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Prophylactic percutaneous endoscopic gastrostomy in patients with head and neck cancer: Influence on nutritional status, utilisation rate and complications

Johannes Hausmann[1] | Alica Kubesch[1] | Jens Müller von der Grün[2] | Carmen M. Goettlich[1] | Natalie Filmann[3] | Andrea Oliver Tal[1] | Johannes Vermehren[1] | Mireen Friedrich-Rust[1] | Astrid Wächtershäuser[1] | Jörg Bojunga[1] | Irina Blumenstein[1]

[1]Department of Internal Medicine 1, University Hospital Frankfurt, Frankfurt am Main, Germany

[2]Department of Radiotherapy and Oncology, University Hospital Frankfurt, Frankfurt am Main, Germany

[3]Institute of Biostatistics and Mathematical Modeling, University Hospital Frankfurt, Frankfurt am Main, Germany

Correspondence
Johannes Hausmann, Department of Medicine 1, University Hospital Frankfurt, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany.
Email: johannes.hausmann@kgu.de

Abstract

Background: Patients with head and neck cancer (HNC) are at high risk for malnutrition because of tumour localisation and therapy. Prophylactic percutaneous endoscopic gastrostomy (PEG) tube placement is common practice to prevent malnutrition.

Objective: To investigate the benefits of prophylactic PEG tube placement for HNC patients in terms of the influence on patients' nutritional status, utilisation rate, complications and to identify the predictors of PEG tube utilisation.

Methods: All consecutive HNC patients who underwent prophylactic PEG tube insertion between 1 January 2011 and 31 December 2012 prior to therapy were enrolled. The PEG tube utilisation rate, complications, the patients' nutritional status and tumour therapy were evaluated with the help of electronic patient charts and telephone interviews.

Results: A total of 181 patients (48 female, median 67.5 years) were included. The PEG utilisation rate in the entire cohort was 91.7%. One hundred and forty-nine patients (82.3%) used the PEG tube for total enteral nutrition, 17 patients (9.4%) for supplemental nutrition and 15 patients (8.3%) made no use of the PEG tube. Peristomal wound infections were the most common complications (40.3%) in this study. A high Nutritional Risk Screening (NRS) score prior to tube insertion was found to be independently associated with PEG utilisation. No significant weight changes were observed across the three patient subgroups.

Conclusions: The overall PEG tube utilisation rate was high in this study. However, given the high rate of infections, diligent patient selection is crucial in order to determine which patients benefit most from prophylactic PEG tube insertion.

1 | INTRODUCTION

Patients with head and neck cancer (HNC) are often at risk for malnutrition during cancer therapy.[1,2] The risk for malnutrition in this patient population is determined by tumour localisation, nutritional state prior to therapy, dysphagia and type of oncologic treatment. A multimodal treatment approach with a combination of surgery and non-surgical/conservative treatment (ie, radiation, chemotherapy) is associated with extensive surgical wounds and mucositis which impairs the ability for oral food intake.[3-5] Although there is an abundance of proposed clinical[6,7] and laboratory parameters,[8] there is still no general consensus how to best assess the nutritional status and consequently stratify the risk for malnutrition. Several studies for instance have shown that a low BMI is associated with poor overall survival.[9-11] Similar observations could be made for bioimpedance analysis (BIA). A recent study was able to show that HNC patients with normal phase angle (PA >5.0) had a significantly better survival.[12] Therefore, both national and international practice guidelines have stressed the importance of maintaining an adequate nutritional status and propose percutaneous endoscopic gastrostomy (PEG) tube insertion for HNC patients prior to starting oncologic therapy.[13-15] However, there is considerable disagreement in whom and when to start PEG-based enteral feeding.[16-19] Overall, enteral nutrition via PEG tube has been shown to be safe and effective in patients with HNC.[20,21] Although PEG insertion is considered a routine procedure, it is not without risk of immediate or delayed complications.[22-25] Approximately, 8%-30% of patients suffer from complications during or after PEG insertion.[22-24,26,27] The aim of the present study was to investigate the influence on patients' nutritional status, the utilisation rate and complication rate after PEG insertion. Moreover, we aimed to investigate whether there are clinical parameters associated with PEG utilisation in this study.

