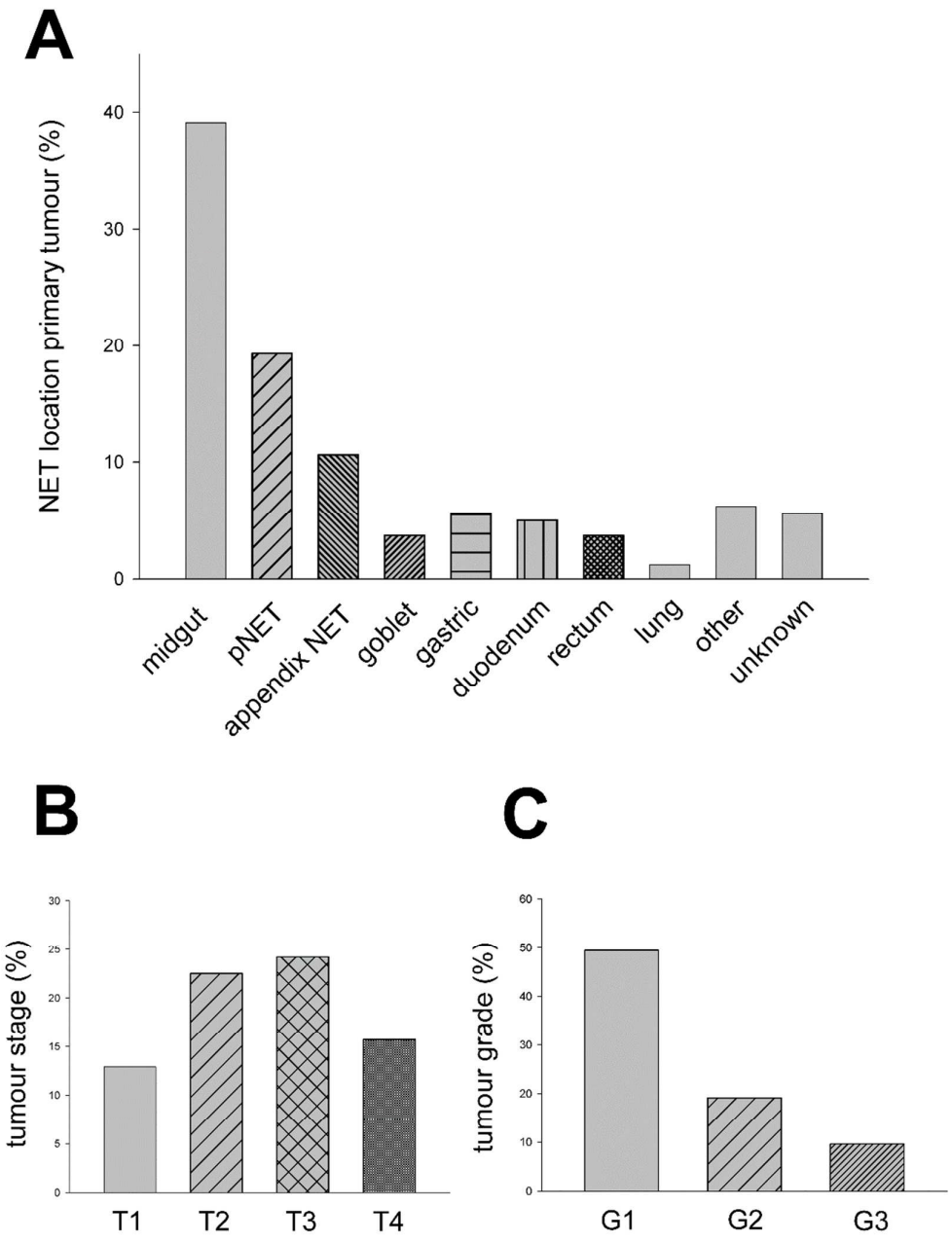
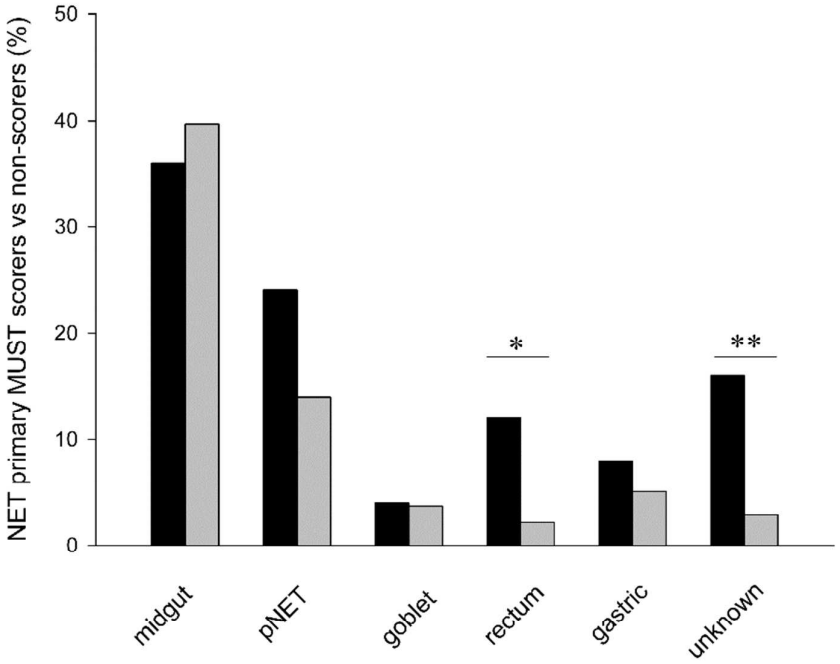


Figure 3



STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	p 2	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
	p 2	(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	p 4	Explain the scientific background and rationale for the investigation being reported
Objectives	p 4	State specific objectives, including any prespecified hypotheses
Methods		
Study design	p 5-6	Present key elements of study design early in the paper
Setting	p 5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	p 5	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	p 5	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	p 5 - 6	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	p 6	Describe any efforts to address potential sources of bias
Study size	p 5	Explain how the study size was arrived at
Quantitative variables	p 6	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	p 6	(*a*) Describe all statistical methods, including those used to control for confounding
	p 6	(*b*) Describe any methods used to examine subgroups and interactions
	p 5, 6	(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants	p 7	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	p 7; 9-10; figures 2 and 3	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	p 7; 10; table 1; figure 3	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	p 7 – 10; table 1; figure 3	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	p 9 -10	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	p 11	Summarise key results with reference to study objectives
Limitations	p 3; 12	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	p 11 - 12	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	p 12-13	Discuss the generalisability (external validity) of the study results
Other information		
Funding	p 14	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Screening for malnutrition in patients with gastro-entero-pancreatic neuroendocrine tumours - a cross-sectional study

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Screening for malnutrition in patients with gastro-entero-pancreatic neuroendocrine tumours – a cross-sectional study

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ABSTRACT

Objectives: To investigate whether screening for malnutrition using the validated malnutrition universal screening tool (MUST) identifies specific characteristics of patients at risk, in patients with gastro-entero-pancreatic neuroendocrine tumours (GEP-NET)

Design: Cross-sectional study

Setting: University Hospitals Coventry & Warwickshire NHS Trust; European Neuroendocrine Tumour Society (ENETS) Centre of Excellence

Participants: Patients with confirmed GEP-NET (n = 161) of varying primary tumour sites, functioning status, grading, staging and treatment modalities.

Main outcome measure: To identify disease and treatment-related characteristics of patients with GEP-NET who score using MUST and should be directed to detailed nutritional assessment.

Results: MUST score was positive (≥1) in 14% of outpatients with GEP-NET. MUST-positive patients had lower faecal elastase concentrations compared to MUST negative patients (244 ± 37 vs 383 ± 20 µg/g stool; p = 0.018) and were more likely to be on treatment with long acting somatostatin analogues (65 vs 38%, p = 0.021). MUST positive patients were also more likely to have rectal or unknown primary NET, whereas frequencies of other GEP-NET including pancreatic NET were comparable between MUST positive and MUST negative patients.

Conclusions: Given the frequency of patients identified at malnutrition risk using MUST in our relatively large and diverse GEP-NET cohort and the clinical implications of detecting malnutrition early, we recommend routine use of malnutrition screening in all patients with GEP-NET and particularly in patients who are treated with long acting somatostatin analogues.

