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The assessment of Ki-67 as a prognostic marker in neuroendocrine tumours: a systematic review and meta-analysis

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ABSTRACT

Introduction Gastroenteropancreatic neuroendocrine tumours (GEP NETs) are classified according to tumour mitotic count or Ki-67 labelling index (LI).

Aim(s) To systematically review articles reporting the prognosis of patients by Ki-67 LI and thereby improve the ability of clinicians to prognosticate for their patients.

Method 265 abstracts were identified relating Ki-67 and survival. After exclusion criteria were applied, 22 articles remained. Articles were excluded if they described non-human specimens, were non-English language, published prior to 2000, reported non-GEP NETs, reported subgroups selected by treatment modality or included <20 cases. Random-effects meta-analysis was used to combine studies to estimate survival proportions.

Results Authors used varied methods in which to present 5-year survival, with often limited survival information. This reduced the number of studies that could be included in the meta-analysis. 5-year survival for patients with grade 1 and 2 GEP NETs were estimated to be 89% (95% CI 85% to 92%, m=12 studies, n=977 participants) and 70% (95% CI 62% to 79%, m=9, n=726), respectively. Using an alternative grade 1/2 boundary of 5%, 5-year survival rates for Ki-67≤5% and 5–20% were estimated as 89% (95% CI 84% to 94%, m=7, n=654) and 51% (95% CI 44% to 59%, m=4, n=183), respectively. For Ki-67>20%, 5-year survival was estimated to be 25% (95% CI 12% to 38%, m=10, n=208).

Conclusions Standardisation of grade boundaries has allowed us to combine data from multiple studies and amass a body of evidence linking Ki-67 and survival.

INTRODUCTION

Gastroenteropancreatic neuroendocrine tumours (GEP NETs) have a UK incidence of 2–3 per 100 000 persons per year, although the incidence is seen to be rising.[1] They comprise a large heterogeneous group, with differing primary sites and functionality. Both factors are seen to impact on prognosis, but of greater significance is the biological behaviour of the NET. Various prognostic markers are used to assess NETs; these include proliferation rate, presence of vascular invasion, tumour size and tumour, node, metastases stage. Several newer techniques such as identification of circulating tumour cells are also under investigation.[2]

Ki-67 is a biomarker/antigen expressed by cells during distinct phases of the cell cycle, including mitosis, G1, S, G2. Therefore, this antigen is evident when cells are proliferating. The Ki-67 antibody, used to produce a Ki-67 labelling index (LI), stains for the Ki-67 antigen. A high Ki-67 LI identifies abnormal proliferation, and therefore broadly speaking, the aggressiveness of a tumour.[3] Ki-67 has been shown to be an accurate marker of proliferation,[1] although certain tumours do show some inconsistency between Ki-67 and proliferation.[4]

Proliferation rate as assessed by Ki-67 LI has been shown repeatedly to be strongly associated with prognosis. As such, proliferation rate/index is included in a wide range of NET classification systems, including European Neuroendocrine Tumor Society (ENETS),[5] WHO[6] and North American Neuroendocrine Tumor Society.[7] The former described immunostaining the specimens for the cellular proliferation marker Ki-67 LI as a 'must' when assessing NET specimens.[5] The 2007 ENETS grading system describes the use of ≤2% (grade 1), 3–20% (grade 2) and >20% (grade 3);[5] however, there is some debate regarding the most suitable cut-off values. Other authors have suggested cut-off values of 5% or 10% may provide more discriminative prognostic information.[8]

A number of techniques can be used to assess Ki-67 LI and can be seen to affect its accuracy. The first method involves 'eyeballing' the highest density of stained cells and estimating a proliferation index. The second method is more reproducible, involving the manual counting of 2000 cells, comparing the number of positively stained cells with the number of negatively stained cells. Young *et al*[9] demonstrated that manual counting of cells had a higher degree of accuracy compared with eyeballing estimates; they showed that 37 out of 93 cases were misclassified according to the ENETS grading system using the eyeballing method. [9] The accuracy of Ki-67 measurement can also be affected by tumour heterogeneity, caused by intratumour and intertumour Ki-67 variation.[10]

Much importance has been placed on Ki-67 LI, and a number of studies have documented its validity and reliability. Since the ENETS guidelines were published for Ki-67 LI, further case series have been reported.[5] Our aim is to systematically review these papers and summate data on 5-year survival rates. To our knowledge, this is the first attempt at a meta-analysis of these data.

METHOD

Literature search

A literature search was undertaken using the search engines Medline Ovid, PubMed, Google Scholar

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and Web of Knowledge. Terms used were 'neuroendocrine' AND 'tumors OR tumours OR tumor OR tumour' AND 'Ki-67 OR Ki67 OR mib1 OR mib-1' AND 'prognosis OR survival OR mortality'. This literature search was carried out on 25 November 2014.

Study selection

Two independent investigators (SR-T and JC) reviewed the search results. If the article met the inclusion criteria, the full text and their citations were assessed. Data were extracted from these articles including the patient demographic, 5-year overall survival rate and Ki-67 LI.