What is already known about this topic?

- Head and neck cancer patients are at risk for weight loss during cancer treatment
- Percutaneous endoscopic gastrostomy (PEG) insertion is recommended by International Guidelines
- Lack of consensus concerning time point of insertion
- Lack of reliable predictive markers for later PEG utilisation

What does this article add?

- A high Nutritional Risk Screening score prior to tube insertion was independently associated with PEG utilisation
- High overall utilisation rate in the cohort (92%)
- Peristomal wound infections were the most common complications in our cohort

2 | PATIENTS AND METHODS

2.1 | Study population and study design

In this retrospective single-centre study all consecutive patients with a diagnosed HNC who underwent prophylactic endoscopic PEG tube insertion between 1 January 2011 and 31 December 2012 at the University Hospital Frankfurt were included. Patients had to be aged 18 years and older and had to have an estimated life expectancy of greater than 12 weeks. A prophylactic tube placement was defined as PEG insertion prior to cancer treatment and when oral food intake was still possible.[28] The primary endpoint of this study was to assess the utilisation rate of prophylactically inserted PEG tubes and the impact on the nutritional status.

2.2 | Ethics statement

Ethics approval for this retrospective study was obtained from the local Ethics Committee of the University Hospital Frankfurt (file number 89/13). Informed consent was obtained from all patients prior to enrolment in the study.

2.3 | Nutritional status

For nutritional status assessment several parameters including the Karnofsky Index at the beginning of the treatment, state of dysphagia at the time of tube insertion, presence of a tracheostomy and serum albumin levels were taken into consideration. For all patients, the body weight was documented at the time of tube insertion, during and at the end of the tumour therapy. The body mass index was calculated for each patient at each time point. The Nutritional Risk Screening (NRS 2002), a well-established nutritional screening tool in clinical practice[29] and recommended by the European Society for Clinical Nutrition and Metabolism (ESPEN) and others,[30] was performed at the time of the PEG tube insertion. A patient with a score ≥3 was considered at increased risk for malnutrition and a nutritional care plan was initiated.[30] Albumin deficiency was defined as albumin values <3.5 g/dL.[15]

2.4 | PEG tube utilisation

The study population was divided into different groups according to the utilisation of the feeding tube for enteral nutrition. Patients received either total enteral nutrition via the feeding tube (Group A), supplemental tube nutrition while oral food intake was still possible (Group B) and no utilisation of the feeding tube (Group C). The starting date of enteral nutrition via the feeding tube was determined based on electronic patient chart data. The duration of enteral tube utilisation was determined via structured telephone interviews.

2.5 | Telephone interviews

The interviews were conducted between October and November 2013. All patients received an informational letter concerning the background and intent of the study at least 1 week prior to the planned telephone interview. Informed consent was obtained at the beginning of the interview. If the patient was not able to partake in the telephone interview, he or she was asked to name a third person (family member, legal guardian) to answer the questions during the interview.

2.6 | Complications

In order to evaluate PEG-associated complications and their respective severity and need for treatment the following classification was applied[27,31,32]:

2.6.1 | Minor complications

Peristomal wound infection (no antibiotic treatment needed), pain at insertion site, gastritis, tube dislocation, bleeding at insertion site, hypergranulation tissue at insertion site, occluded tube, tube leakage, abdominal pain caused by proximity to liver capsule.

2.6.2 | Major complications

Severe peristomal wound infection (antibiotic treatment needed), severe bleeding at insertion site, peritonitis, gastric perforation, intrahepatic tube placement, aspiration pneumonia, buried bumper syndrome as well as complications during PEG insertion such as bleeding and decreased oxygenation levels during sedation.[27,32]

2.7 | Follow up

Follow up for this study was conducted until the end of November 2013. After this time point, the patients received follow up at their treating physician's discretion.