Strenghts and limitations of this study

- This study investigates the important clinical problem of malnutrition screening in patients with gastro-entero-pancreatic neuroendocrine tumours (GEP-NET).
- Possible implications of the use of somatostatin analogues on the risk of malnutrition in patients with GEP-NET have not been reported in previous studies.
- Strengths of the study include the relatively large size of a well characterised diverse cohort of patients with GEP-NET, including information about tumour grading, staging, functioning status, biomarkers and treatment modalities.
- Limitations include the observational, real-world nature of this study, with attendant limitations on availability of data subsets and power in regression analyses.

INTRODUCTION

Malnutrition is caused by insufficient delivery of nutrients or increased catabolism and is linked to major negative outcomes including excess morbidity, mortality and higher treatment costs [1-4]. The prevalence of malnutrition in cancer patients has been reported to range between 30 and 70%, depending on tumour type, stage and treatment modalities [5], but might be different in patients with gastro-entero-pancreatic neuroendocrine tumours (GEP-NET). GEP-NET comprise a complex group of often slow growing neoplasms that are derived from primitive endocrine and neural cells. The annual incidence of GEP-NET has recently tripled to 40-50 cases per million, which is thought to be at least in part related to increased awareness and improved diagnostic modalities [6]. Malnutrition in patients with GEP-NET might be frequent for various reasons which include functioning tumours producing hormones that affect gut transit[7-9], pancreatic masses, tumour infiltration of the mesentery in midgut NET[10 11], prior abdominal surgery, or treatment with somatostatin analogues [12-14]. The National Institute for Health and Care Excellence (NICE) recommends malnutrition screening in all adult inpatients and outpatients in at risk groups [2]. Several screening tools have been developed of varying complexity [5]. The malabsorption universal screening tool (MUST) is one of the more commonly used screening methods in UK NHS Trusts, due to its simplicity and previous validation in multiple settings including use in cancer patients [2 15 16] (figure 1). However, the potential utility of malnutrition screening in patients with GEP-NET was only reported in a single very recent study to date [17].

Here, we explored in a cohort of 161 patients with confirmed GEP-NET of varying primary tumour sites, grading, staging, functioning status and treatment modalities, the prevalence of malnutrition, and whether MUST positive GEP-NET patients showed specific disease or treatment related characteristics.

MATERIALS AND METHODS

Participants and sample collection

The University Hospitals Coventry and Warwickshire NHS Trust (UHCW) audit department approved the study (audit number 1133/2015; July 2015). Data were obtained from the local database at the ARDEN NET centre, European Neuroendocrine Tumour Society (ENETS) Centre of Excellence (CoE) in UHCW. All patients with GEP-NET who attend their routine clinical appointments in the ARDEN NET Centre are screened using MUST since May 2015; and were eligible for inclusion.

Patients had physical examination as part of routine clinical care and were characterised according to age, gender, body weight, body height, body mass index (BMI), the location of the primary tumour, staging, histological grading (surgical sample or diagnostic biopsy), presence or absence of functioning symptoms (i.e. flushing or diarrhoea), treatment modalities received, for example treatment with somatostatin analogues, information about prior abdominal surgery GEP-NET related or for any reason, and GEP-NET related biomarkers (most recent overnight fasted gut hormone profile from within the previous 6 months including Chromogranin A; other biomarkers such as Chromogranin B, gastrin, vasoactive intestinal polypeptide (VIP), somatostatin, glucagon and pancreatic polypeptide were available but only used for clinical decision making when appropriate. Samples for 24-hour urine were obtained following restriction of known factors that can cause false high measurements of urinary 5-HIAA. Further characteristics such as biomarkers for screening for exocrine pancreatic insufficiency or heart failure were used if available for clinical reasons. Using the available data a MUST score was calculated. A simplified scheme of the use of the MUST score is depicted in (figure 1).

Measurement of biomarkers

Routine biochemical markers were performed in the Biochemistry laboratory at UHCW. Plasma gut hormone profiles were sampled after at least 10 hours of overnight fast. Analyses were performed by radioimmunoassay at Hammersmith Hospital. Analyses for 5-HIAA were performed using HPLC at Heartlands Hospital, Birmingham. Faecal elastase-1 concentration in stools was determined using an enzyme-linked immunosorbent assay (ScheBo® Pancreatic Elastase-1 Stool Test), measured at City Hospital, Birmingham, UK. The human faecal elastase-1 antibody used here is immunologically specific and is not affected by enzyme replacement therapies. A faecal elastase concentration < 200 mcg/g stool indicates moderate and a concentration < 100 mcg/g stool indicates severe exocrine pancreatic insufficiency.

Statistical analyses

Data are presented as mean ± SE. Metric values were tested for normal distribution using the Kolmogorov-Smirnov test and further analysed using the paired t test. Non-normally distributed metric variables and ordinally scaled variables were analysed using the Mann-Whitney U test. For nominally scaled variables, Chi-square tests were applied. Ordinal data were correlated using Spearman's analyses (based on 1000 bootstrap samples) to assess the associations between variables. Due to the relatively small number of patients in the respective sub-groups, all MUST positive patients were pooled for comparison with MUST negative patients. Breusch Pagan test and auxiliary regressions were used to investigate for heteroscedasticity and significant relationships between fitted predicted values and squared residuals. Bootstrapped ordinal regression analyses (set as 1000 bootstrap samples) were performed with MUST score (positive versus negative) as the dependent variable and age, tumour stage, prior abdominal surgery (separately for any prior abdominal surgery or GEP-NET related surgery, i.e. ileocaecal resection or right hemicolectomy), functioning status,

treatment and duration of treatment (in month) with somatostatin analogues and GEP-NET related biomarkers (normal or pathological) as the independent variables, based on biological plausibility to potentially cause malabsorption; bootstrapped p-values are provided. Backward stepwise binary logistic regression analyses were additionally used to assess the influence of individual variables on MUST score. A p value < 0.05 was considered statistically significant. Data analyses were performed using IBM SPSS Statistics version 22 (Chicago, Illinois).

RESULTS

Baseline characteristics

MUST data were available in n = 161 patients of the GEP-NET cohort in the ARDEN NET centre. The cohort comprised 74 males and 87 females. Age was 63.2 ± 1.2 years, body weight 75 ± 1.4 kg, body height 167 ± 0.01 cm, BMI 26.7 ± 0.4 kg/m^2, and serum creatinine 85.1 ± 3.5 µmol/l. Previous abdominal surgery for any reason had been performed in n = 96 (59.6%) of the patients. Previous ileocaecal resection was done in n = 17 (10.6%) and right hemicolectomy in n = 14 (8.7%) of the patients. Histological grading was available in n = 133 (82.6%) of the patients, if performed for clinical reasons; of those, n = 86 (64.7%) had a grade 1 well differentiated (G1) GEP-NET, n = 32 (24.1%) had a grade 2 well differentiated (G2) GEP-NET, and n = 15 (11.3%) had a poorly differentiated (G3) neuroendocrine carcinoma of gastro-entero-pancreatic origin. Out of the 161 NET patients in this cohort, n = 67 (41.6%) were on treatment with somatostatin analogues (Sandostatin LAR 30 mg once monthly; or Somatuline Autogel 120 mg once monthly); of those, n = 43 (65.2%) had a well differentiated midgut NET and n = 14 (21.2%) had a well differentiated pancreatic NET. Mean duration of treatment with somatostatin analogues in the n = 67 treated patients was 19.5 ± 3.3 month at the time of data collection. Out of the 15 patients in the GEP NET cohort with pathological faecal elastase concentrations (< 200 mcg/g stool; 96.1 ± 18.9 mcg/g stool), n = 12 had previous abdominal surgery (any), n = 7 had a pNET, n = 3 had previous ileocaecal resection and n = 1 had previous right hemicolectomy. Further tumour characteristics of the cohort are shown in (figure 2 a, b and c).

Patients scoring positive using MUST

Fourteen percent of the GEP-NET patients (n = 23/161) scored ≥1 using MUST, which classifies the patient as “medium risk for malnutrition” and should trigger a recommendation

of "further observation" of nutrition status according to BAPEN/NICE guidelines [2 15]; and 5.5% of the patients scored ≥ 2 (n = 9/161), which classifies the patient as "high risk for malnutrition" and should trigger treatment of malnutrition and ideally referral to the dieticians or multidisciplinary nutrition team [15].