Inclusion/exclusion criteria

For inclusion in this systematic review, articles were required to describe a 5-year survival rate for a cohort of patients with GEP NETS and to relate survival to Ki-67 LI. We excluded articles published prior to 2000 because we felt the methodology for measuring Ki-67 LI was likely to be unacceptably heterogeneous. We also excluded articles that described non-human specimens, reported non-GEP NETs, reported subgroups selected by treatment modality, included <20 cases or were not published in English.

The results are presented in two parts: for part 1 of the systematic review, we included only articles reporting the ENETS grading systems[5]; for part 2, we included articles reporting an alternative system of grade 1 (<5%) and grade 2 (5–20%). Results were combined with those of the ENETS grading system.

Analysis and statistical methodology

Random-effects meta-analysis is used to calculate the combined estimates of survival proportions[11]; this method was chosen a priori due to the different patient characteristics across studies. A user-built file in Excel was constructed to run the analysis (discussed later).

RESULTS

Details of the inclusion/exclusion of articles considered are given in figure 1. The commonest reason for exclusion was articles not relating survival to Ki-67 LI. All authors suggested that Ki-67 provided prognostic value and that the Ki-67 LI was associated with the prognosis of patients. This association was seen across a broad range of GEP NETs and situations.

Attempts to perform a meta-analysis on the survival within each grade were hampered by the limited information provided by the majority of articles. Often, only initial sample size and proportion surviving were reported; attempts to calculate the actual number surviving 5 years often returned fractions of people. We assume this is due to (unreported) drop-out and/or incomplete follow-up to 5 years, and not solely rounding error, due to the values we observed. Furthermore, although some articles reported Kaplan–Meier curves,[12 13] the necessary information could not be extracted due to the lack of detail on the axes; in these cases, the results were not used to calculate the combined estimate but estimates are presented in the tables (as discussed later).

A number of articles reported survival but not using the complete grading system[14–18]; where possible, these results were used to calculate the combined estimates for the appropriate grades. Articles also reported inconsistent use of grading boundaries (eg, ≤2%, <2% and <3% were all used as the lower grading limit), but these are not distinguished in our analysis and are treated as ≤2%.

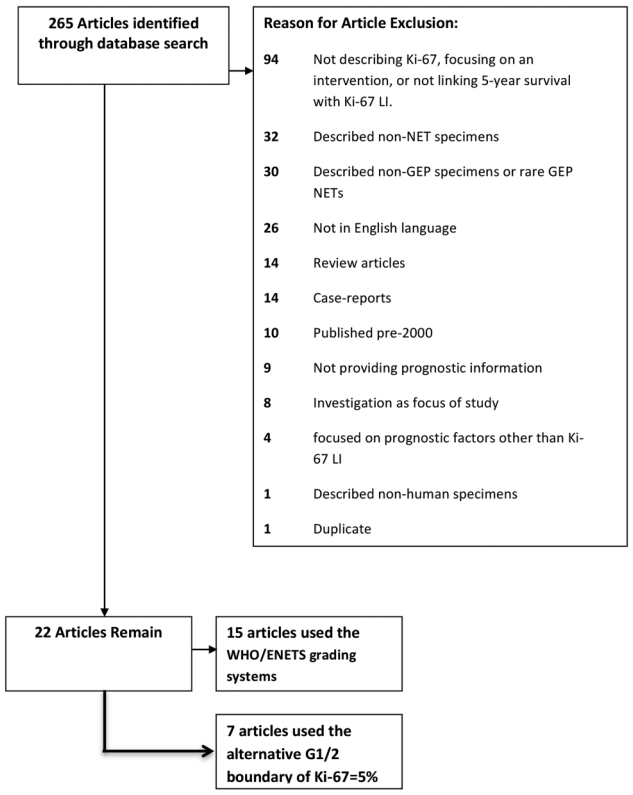

Figure 1 Consort diagram depicting the excluded and included articles. ENETS, European Neuroendocrine Tumor Society; GEP NET, gastroenteropancreatic neuroendocrine tumours; LI, labelling index.

We took each article's sample size and proportion surviving at face value (despite not always being consistent with an integer number) in order to summarise the data. The effect of this approach is to potentially overestimate the precision of each article's estimate (as presented in the forest plots, where the lines representing CIs are artificially short). Furthermore, to

Table 1 Internal validity of the articles

Feature	Qualities found in studies reviewed
Samples of patients	Since these case series are based on diagnostic biopsies, they are inception cohorts with a gold standard diagnostic criteria (a biopsy) and well-described demographic characteristics. Not every patient can be included due to not having been assessed for Ki-67 labelling index(LI)
Follow-up of patients	An uncertain number of patients in each cohort were censored before 5 years of follow-up was complete
Outcome	The outcome of 5-year survival is objective and non-biased
Prognostic variable	The Ki-67 LI is well defined by the European Neuroendocrine Tumor Society guidelines, but the details of the measurement methods used (eg, eyeball or counting) are not available from the studies
Analysis	None of the articles reviewed have adjusted for any other prognostic factor
Treatment subsequent to inclusion in cohort	The treatment subsequent to diagnosis is only described in a minority of cases and varies series by series. It is also likely to have changed significantly over time. The impact of any treatments for neuroendocrine tumour on the outcome of interest (survival) is not known