2.8 | Statistical analyses

Nominal data were presented as raw numbers and percentages, ordinal data as median and Interquartile range (IQR) and quantitative data as mean and standard deviation (sd) or –if skewed—as median and IQR. In order to determine the impact of quantitative parameters on type of PEG tube utilisation the Wilcoxon-Mann-Whitney-*U*-test ('PEG utilisation' vs 'No PEG utilisation' group) and the Jonckheere-Terpstra test and Kruskal-Wallis test for groups A–C were applied. To analyse the results of the PEG utilisation during follow up—determined through telephone interviews—and to determine the nutritional status, the same statistical methods were applied. For nominal parameters, the Fisher's exact test was applied for groups A-C and 'utilisation' vs 'no utilisation' group. $P<.05$ were considered to be statistically significant. Associations of PEG utilisation with continuous or dichotomic variables were assessed in linear and logistic regression models, respectively. After univariate analyses, multivariate analyses were performed for significant associations. Multivariate models were obtained by backward selection, using a $P > .15$ for removal from the model. Statistical analyses were conducted using IBM SPSS Statistics Version 22.0 (International Business Machine Corporation).

3 | RESULTS

3.1 | Patient characteristics

A total of 181 patients diagnosed with HNC who received prophylactic PEG tube insertion between 1 January 2011 and 31 December 2012 prior to cancer treatment were included in this study. Baseline characteristics of the study population are shown in Table 1. The overall PEG utilisation rate was 91.7% (n = 166/181). About 82.3% (n = 149/181) used the PEG tube for total enteral nutrition, 9.4% (n = 17/181) required supplemental nutrition and 8.3% (n = 15/181) made no use of the feeding tube. A total of 73.5% (n = 133/181) of patients were male and 26.5% (n = 48/181) were female. The mean age at tube insertion was 64 years (range, 38-93 years). Women were significantly older than men at the time of tube insertion (67 vs 62.7 years; $P = .007$). However, age and sex distribution across the three groups (A-C) were not statistically different ($P = .3$ and $P = .19$, respectively). Liver cirrhosis, diabetes (type 1 and 2), chronic renal insufficiency, immunosuppression, second active malignant disease and chronic neurological diseases were defined as relevant concomitant diseases. A total of 178 patients were included in this evaluation, as for three patients (Group A) no sufficient patient history data were available.

3.2 | Nutritional status

The Karnofsky Index, body weight, BMI, presence of tracheostomy, dysphagia at time of tube insertion as well as serum albumin levels were considered relevant parameters to determine the nutritional status of the patients. In a univariate analysis, dysphagia at time of PEG insertion ($P = .04$), low serum albumin levels ($P = .04$) and a high NRS score ($P = .0001$) were associated with PEG tube utilisation. However, in a multivariate model, only the NRS score remained independently associated with PEG tube utilisation ($P = .001$; OR 0.45; 95% CI 0.28-0.70). Other factors, including Karnofsky index ($P = .77$), presence of a tracheostomy ($P = .3$), total body weight ($P = .12$), surgical treatment ($P = .99$) and BMI ($P = .195$) were not associated with PEG tube utilisation in this study (Table 2).

3.3 | Weight changes after tube insertion

Weight changes were documented for a total of 168 out of 181 patients (92.8%). The median weight change was −1.6kg (range, −30.0 to +11.5 kg) over the course of the study. The highest weight loss was observed in Group B (median: −3.0 kg; range, −15 to +5.4 kg).