Correlation analyses

Spearman analyses (based on 1000 bootstrap samples) showed a moderate but statistically significant negative correlation of total MUST score (positive vs negative) with faecal elastase concentrations (r = - 0.32, p = 0.005) and a weak but statistically significant positive correlation with treatment with somatostatin analogues (r = 0.20, p = 0.013). Duration of treatment with somatostatin analogues showed a weak trend with total MUST score (r = 0.14; p = 0.078). None of the remaining markers significantly correlated with MUST total score in bootstrapped regression analyses, which included age, gender, functioning status, tumour grade, tumour stage, biomarkers in blood and urine, serum creatinine, BNP, prior abdominal surgery (any), and prior ileocaecal resection or right hemicolectomy (all p > 0.13).

Regression analyses

Bootstrapped binary logistic regression analyses in the complete model identified use of somatostatin analogues [exp(B) = 0.022; 95% CI (0.000; 1.554); p = 0.004], faecal elastase concentrations [exp(B) = 0.992; 95% CI (0.984; 1.001); p = 0.009)], age [exp(B) = 0.901; 95% CI (0.781; 1.038; p = 0.010] and tumour stage [≥ T2; p < 0.044); and presence of distant metastatic disease M1 (p = 0.037); but not N1] as significant predictors of MUST score. The regression model was statistically significant (chi-square 26.58; p = 0.046). The model explained 57% (Nagelkerke R^2) of the variance in MUST and correctly identified 95.5% of MUST negative and 44.4% of MUST positive subjects.

To obtain additional information about the influence of individual dependent variables, additional backward stepwise binary logistic regression analyses were tested. In step 6 of this stepwise model, again 44.4% percent of MUST positive patients and 97% of MUST negative patients were correctly identified, with use of somatostatin analogues, but not other factors including the duration of treatment with somatostatin analogues remaining a statistically significant predictor ($p < 0.001$; chi-square test 21.98, $p = 0.009$) and explaining 49% (Nagelkerke R^2) of the variance in MUST.

Use of somatostatin analogues (yes vs no) in bootstrapped analyses with location of the primary tumour, tumour grade, tumour stage, and functioning status as the independent variables was predicted by functioning status ($p = 0.011$), tumour grade ($p < 0.032$) and presence of distant metastases (M1, $p = 0.035$), with location of the primary tumour showing a trend. The model explained 55% of the variance in use of somatostatin analogues and reasonably correctly predicted both treated (84.2%) and untreated (78.3%) patients (chi-square 66.35, $p < 0.001$).

Characteristics of MUST positive compared with MUST negative patients

Specific characteristics of GEP-NET patients with a positive as compared with a negative MUST scores are shown in (table 1). GEP-NET patients who scored ≥ 1 using MUST were significantly more frequently treated with somatostatin analogues as compared to patients who did not score using MUST (65 vs 38%; $p = 0.021$) (table 1).

When stratifying the entire cohort according to treatment with somatostatin analogues, 22.4% (n = 15 of 67 patients) who were treated with somatostatin analogues scored ≥ 1 using MUST, as compared to 8.5% (n = 8 of 94 patients) who were not on treatment with somatostatin analogues ($p = 0.013$). MUST positive patients showed significantly lower faecal elastase levels, as compared with MUST negative patients (table 1). Faecal elastase

concentrations also tended to be lower in patients who were on treatment with somatostatin analogues, as compared with patients who were not on treatment with somatostatin analogues (335 ± 26 vs 402 ± 27 µg/g stool; p = 0.075). Finally, patients who scored using MUST had significantly more often NET of the rectum or of unknown origin, as compared with patients who did not score (figure 3). Frequencies of midgut NET (p = 0.688), pancreatic NET (p = 0.195) and gastric NET (p = 0.443) were not significantly different between MUST positive and negative patients (figure 3).

DISCUSSION

Malnutrition is an adjustable risk factor [18], but associated with severe adverse clinical outcomes if not addressed [1 2]. Patients with GEP-NET do not typically present with major weight loss or acute illness before reaching the very final stages with extensive metastatic disease or carcinoid heart disease. This is related to the fact that GEP-NET are often slower growing and less aggressive tumours at least when well differentiated, as compared with other types of cancer [19]. Nevertheless, malnutrition in patients with GEP-NET could be present for other reasons, including chronic loose stools, osmotic diarrhoea [19], and excess secretion of serotonin precursors stimulating small bowel motility [7-9]. Pancreatic mass effects, tumour infiltration of the mesentery [10 11] and GEP-NET related treatment [12-14] are further potential risk factors.

In our outpatient cohort of patients with various types of GEP-NET, 14% had a MUST score of > 1, which should trigger referral to the dietitians and the nutrition support team [2 15]. When comparing the cohort of patients who scored using MUST with the patients who did not score, we identified distinct characteristics of MUST positive patients with GEP-NET. MUST positive patients were more likely to have unknown primary or rectal NET, which might be related to delayed diagnosis and widespread disease in these patients. Furthermore, MUST positive patients showed significantly lower faecal elastase concentrations, although the frequency of pancreatic NET was not significantly different between groups, arguing against possible pancreatic mass effects as the main driving factor. Most importantly, MUST positive patients were some 2-fold more likely to be on treatment with long acting somatostatin analogues. This relationship could be causal, related to rapid onset suppression of pancreatic exocrine secretion by somatostatin analogues [20 21] and consequent steatorrhea [22]. Further known mechanisms are in agreement with a possible role of somatostatin analogues

in conveying malnutrition in patients with GEP-NET. Impairment of hepatic bile acid physiology by somatostatin analogues has been reported [23]. Furthermore, intravenous somatostatin inhibits glucose, triglyceride, amino acid, and calcium absorption by direct effects on the intestinal mucosa [12 13 24]; and decreases gastric acid secretion by 90% in healthy volunteers [24]. In addition, suppression of various gut hormones such as cholecystokinin and glucagon like peptide-1 by somatostatin analogues are well described [22 25 26], and diarrhoea, steatorrhea and weight loss are key features of excess hormone producing somatostatinomas [27]. Possible implications of treatment with somatostatin analogues on other aspects such as loss of fat soluble vitamins in the faeces have been also reported [28]. It might be argued that patients who were treated with long acting somatostatin analogues were more prone to score using MUST related to functioning status and advanced disease progression, as well as general risk factors such as age and disease related depression. However, treatment with somatostatin analogues remained a statistically significant predictor of MUST in all tested models, further supporting a potentially causal involvement.

Our observed total rate of patients at risk of malnutrition was somewhat lower than the prevalence very recently reported by Maasberg and colleagues, in a neuroendocrine cohort of comparable size [17]. Authors identified some 21-25% of the patients at risk [17], as compared to 14% in our study; however, the cohort in the mentioned study comprised 87% inpatients and also included patients with neuroendocrine tumours of the lung, as compared to our study which was exclusively assessed in outpatients with GEP-NET. This may explain the lower frequency of patients at risk of malnutrition in our cohort.

The observational nature of this study needs to be mentioned as a limitation, as well as relatively small sample sizes when including not routinely measured biomarkers in the regression models. Our study confirms the importance of screening for malnutrition in patients with GEP-NET. This is directly clinically relevant, considering that malnutrition in

patients with neuroendocrine tumours could be an independent prognostic factor [17]. Confirmation of our findings in multicentre settings with access to large and diverse GEP-NET patient cohorts will be useful.

In summary, somatostatin analogues are key treatment modalities in patients with well differentiated GEP-NET but may cause transient or permanent gastrointestinal side effects such as bloating and cramping in up to 30% of the patients [29-31]; and, based on our findings, appear to increase malnutrition risk as identified by MUST. Without systematically screening GEP-NET patients for malnutrition, mild impairment of digestive processes might be missed or attributed to functioning aspects of the GEP-NET, rather than recognised and treated as a possible side effect of the treatment. Referring these patients for early nutritional intervention could lead to improvement of the nutritional status and quality of life [32].

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Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no competing interests

Ethical approval: The UHCW audit department had approved this study (audit number 1133/2015; July 2015). All study data were accessed using techniques compliant with the Health Insurance Portability and Accountability Act of 1996 (HIPAA), and, because this study involved analysis of pre-existing, de-identified data, it was exempt from institutional review board approval.

Transparency: The lead author (the manuscript's guarantor) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Data sharing: No additional data available.

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Tables

Table 1 Characteristics of MUST positive compared with MUST negative patients with gastro-entero-pancreatic neuroendocrine tumours (GEP-NET) of varying primaries, tumour grading, staging and functioning status. Data are given as mean ± SE. SSA, long acting somatostatin analogues

	MUST positive n = 23	MUST negative n = 138	p value
Treatment with SSA (%)	65	38	**0.021**
Faecal elastase (μg/g stool)	244 ± 37	383 ± 20	**0.018**

Figure legends

Figure 1: Simplified scheme of use of the MUST score (adapted from [33]). MUST was positive in 14.2% of the screened patients (23/161 patients with GEP-NET). The majority of the patients with positive MUST scored 1 (n = 14) or 2 (n = 7), mostly related to BMI < 20 kg/m^2 (n = 16) and/or, less frequently, recent weight loss (n = 9). Only n = 2 of the patients in the entire cohort had a MUST score of ≥ 3.

Figure 2: Characteristics of the GEP-NET cohort. (A) location of the primary tumour, (B) distribution of tumour staging, with the remaining 11.2% of the patients being classified as Tx (no signs of primary tumour), (C) histological grading (well differentiated, grade 1 and 2; poorly differentiated, grade 3).

Figure 3: MUST positive compared with MUST negative patients with GEP-NET. Patients who scored using MUST were significantly more likely to have rectum NET ($p < 0.017$) or a NET with an unknown primary ($p < 0.017$). Other types of NET were not significantly different between MUST positive and MUST negative patients, which included pancreatic NET ($p = 0.195$). Black bars: MUST positive patients; grey bars: MUST negative patients. pNET, pancreatic neuroendocrine tumour