TABLE 1 Baseline characteristics of included patients

	Entire cohort N = 181	Group A N = 149	Group B N = 17	Group C N = 15
Female gender, N (%)	48 (26.5)	43 (28.9)	4	1
Age (y), median (range)	64 (38-93)	64 (38-87)	67 (51-93)	60 (47-79)
Concomitant disease N (%)[a]	178 (98.3)	146 (98%)	17 (100%)	15 (100%)
Liver cirrhosis, N (%)	6 (3.3)	6 (4.1%)	0	0
Renal insufficiency, N (%)	10 (5.6)	9 (6.2)	0	1
Diabetes, N (%)	28 (15.5)	24 (16.1)	2	2
Chronic neurological disease, N (%)	19 (9.6)	11 (7.5)	3	3
Second active malignant disease, N (%)	6 (3.4)	3	3	0
Karnofsky index, % (range)	90 (40-100)	90 (40-100)	90 (50-100)	90 (50-100)
Weight in kg, median (range)	70 (38-119.8)	68 (38-119.8)	70 (40.8-93)	76 (48-96)
BMI (kg/m^2), median (range)	23.8 (12.8-37.8)	23.2 (12.8-37.8)	24.4 (16.7-28-8)	25.4 (18.2-30.6)
Dysphagia prior to PEG insertion, N (%)	85 (47)	75 (50.3)	7	3
Tracheostomy, N (%)	58 (32)	50 (33.6)	5	3
Albumin, g/dl, median (range)	4.1 (2.0-4.9)	4.0 (2.0-4.9)	4.0 (2.5-4.7)	4.4 (2.7-4.9)
NRS median (IQR)	3.0 (0.0-6.0)	3 (0.0-6.0)	2.0 (0.0-5.0)	1 (0-0-4.0)
Weight after PEG insertion kg, median (range)	−1.6 (−30- +11.5)	−1.4 (−30-+11.5)	−3.0 (−15-+5.4)	−1.5 (−12.3-+4.1)
Radiation, N (%)	169 (94.9)	141 (95.9)	16 (94.1)	12 (80)

Abbreviations: BMI, body mass index; PEG, percutaneous endoscopic gastrostomy; NRS, nutritional risk screening.
[a]For two patients, no information concerning concomitant diseases was provided in Group A.

TABLE 2 Logistic regression analyses for PEG utilisation

	Univariate analysis		Multivariate analysis	
	P-value	OR (95% CI)	*P*-value	OR (95% CI)
Entire cohort				
Dysphagia prior to PEG insertion	.04	0.25 (0.07-0.94)		
Albumin	.04	3.5 (1.05-11.61)		
NRS	<.0001	0.45 (0.28-0.70)	.001	0.45 (0.28-0.70)

Abbreviations: NRS, nutritional risk screening; PEG, percutaneous endoscopic gastrostomy.

However, overall weight changes were not associated with PEG tube utilisation (*P* = .88).

3.4 | Tumour entities, size and treatment

Hypopharyngeal cancer was the most common tumour entity in this study (n = 26; 14.4%). Hypopharyngeal cancer was also the most common cancer among patients who used their PEG tube for enteral nutrition (n = 25; 15.1%) whereas laryngeal cancer was the most frequent diagnosis (n = 4 26.7%) in patients who did not make use of the feeding tube. Tumour size at the time of tube insertion was determined with the help of the TNM classification and correlated with the probability of tube utilisation. Tumour stage T4 was most commonly seen in group A (n = 70; 51.5%), whereas tumour stages T2, T3 and T4 were all equally represented in Group B (n = 4 per/T stadium). Patients who did not make use of the feeding tube had less advanced tumour stages (T3 was present in n = 5; 41.7% of patients), however, this difference did not reach statistical significance (*P* = .052). The most commonly applied cancer treatment was combined chemoradiotherapy (CRT); n = 144/181; 80.5%). Overall, 169 (94.9%) patients received radiotherapy (RT) during their treatment. In groups A and B, 119 (79.9%) and 13 patients (76.5%) received CRT, respectively. In both groups combined, 94.6% (n = 157) of patients received radiation therapy. In group C, 12 patients (80%) underwent CRT therapy, whereas 6.7% (n = 1) received adjuvant CRT and the remaining 13.3% (n = 2) received isolated chemotherapy. Radiation mode (*P* = .17) and dosage (*P* = .37) were not significantly different across the different PEG utilisation groups. A total of 152 patients (84%) received chemotherapy in this study, mainly in combination with a radiation therapy. The most common applied regimen was a platinum-based chemotherapy in 107 patients (70.4%). Only 11 patients (6%) underwent either isolated surgery (n = 4, 2.2%) or combined with CRT (n = 7, 3.9%). Interestingly, the majority of

patients undergoing surgery (n = 8, 72.7%) reported dysphagia at the time of PEG insertion.