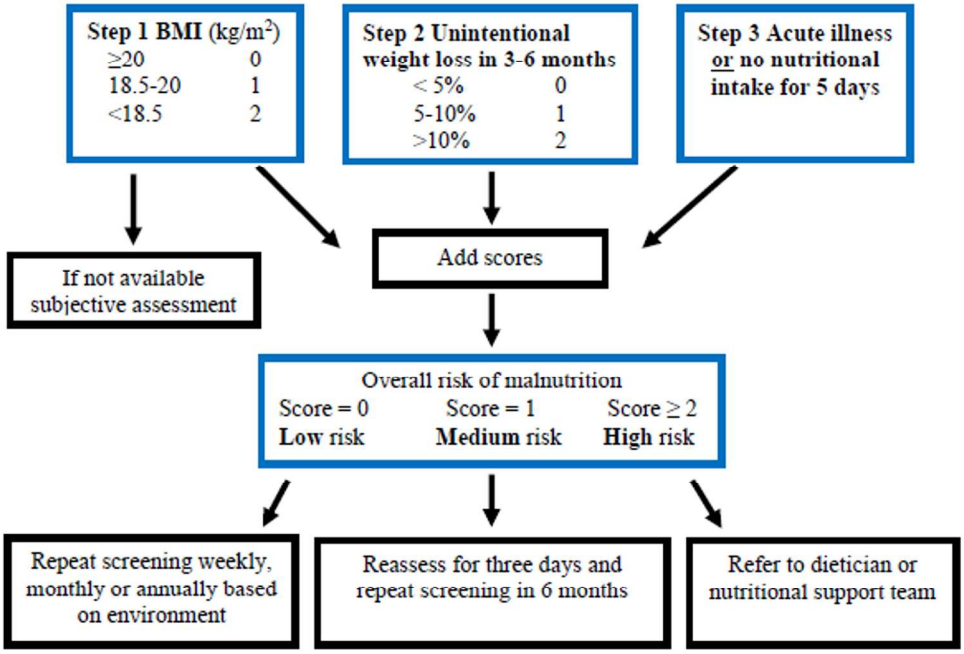

Figure 1: Simplified scheme of the use of the MUST score

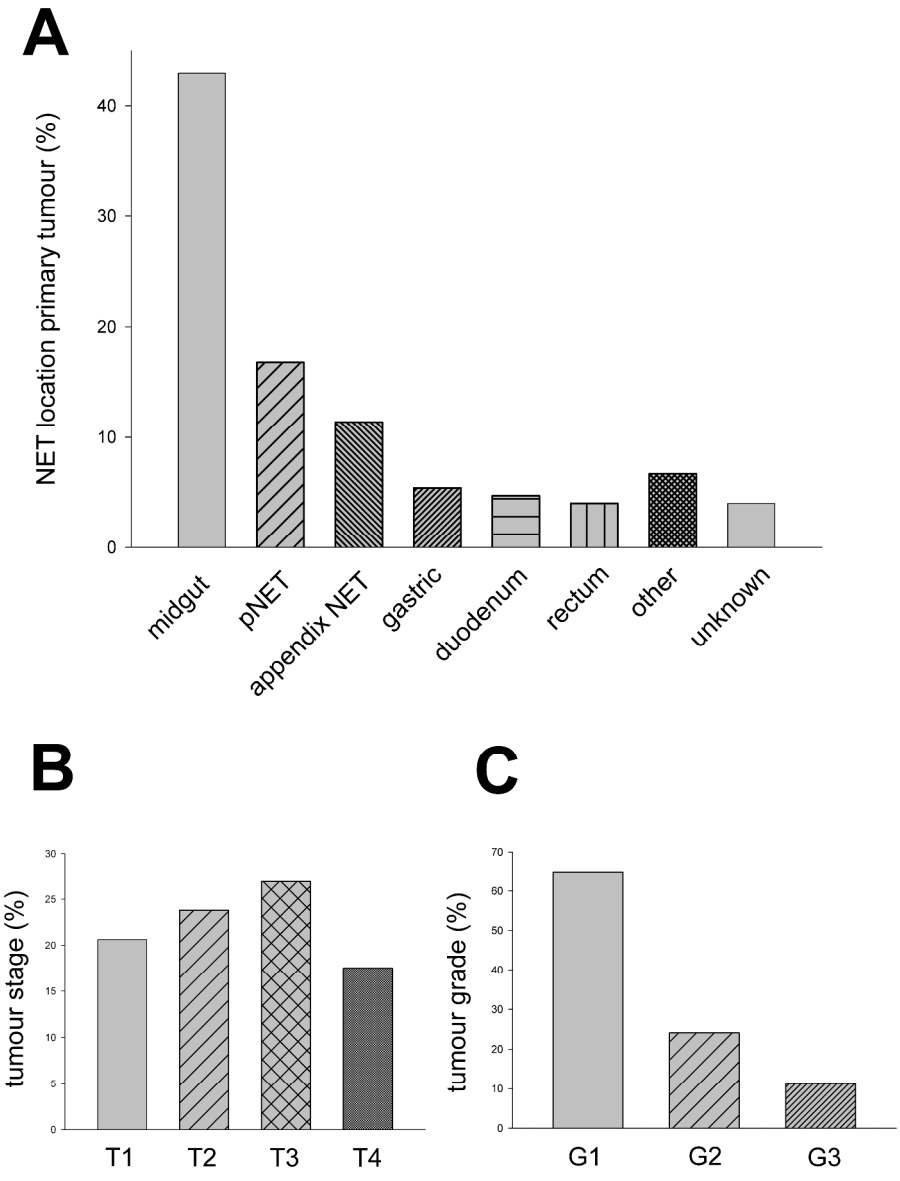

Figure 2: Characteristics of the GEP-NET cohort

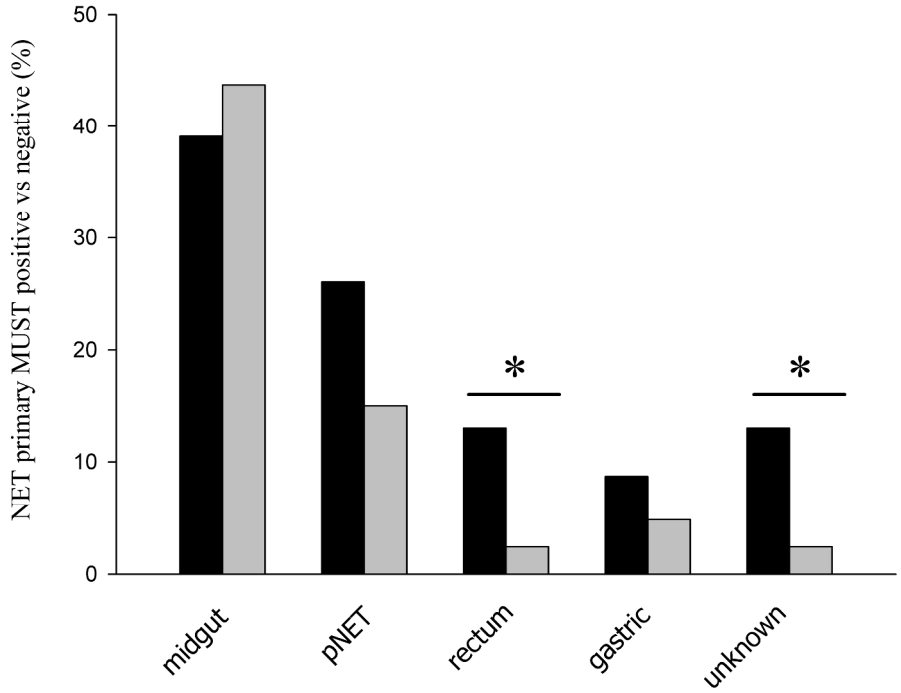

Figure 3: MUST positive compared with MUST negative patients with GEP-NET

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	p 3	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
	p 3	(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	p 5	Explain the scientific background and rationale for the investigation being reported
Objectives	p 5	State specific objectives, including any prespecified hypotheses
Methods		
Study design	p 5-6	Present key elements of study design early in the paper
Setting	p 6	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	p 6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	p 3	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	p 6	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	p 6	Describe any efforts to address potential sources of bias
Study size	p 6	Explain how the study size was arrived at
Quantitative variables	p 6	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	p 7-8	(*a*) Describe all statistical methods, including those used to control for confounding
	p 7	(*b*) Describe any methods used to examine subgroups and interactions
	p 6, 7-8	(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants	p 6	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	p 6; 9-12; figures 2 and 3	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	p 3; 9-12; table 1; figure 3	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	p 9 – 12; table 1; figure 3	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	p 9 -12	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	p 9-12	Summarise key results with reference to study objectives
Limitations	p 3; 14	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	p 13 - 15	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	p 14-15	Discuss the generalisability (external validity) of the study results
Other information		
Funding	p 16	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Screening for malnutrition in patients with gastro-entero-pancreatic neuroendocrine tumours - a cross-sectional study

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Screening for malnutrition in patients with gastro-entero-pancreatic neuroendocrine tumours – a cross-sectional study

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ABSTRACT

Objectives: To investigate whether screening for malnutrition using the validated malnutrition universal screening tool (MUST) identifies specific characteristics of patients at risk, in patients with gastro-entero-pancreatic neuroendocrine tumours (GEP-NET)

Design: Cross-sectional study

Setting: University Hospitals Coventry & Warwickshire NHS Trust; European Neuroendocrine Tumour Society (ENETS) Centre of Excellence

Participants: Patients with confirmed GEP-NET (n = 161) of varying primary tumour sites, functioning status, grading, staging and treatment modalities.

Main outcome measure: To identify disease and treatment-related characteristics of patients with GEP-NET who score using MUST and should be directed to detailed nutritional assessment.

Results: MUST score was positive (≥1) in 14% of outpatients with GEP-NET. MUST-positive patients had lower faecal elastase concentrations compared to MUST negative patients (244 ± 37 vs 383 ± 20 µg/g stool; p = 0.018) and were more likely to be on treatment with long acting somatostatin analogues (65 vs 38%, p = 0.021). MUST positive patients were also more likely to have rectal or unknown primary NET, whereas frequencies of other GEP-NET including pancreatic NET were comparable between MUST positive and MUST negative patients.

Conclusions: Given the frequency of patients identified at malnutrition risk using MUST in our relatively large and diverse GEP-NET cohort and the clinical implications of detecting malnutrition early, we recommend routine use of malnutrition screening in all patients with GEP-NET and particularly in patients who are treated with long acting somatostatin analogues.

Strenghts and limitations of this study

- This study investigates the important clinical problem of malnutrition screening in patients with gastro-entero-pancreatic neuroendocrine tumours (GEP-NET).
- Possible implications of the use of somatostatin analogues on the risk of malnutrition in patients with GEP-NET have not been reported in previous studies.
- Strengths of the study include the relatively large size of a well characterised diverse cohort of patients with GEP-NET, including information about tumour grading, staging, functioning status, biomarkers and treatment modalities.
- Limitations include the observational, real-world nature of this study, with attendant limitations on availability of data subsets and power in regression analyses.

INTRODUCTION

Malnutrition is caused by insufficient delivery of nutrients or increased catabolism and is linked to major negative outcomes including excess morbidity, mortality and higher treatment costs [1-4]. The prevalence of malnutrition in cancer patients has been reported to range between 30 and 70%, depending on tumour type, stage and treatment modalities [5], but might be different in patients with gastro-entero-pancreatic neuroendocrine tumours (GEP-NET). GEP-NET comprise a complex group of often slow growing neoplasms that are derived from primitive endocrine and neural cells. The annual incidence of GEP-NET has recently tripled to 40-50 cases per million, which is thought to be at least in part related to increased awareness and improved diagnostic modalities [6]. Malnutrition in patients with GEP-NET might be frequent for various reasons which include functioning tumours producing hormones that affect gut transit[7-9], pancreatic masses, tumour infiltration of the mesentery in midgut NET[10 11], prior abdominal surgery, or treatment with somatostatin analogues [12-14]. The National Institute for Health and Care Excellence (NICE) recommends malnutrition screening in all adult inpatients and outpatients in at risk groups [2]. Several screening tools have been developed of varying complexity [5]. The malabsorption universal screening tool (MUST) is one of the more commonly used screening methods in UK NHS Trusts, due to its simplicity and previous validation in multiple settings including use in cancer patients [2 15-17] (figure 1). However, the potential utility of malnutrition screening in patients with GEP-NET was only reported in a single very recent study to date [18].

Here, we explored in a cohort of 161 patients with confirmed GEP-NET of varying primary tumour sites, grading, staging, functioning status and treatment modalities, the prevalence of malnutrition, and whether MUST positive GEP-NET patients showed specific disease or treatment related characteristics.

MATERIALS AND METHODS

Participants and sample collection

The University Hospitals Coventry and Warwickshire NHS Trust (UHCW) audit department approved the study (audit number 1133/2015; July 2015). Data were obtained from the local database at the ARDEN NET centre, European Neuroendocrine Tumour Society (ENETS) Centre of Excellence (CoE) in UHCW. All patients with GEP-NET who attend their routine clinical appointments in the ARDEN NET Centre are screened using MUST since May 2015; and were eligible for inclusion.

Patients had physical examination as part of routine clinical care and were characterised according to age, gender, body weight, body height, body mass index (BMI), the location of the primary tumour, staging, histological grading (surgical sample or diagnostic biopsy), presence or absence of functioning symptoms (i.e. flushing or diarrhoea), treatment modalities received, for example treatment with somatostatin analogues, information about prior abdominal surgery GEP-NET related or for any reason, and GEP-NET related biomarkers (most recent overnight fasted gut hormone profile from within the previous 6 months including Chromogranin A; other biomarkers such as Chromogranin B, gastrin, vasoactive intestinal polypeptide (VIP), somatostatin, glucagon and pancreatic polypeptide were available but only used for clinical decision making when appropriate. Samples for 24-hour urine were obtained following restriction of known factors that can cause false high measurements of urinary 5-HIAA. Further characteristics such as biomarkers for screening for exocrine pancreatic insufficiency or heart failure were used if available for clinical reasons. Using the available data a MUST score was calculated. A simplified scheme of the use of the MUST score is depicted in (figure 1).