3.5 | Complications during PEG tube insertion and later utilization

Complications were defined as minor and major complications as outlined above (Table 3). Ninety-three patients (51.4%) experienced PEG tube-associated complications. Among these, 67 (72%) patients experienced a single minor complication and 11 (11.8%) patients experienced repeated minor complications. Thirty-three patients (18.2%) experienced both minor and major complications. A 59-year-old patient with HNC suffered from recurring buried bumper syndrome and associated complications. PEG tube removal as a result of complications was necessary in 21 patients (11.6%). The most frequent minor complications were either directly associated to tube feeding (36%; 65.4% nausea/vomiting, 3.8% obstipation, 30.8% diarrhoea) or non-antibiotic treatment-dependent peristomal insertion site infections (n = 21, 29%). Peristomal wound infections, both with and without the need for antibiotic treatment, were reported in 73 patients (40.3%; Figure 1). Of these, 51 (71.2%) required antibiotic treatment and were considered as major complications. The risk of getting a peristomal wound infection was comparable across all groups (Group A: n = 61, 40.9%; Group B: n = 6, 35.3%; Group C: n = 4, 40%). Peristomal infections were not associated with PEG tube utilisation in this study (P = .978).

3.6 | Incidental findings during endoscopy at tube insertion

In 38.7% of the patients (n = 70), incidental pathologies were detected during the routinely performed oesophagogastroduodenoscopy (EGD) at tube insertion. The most common findings were gastritis and oesophagitis (see Table 4). However, it is noteworthy that in one patient an early stage malignant duodenal tumor was detected which could be resected surgically.

TABLE 3 Complications during and after percutaneous endoscopic gastrostomy tube insertion

	Entire cohort N = 181
Minor Complication, N[a]	73
Peristomal wound infection, N (%)	21 (29)
Hypergranulation tissue at insertion site, N (%)	5 (7)
Pain at insertion site, N (%)	8 (11)
Gastritis, N (%)	0 (0)
Tube dislocation, N (%)	9 (12)
Bleeding at insertion site, N (%)	2 (3)
Occluded tube, N (%)	2 (3)
Tube leakage, N (%)	0 (0)
Abdominal pain to proximity to liver capsule, N (%)	0 (0)
Complications associated with tube feeding, N (%)	26 (36)
Major Complication, N[a]	68
Antibiotic-treated peristomal wound infection, N (%)	52 (72.4)
Severe bleeding at insertion site, N (%)	0 (0)
Peritonitis, N (%)	3 (4.3)
Gastric perforation, N (%)[b]	1 (1.4)
Intrahepatic tube placement, N (%)	0 (0)
Aspiration pneumonia, N (%)	3 (4.3)
Buried-bumper-syndrome, N (%)[b]	3 (4.3)
Decreased oxygenation levels during sedation, N (%)	6 (8.6)

[a]Minor and Major complications are described in Materials and Methods.
[b]Two of three buried bumper syndrome patients were reported in the same patient as well as the gastric perforation.

FIGURE 1 Antibiotic-treated peristomal wound infection

TABLE 4 List of incidental diagnosis during oesophagogastroduodenoscopy (EGD) at tube insertion

	Entire cohort N = 161
Amount of total positive findings, N (%)	70 (43.48)
Diagnosis during EGD	
Gastritis, N(%)	24 (34.29)
Gastric erythema, N (%)	19 (27.14)
Reflux oesophagitis, N (%)	4 (5.71)
Gastric ulcus, N (%)	6 (8.57)
Duodenal ulcus, N (%)	3 (4.29)
Malignant duodenal tumour, N (%)	1 (1.43)
Hiatal hernia, N (%)	6 (8.57)
Erosive oesophagitis, N (%)	4 (5.71)
Glycogen acanthosis N (%)	3 (4.29)

4 | DISCUSSION

Patients with HNC are at high risk for malnutrition and associated complications. Prophylactic PEG tube insertion can contribute to the therapeutic success, as it may aid in maintaining an adequate nutritional state and weight during therapy.[1,2] As PEG tube insertion is an invasive procedure that might not solely be to the patient's benefit, it is of major clinical relevance to identify the patients who benefit most of prophylactic enteral feeding tube insertion. To date, there are no established parameters to reliably identify these patients.[16-19]

In our study, a high NRS score was independently associated with PEG tube utilisation (P = .001; OR 0.45; CI 0.28-0.70). Other parameters, including dysphagia prior to treatment, low serum albumin levels, proved to be at least indicative for PEG tube utilisation.