Measurement of biomarkers

Routine biochemical markers were performed in the Biochemistry laboratory at UHCW. Plasma gut hormone profiles were sampled after at least 10 hours of overnight fast. Analyses were performed by radioimmunoassay at Hammersmith Hospital. Analyses for 5-HIAA were performed using HPLC at Heartlands Hospital, Birmingham. Faecal elastase-1 concentration in stools was determined using an enzyme-linked immunosorbent assay (ScheBo® Pancreatic Elastase-1 Stool Test), measured at City Hospital, Birmingham, UK. The human faecal elastase-1 antibody used here is immunologically specific and is not affected by enzyme replacement therapies. A faecal elastase concentration < 200 mcg/g stool indicates moderate and a concentration < 100 mcg/g stool indicates severe exocrine pancreatic insufficiency.

Statistical analyses

Data are presented as mean ± SE. Metric values were tested for normal distribution using the Kolmogorov-Smirnov test and further analysed using the paired t test. Non-normally distributed metric variables and ordinally scaled variables were analysed using the Mann-Whitney U test. For nominally scaled variables, Chi-square tests were applied. Ordinal data were correlated using Spearman's analyses (based on 1000 bootstrap samples) to assess the associations between variables. Due to the relatively small number of patients in the respective sub-groups, all MUST positive patients were pooled for comparison with MUST negative patients. Breusch Pagan test and auxiliary regressions were used to investigate for heteroscedasticity and significant relationships between fitted predicted values and squared residuals. Bootstrapped ordinal regression analyses (set as 1000 bootstrap samples) were performed with MUST score (positive versus negative) as the dependent variable and age, tumour stage, prior abdominal surgery (separately for any prior abdominal surgery or GEP-NET related surgery, i.e. ileocaecal resection or right hemicolectomy), functioning status,

treatment and duration of treatment (in month) with somatostatin analogues and GEP-NET related biomarkers (normal or pathological) as the independent variables, based on biological plausibility to potentially cause malabsorption; bootstrapped p-values are provided. Backward stepwise binary logistic regression analyses were additionally used to assess the influence of individual variables on MUST score. A p value < 0.05 was considered statistically significant. Data analyses were performed using IBM SPSS Statistics version 22 (Chicago, Illinois).

RESULTS

Baseline characteristics

MUST data were available in n = 161 patients of the GEP-NET cohort in the ARDEN NET centre. The cohort comprised 74 males and 87 females. Age was 63.2 ± 1.2 years, body weight 75 ± 1.4 kg, body height 167 ± 0.01 cm, BMI 26.7 ± 0.4 kg/m^2, and serum creatinine 85.1 ± 3.5 µmol/l. Previous abdominal surgery for any reason had been performed in n = 96 (59.6%) of the patients. Previous ileocaecal resection was done in n = 17 (10.6%) and right hemicolectomy in n = 14 (8.7%) of the patients. Histological grading was available in n = 133 (82.6%) of the patients, if performed for clinical reasons; of those, n = 86 (64.7%) had a grade 1 well differentiated (G1) GEP-NET, n = 32 (24.1%) had a grade 2 well differentiated (G2) GEP-NET, and n = 15 (11.3%) had a poorly differentiated (G3) neuroendocrine carcinoma of gastro-entero-pancreatic origin. Out of the 161 NET patients in this cohort, n = 67 (41.6%) were on treatment with somatostatin analogues (Sandostatin LAR 30 mg once monthly; or Somatuline Autogel 120 mg once monthly); of those, n = 43 (65.2%) had a well differentiated midgut NET and n = 14 (21.2%) had a well differentiated pancreatic NET. Mean duration of treatment with somatostatin analogues in the n = 67 treated patients was 19.5 ± 3.3 month at the time of data collection. Out of the 15 patients in the GEP NET cohort with pathological faecal elastase concentrations (< 200 mcg/g stool; 96.1 ± 18.9 mcg/g stool), n = 12 had previous abdominal surgery (any), n = 7 had a pNET, n = 3 had previous ileocaecal resection and n = 1 had previous right hemicolectomy. Further tumour characteristics of the cohort are shown in (figure 2 a, b and c).

Patients scoring positive using MUST

Fourteen percent of the GEP-NET patients (n = 23/161) scored ≥1 using MUST, which classifies the patient as “medium risk for malnutrition” and should trigger a recommendation

of "further observation" of nutrition status according to BAPEN/NICE guidelines [2 16]; and 5.5% of the patients scored ≥ 2 (n = 9/161), which classifies the patient as "high risk for malnutrition" and should trigger treatment of malnutrition and ideally referral to the dieticians or multidisciplinary nutrition team [16].

Correlation analyses

Spearman analyses (based on 1000 bootstrap samples) showed a moderate but statistically significant negative correlation of total MUST score (positive vs negative) with faecal elastase concentrations (r = - 0.32, p = 0.005) and a weak but statistically significant positive correlation with treatment with somatostatin analogues (r = 0.20, p = 0.013). Duration of treatment with somatostatin analogues showed a weak trend with total MUST score (r = 0.14; p = 0.078). None of the remaining markers significantly correlated with MUST total score in bootstrapped regression analyses, which included age, gender, functioning status, tumour grade, tumour stage, biomarkers in blood and urine, serum creatinine, BNP, prior abdominal surgery (any), and prior ileocaecal resection or right hemicolectomy (all p > 0.13).

Regression analyses

Bootstrapped binary logistic regression analyses in the complete model identified use of somatostatin analogues [exp(B) = 0.022; 95% CI (0.000; 1.554); p = 0.004], faecal elastase concentrations [exp(B) = 0.992; 95% CI (0.984; 1.001); p = 0.009)], age [exp(B) = 0.901; 95% CI (0.781; 1.038; p = 0.010] and tumour stage [≥ T2; p < 0.044); and presence of distant metastatic disease M1 (p = 0.037); but not N1] as significant predictors of MUST score. Duration of treatment with somatostatin analogues was not a significant predictor, neither in bootstrapped nor in conventional analyses (p = 0.11 and p = 0.63, respectively). The regression model was statistically significant (chi-square 26.58; p = 0.046). The model

explained 57% (Nagelkerke R^2) of the variance in MUST and correctly identified 95.5% of MUST negative and 44.4% of MUST positive subjects.

To obtain additional information about the influence of individual dependent variables, additional backward stepwise binary logistic regression analyses were tested. In step 6 of this stepwise model, again 44.4% percent of MUST positive patients and 97% of MUST negative patients were correctly identified, with use of somatostatin analogues, but not other factors including the duration of treatment with somatostatin analogues remaining a statistically significant predictor ($p < 0.001$; chi-square test 21.98, $p = 0.009$) and explaining 49% (Nagelkerke R^2) of the variance in MUST. Again, duration of treatment with somatostatin analogues did not influence the variance in the MUST score ($p > 0.595$ in all tested models). Use of somatostatin analogues (yes vs no) in bootstrapped analyses with location of the primary tumour, tumour grade, tumour stage, and functioning status as the independent variables was predicted by functioning status ($p = 0.011$), tumour grade ($p < 0.032$) and presence of distant metastases (M1, $p = 0.035$), with location of the primary tumour showing a trend. The model explained 55% of the variance in use of somatostatin analogues and reasonably correctly predicted both treated (84.2%) and untreated (78.3%) patients (chi-square 66.35, $p < 0.001$).

Characteristics of MUST positive compared with MUST negative patients

Specific characteristics of GEP-NET patients with a positive as compared with a negative MUST scores are shown in (table 1). GEP-NET patients who scored ≥ 1 using MUST were significantly more frequently treated with somatostatin analogues as compared to patients who did not score using MUST (65 vs 38%; $p = 0.021$) (table 1).

When stratifying the entire cohort according to treatment with somatostatin analogues, 22.4% (n = 15 of 67 patients) who were treated with somatostatin analogues scored ≥ 1 using

MUST, as compared to 8.5% (n = 8 of 94 patients) who were not on treatment with somatostatin analogues (p = 0.013). MUST positive patients showed significantly lower faecal elastase levels, as compared with MUST negative patients (table 1). Faecal elastase concentrations also tended to be lower in patients who were on treatment with somatostatin analogues, as compared with patients who were not on treatment with somatostatin analogues (335 ± 26 vs 402 ± 27 µg/g stool; p = 0.075). Finally, patients who scored using MUST had significantly more often NET of the rectum or of unknown origin, as compared with patients who did not score (figure 3). Frequencies of midgut NET (p = 0.688), pancreatic NET (p = 0.195) and gastric NET (p = 0.443) were not significantly different between MUST positive and negative patients (figure 3).

DISCUSSION

Malnutrition is an adjustable risk factor [19], but associated with severe adverse clinical outcomes if not addressed [1 2]. Patients with GEP-NET do not typically present with major weight loss or acute illness before reaching the very final stages with extensive metastatic disease or carcinoid heart disease. This is related to the fact that GEP-NET are often slower growing and less aggressive tumours at least when well differentiated, as compared with other types of cancer [20]. Nevertheless, malnutrition in patients with GEP-NET could be present for other reasons, including chronic loose stools, osmotic diarrhoea [20], and excess secretion of serotonin precursors stimulating small bowel motility [7-9]. Pancreatic mass effects, tumour infiltration of the mesentery [10 11] and GEP-NET related treatment [12-14] are further potential risk factors.

In our outpatient cohort of patients with various types of GEP-NET, 14% had a MUST score of > 1, which should trigger referral to the dietitians and the nutrition support team [2 16]. When comparing the cohort of patients who scored using MUST with the patients who did not score, we identified distinct characteristics of MUST positive patients with GEP-NET. MUST positive patients were more likely to have unknown primary or rectal NET, which might be related to delayed diagnosis and widespread disease in these patients. Furthermore, MUST positive patients showed significantly lower faecal elastase concentrations, although the frequency of pancreatic NET was not significantly different between groups, arguing against possible pancreatic mass effects as the main driving factor. Most importantly, MUST positive patients were some 2-fold more likely to be on treatment with long acting somatostatin analogues. After acute, short term administration, rapid onset suppression of pancreatic exocrine secretion by somatostatin analogues [21 22] and consequent steatorrhea [23] have been reported. Further known mechanisms are in agreement with a possible role of

somatostatin analogues in conveying malnutrition in patients with GEP-NET, although it is important to mention that most previous reports refer to effects of acute administration of somatostatin analogues [12 13 24 25] or *in vitro* studies [26], whereas effects after chronic administration [23] might be different related to possible adaptive mechanisms. Impairment of hepatic bile acid physiology by somatostatin analogues has been reported after both short term [27] and more prolonged administration and is causally involved in gall stone formation [28]. In the acute setting, intravenous somatostatin inhibits glucose, triglyceride, amino acid, and calcium absorption by direct effects on the intestinal mucosa [12 13 24]; and decreases gastric acid secretion by 90% in healthy volunteers [24]. In addition, acute suppression or *in vitro* effects of various gut hormones such as cholecystokinin and glucagon like peptide-1 by somatostatin analogues are well described [23 25 26], and diarrhoea, steatorrhea and weight loss are key features of excess hormone producing somatostatinomas [29]. Possible implications of treatment with somatostatin analogues on other aspects such as loss of fat soluble vitamins in the faeces have been also reported [30]. It might be argued that patients who were treated with long acting somatostatin analogues were more prone to score using MUST related to functioning status and advanced disease progression, as well as general risk factors such as age and disease related depression. However, treatment with somatostatin analogues remained a statistically significant predictor of MUST in all tested models. Importantly, duration of treatment with somatostatin analogues showed no significant influence in our analyses, indicating that acute effects of the administration of somatostatin analogues on the likelihood scoring positive in the MUST score were sustained after longer term treatment and somewhat arguing against adaptive mechanisms in this context.

Our observed total rate of patients at risk of malnutrition was somewhat lower than the prevalence very recently reported by Maasberg and colleagues, in a neuroendocrine cohort of comparable size [18]. Authors identified some 21-25% of the patients at risk [18], as compared to

14% in our study; however, the cohort in the mentioned study comprised 87% inpatients and also included patients with neuroendocrine tumours of the lung, as compared to our study which was exclusively assessed in outpatients with GEP-NET. This may explain the lower frequency of patients at risk of malnutrition in our cohort.

The observational nature of this study needs to be mentioned as a limitation, as well as relatively small sample sizes when including not routinely measured biomarkers in the regression models. Our study confirms the importance of screening for malnutrition in patients with GEP-NET. This is directly clinically relevant, considering that malnutrition in patients with neuroendocrine tumours could be an independent prognostic factor [18]. Confirmation of our findings in multicentre settings with access to large and diverse GEP-NET patient cohorts will be useful.

In summary, somatostatin analogues are key treatment modalities in patients with well differentiated GEP-NET but may cause transient or permanent gastrointestinal side effects such as bloating and cramping in up to 30% of the patients [31-33]; and, based on our findings, appear to increase malnutrition risk as identified by MUST. Without systematically screening GEP-NET patients for malnutrition, mild impairment of digestive processes might be missed or attributed to functioning aspects of the GEP-NET, rather than recognised and treated as a possible side effect of the treatment. Referring these patients for early nutritional intervention could lead to improvement of the nutritional status and quality of life [34].

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Contributors: MOW, NB and SAQ were involved in the study concept and design. SK, KG, JLHW, LD, SF, WS and SS were involved in the acquisition of clinical data. CD was involved in the analyses of biochemical markers. SQ, NB, LD, GKD and MOW were involved in the collection of MUST data. MOW and JH did the statistical analyses. MOW and MD supervised the MSc project of SAQ. MD provided important intellectual content and critical review of the manuscript. SAQ provided input to the first draft of the manuscript. MOW drafted the manuscript, and all authors critically revised it for important intellectual content. MOW supervised the study and is the guarantor. We thank Ms Josie Goodby for support with data collection.

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Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no competing interests

Ethical approval: The UHCW audit department had approved this study (audit number 1133/2015; July 2015). All study data were accessed using techniques compliant with the Health Insurance Portability and Accountability Act of 1996 (HIPAA), and, because this study involved analysis of pre-existing, de-identified data, it was exempt from institutional review board approval.

Transparency: The lead author (the manuscript's guarantor) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Data sharing: No additional data available.

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Tables

Table 1 Characteristics of MUST positive compared with MUST negative patients with gastro-entero-pancreatic neuroendocrine tumours (GEP-NET) of varying primaries, tumour grading, staging and functioning status. Data are given as mean ± SE. SSA, long acting somatostatin analogues

	MUST positive n = 23	MUST negative n = 138	p value
Treatment with SSA (%)	65	38	**0.021**
Faecal elastase (μg/g stool)	244 ± 37	383 ± 20	**0.018**

Figure legends

Figure 1: Simplified scheme of use of the MUST score (adapted from [15]). MUST was positive in 14.2% of the screened patients (23/161 patients with GEP-NET). The majority of the patients with positive MUST scored 1 (n = 14) or 2 (n = 7), mostly related to BMI < 20 kg/m^2 (n = 16) and/or, less frequently, recent weight loss (n = 9). Only n = 2 of the patients in the entire cohort had a MUST score of ≥ 3.

Figure 2: Characteristics of the GEP-NET cohort. (A) location of the primary tumour, (B) distribution of tumour staging, with the remaining 11.2% of the patients being classified as Tx (no signs of primary tumour), (C) histological grading (well differentiated, grade 1 and 2; poorly differentiated, grade 3).

Figure 3: MUST positive compared with MUST negative patients with GEP-NET. Patients who scored using MUST were significantly more likely to have rectum NET ($p < 0.017$) or a NET with an unknown primary ($p < 0.017$). Other types of NET were not significantly different between MUST positive and MUST negative patients, which included pancreatic NET ($p = 0.195$). Black bars: MUST positive patients; grey bars: MUST negative patients. pNET, pancreatic neuroendocrine tumour