A retrospective study from the United States was able to show that low albumin levels were associated with poorer overall survival and increased risk for wound healing in HNC patients[33] In another retrospective cohort study from the UK, low serum albumin levels (<4.0 g/L) were found to be predictive for the need of enteral nutrition in HCN patients.[34] The latter observation could be confirmed in our study. However, in a multivariate model, only a high NRS score was independently associated with PEG tube utilisation. As there is no general consensus how to best determine the nutritional status, we chose to assess it with the help of a combination of clinical and laboratory parameters. However, as it is not always easy to document a wide variety of parameters in the daily clinical life, we elected parameters that were easy to obtain and that would help us in getting a comprehensive overview of the patient's nutritional state.

We observed a high overall PEG utilisation rate (>91%) in our cohort that was in accordance with the PEG utilisation rate seen in a similarly designed study from Finland.[35]

Interestingly, patients who used the PEG only intermittently for nutrition had the highest weight loss and suffered more often from minor nutrition-associated complications such as nausea or emesis. Refeeding-syndrome (RFS) is significant fear in severely malnourished patients, especially after long periods of starvation. It is defined as imbalances of fluid and electrolytes with associated clinical manifestations[36] and is of especial relevance in HNC patients, who often suffer from dysphagia. In order to avoid RFS occurrence, prophylactic PEG insertion should be advised as well as a step-up regimen for enteral nutrition.

Our study did not show a significant association between other comorbidities, including cirrhosis, neurological disorders or a second active malignant disease and the need for PEG tube utilisation.

Some studies even raised concerns over prophylactic PEG tube insertion. However, these were either small in sample size[37] or evaluated heterogenous patient cohorts.[38] One of the major concerns is the potential risk of prolonged tube dependency and swallow musculature atrophy,[39,40] however a recent prospective study from Sweden was able to show that patients with PEG tube do not have an increased risk in a long-term follow up.[41]

Currently there is no consensus regarding predictive factors that might help to assess the right timing and indication for PEG tube insertion in HNC patients.[42] A study by Machtay et al, identified older age, neck dissection after CRT, tumour localisation and advanced tumour stage as risk factors for late toxicity, that may include long-term severe dysphagia and dependence on a feeding tube.[43] In another US-based study, presence of a tracheostomy, free flap reconstruction, chemotherapy, tumour localisation and advanced tumour stage and patient age were all associated with the need for feeding tube placement.[44] In a more recent study by Karsten et al on HNC patients undergoing primary CRT, a prediction model based on BMI, weight loss, functional oral intake scale and tumour stage