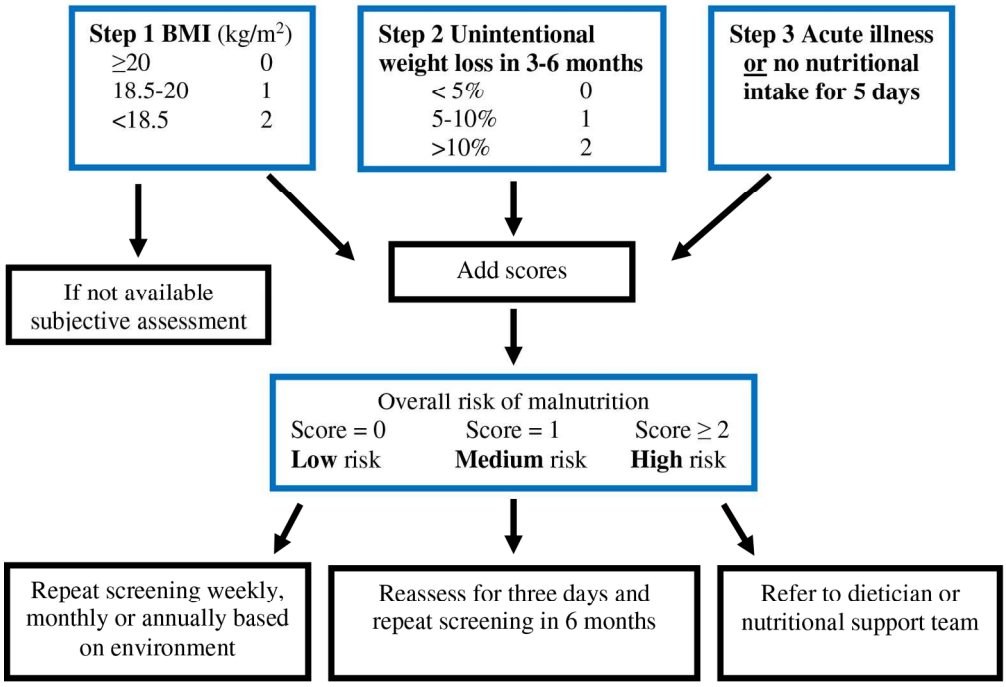

Figure 1: Simplified scheme of use of the MUST score
161x114mm (300 x 300 DPI)

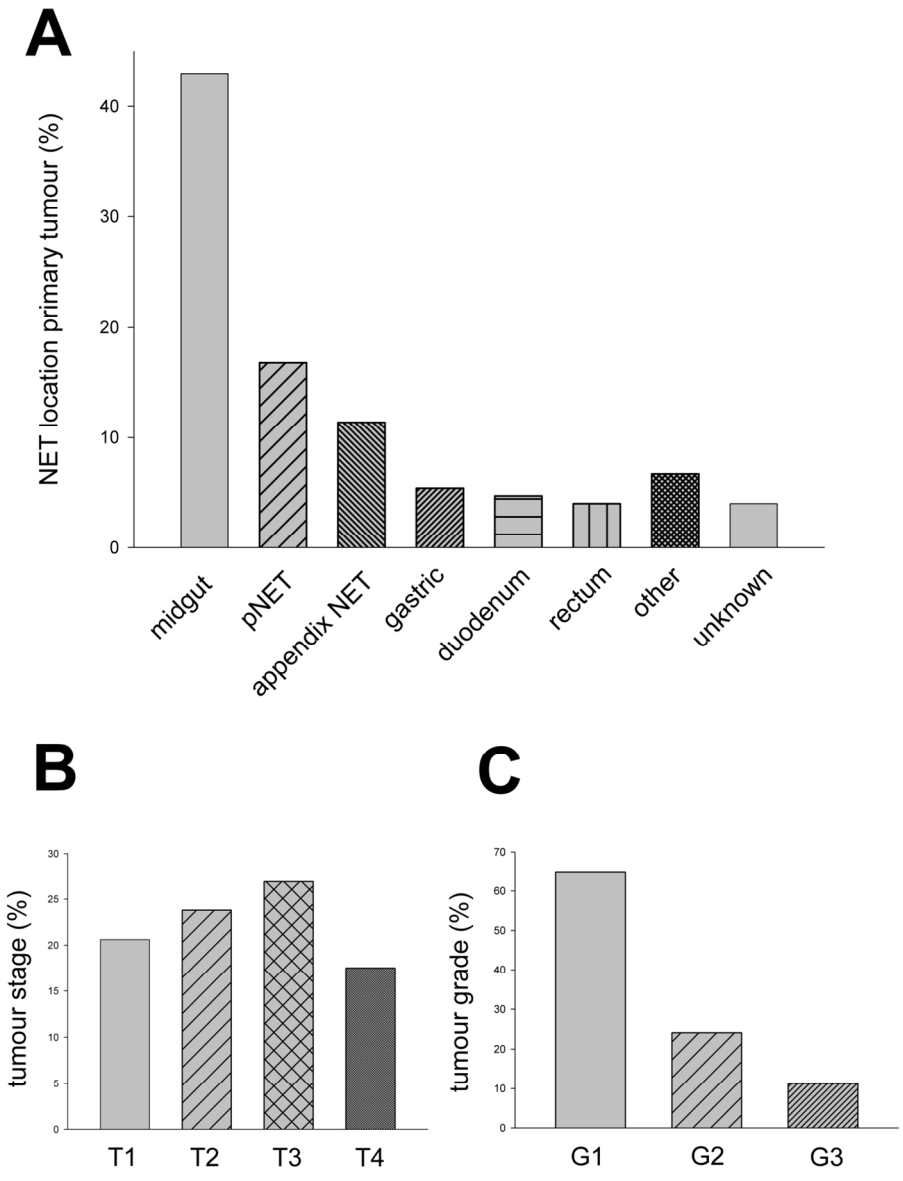

Figure 2: Simplified scheme of use of the MUST score
119x154mm (300 x 300 DPI)

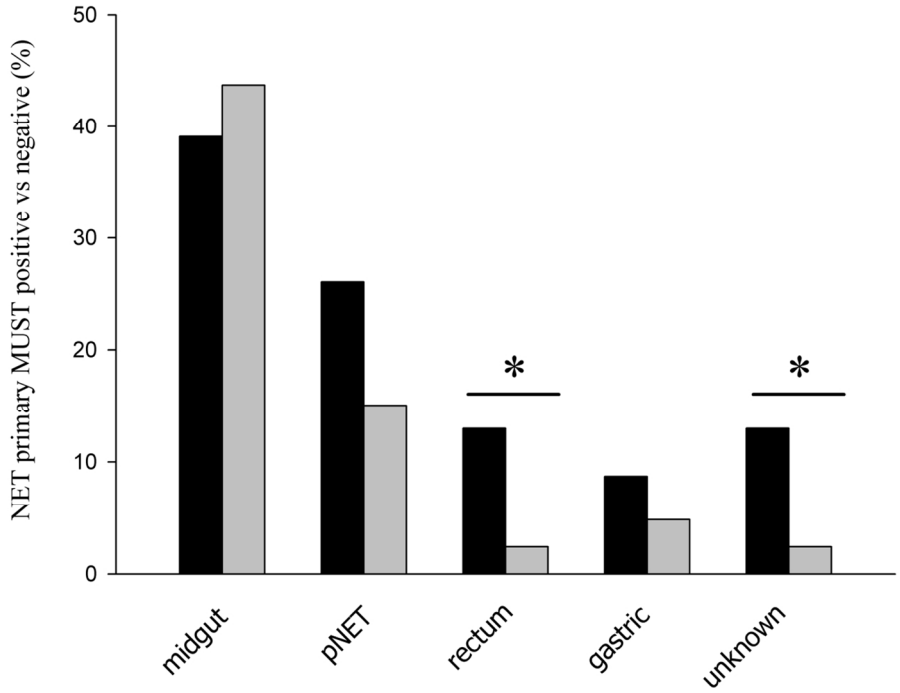

Figure 3: MUST positive compared with MUST negative patients with GEP-NET
119x91mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	p 3	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
	p 3	(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	p 5	Explain the scientific background and rationale for the investigation being reported
Objectives	p 5	State specific objectives, including any prespecified hypotheses
Methods		
Study design	p 5-6	Present key elements of study design early in the paper
Setting	p 6	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	p 6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	p 3	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	p 6	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	p 6	Describe any efforts to address potential sources of bias
Study size	p 6	Explain how the study size was arrived at
Quantitative variables	p 6	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	p 7-8	(*a*) Describe all statistical methods, including those used to control for confounding
	p 7	(*b*) Describe any methods used to examine subgroups and interactions
	p 6, 7-8	(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants	p 6	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	p 6; 9-12; figures 2 and 3	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	p 3; 9-12; table 1; figure 3	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	p 9 – 12; table 1; figure 3	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	p 9 -12	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	p 9-12	Summarise key results with reference to study objectives
Limitations	p 3; 14	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	p 13 - 15	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	p 14-15	Discuss the generalisability (external validity) of the study results
Other information		
Funding	p 16	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.