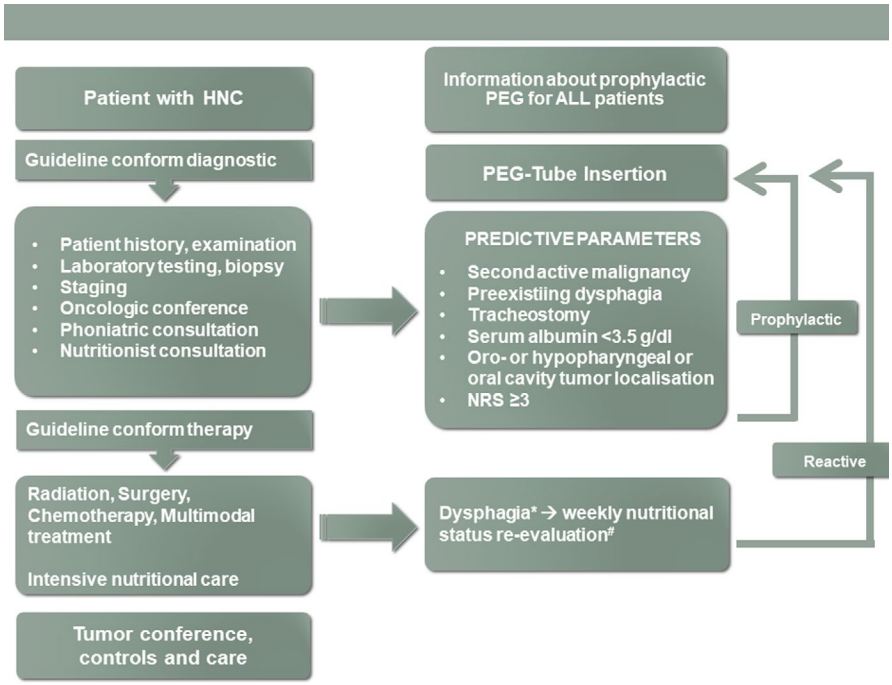

FIGURE 2 Frankfurt Algorithm for percutaneous endoscopic gastrostomy (PEG) Insertion in head and neck cancer (HNC) Patients. *Dysphagia leading to reactive PEG insertion was defined as subjective dysphagia, pain while swallowing, reduced oral food intake. #Weekly evaluation entailed questions targeting signs for dysphagia, physical examination for causes of dysphagia (ie, radioderma or oral mucositis), if available body weight documentation and examination by a swallow specialist

was proposed to identify patients eligible for proactive feeding tube placement.[45] Lastly Jack and colleagues demonstrated that RT was an independent predictor of PEG tube utilisation.[46] Interestingly, we could not confirm this association in our cohort. Despite these heterogeneous study data, most centres including ours follow the approach of prophylactic PEG tube placement.

Prophylactic PEG tube insertion seems justifiable, since HNC treatment often includes radiotherapy and even minimal-invasive surgical techniques do not always spare patients from reduced oral food intake.[47]

Factors such as age, tumour size and localisation, tracheostomy, BMI and mode of treatment were not associated with PEG tube utilisation in our study, Instead, our data support the use of nutritional screening before treatment initiation.

Interestingly, the complication rate was relatively high in our study (51.4%). However, it was comparable to that seen in a similar study (43.3%).[35] Other studies have shown variable complication rates between 5% and 20% for minor complications and 1%-23% for major complications with an overall high variability because of different tumour entities and comorbidities.[24,27,31,48,49] Peristomal wound infections were the most common complications (40.3%) in our cohort. This rate is relatively high in comparison to the literature, where peristomal infection is reported in 5%-25% of the patients, with the highest reported percentage being 65%.[24,50,51]

The overall high infection rate strongly supports the use of antimicrobial stewardship in these severely ill patients as well as patient education and PEG care. As a consequence, consultation hours for enteral nutrition support were implemented in our outpatient clinic, aiming to better educate and support patients under enteral tube feeding and to possibly prevent severe complications such as peristomal infections. Furthermore, we established an algorithm—mostly based on the predictors determined in this study—for our clinicians that should aid in deciding when and for whom a prophylactic tube insertion is advisable (Figure 2).

Limitations of our study are the retrospective, single-centre design and the low patient numbers in groups B and C. Thus, observed tendencies and predictive parameters might reflect local practices. However, our study still has its merits as it provides real-world data on PEG utilisation and follow-up via telephone interviews, which is not often assessed. Furthermore, the diagnostic endoscopy during insertion could detect additional pathologies in 70 of our patients. Thus, highlighting that the vast majority of patients possibly benefit from the diagnostic EGD during tube insertion.

In summary, our data support the use of prophylactic PEG tube placement given the overall high utilisation rate during HNC therapy. The patients' nutritional status before treatment initiation as reflected by the NRS score was the single most important predictor for PEG tube utilisation. However, peristomal wound infections were commonly observed and warrant antimicrobial stewardship guidance. Patients would thus greatly benefit from PEG and nutrition-focused consultation hours, aiming to better monitor the patient's nutritional status and to improve PEG-related care and education and aiding in possibly avoiding complications in the future.

DISCLOSURES

All authors have no conflict of interest to disclose.

AUTHOR CONTRIBUTIONS

JB and IB planned the study, CG and AW collected the data, CG, NF, JH, IB, AK and AW analysed and interpreted the data, JH, AK, IB, NF and JMVD prepared the manuscript and all authors revised the manuscript.

ORCID

Johannes Hausmann https://orcid.org/0000-0002-5217-2301

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