Table 2 Articles included in the meta-analysis

Author	Patient demographic	Ki-67 notes		
		Ki-67 labelling index (%)	5-Year survival (%)	No.
Scarpa *et al*[20]	274 consecutive patients (237 patients stained), pancreatic endocrine tumours operated on. Surgical resection and biopsy	≤2	90	130
		>2–20	63	85
		>20	12	22
Hentic *et al*[21]	45 consecutive patients, metastatic digestive endocrine carcinoma. All with liver metastases. Surgical resection and biopsy	0–2	100	6
		3–5	92	13
		6–14	73	9
		15–20	35	14
		>20	33	3
Norlén *et al*[22]	603 consecutive patients (299 stained), small intestinal neuroendocrine tumours (NETs). Surgical resection and biopsy	≤2	82	203
		3–20	54	89
		>20	51	7
Garcia-Carbonero *et al*[23]	837 consecutive patients (288 patients stained), gastroenteropancreatic (GEP) NETs. Surgical resection and biopsy	<2	83.3	126
		3–20	77.1	109
		>20	43.5	53
Hashim *et al*[24]	175 consecutive patients (136 patients stained), pancreatic NETs, 94% non-functioning. Surgical resections only	≤2	90.5	38
		>2–20	88.1	68
		>20	56.9	15
Ellison *et al*[25]	326 consecutive patients (276 patients stained), non-functioning pancreatic NETs. Surgical resection and biopsy	<3	85	150
		3–20	78	108
		>20	9	18
Jann *et al*[26]	270 consecutive patients (189 patients stained), midgut and hindgut NETs. Surgical resection and biopsy	≤2	95.2	117
		>2–20	82.0	61
		>20	51.4	11
Strosberg *et al*[19]	83 consecutive patients with metastatic GEP NETs. Surgical resection and biopsy	≤2	87	27
		>2–20	37	28
		>20	0	28
Pape *et al*[27]	202 consecutive patients (158 patients stained), upper GEP NETs. Surgical resection and biopsy	≤2	95.7	44
		>2–20	73.4	85
		>20	27.7	29
Martin-Perez *et al*[28]	481 consecutive patients (184 stained), pancreatic NETs and peri-pancreatic NETs. Surgical resection and biopsy	≤2	80.4	71
		3–20	68.7	93
		>20	17.4	20

potentially bias the combined estimate of the proportion (in unknown ways, due to uncertainty in how many people were used to estimate the proportion surviving in any given study) and to overestimate the precision of the combined estimate. Although this combined estimate is statistically imperfect, we believe this is a compromise, which makes a small impact on the validity of the study.

Martin-Perez *et al*[28] reported 0% survival in one group; in this instance, 0.5 was added to the empty cell to ensure non-zero variance (and hence finite weight in the meta-analysis).

As statistical software often requires numbers surviving as an input, a user-built Excel file was used to run the analysis; simulated examples were used to confirm the fidelity of the results against the *metaprop* function in Stata V.14.1.

Table 3 Articles excluded from the meta-analysis due to incomplete European Neuroendocrine Tumor Society (ENETS) grading system and/or Kaplan–Meier plots only

Author	Patient demographic	Ki-67 notes		
		Ki-67 labelling index (%)	5-Year survival (%)	No.
Panzuto *et al*[14]	185 consecutive patients (96 patients stained), pancreatic endocrine tumour+gastrointestinal carcinoids. Surgical resection and biopsy	≤2	90.1	58
		>2	53.5	38
Bettini *et al*[15]	180 consecutive patients (49 patients stained), non-functioning pancreatic tumours. Surgical resection only	≤2	93.7	77
		>2	50.2	75
Hamilton *et al*[12]	140 consecutive patients, pancreatic neuroendocrine tumours (NETs). Surgical resection and biopsy	≤2	89	43
		3–20	80	78
		>20	20	19
Hoej *et al*[16]	161 consecutive patients, small intestine NETs tumours. Surgical resection only	≤2	85	
		3–20	50	
		>20	0	
Ahmed *et al*[13]	360 consecutive patients (112 stained) midgut NETs with liver metastases. Surgical resection and biopsy	≤2	82	60
		>2–20	65	45
		>20	0	6

Table 4 Estimated summary data

European Neuroendocrine Tumor Society grade	5-Year survival (%)	95% CI (%)	No.
1	88.5	85.1 to 92.0	977
2	70.3	62.2 to 78.5	726
3	25.1	12.3 to 37.8	208

Internal validity of the articles

The internal validity of the articles is described in table 1. Of particular relevance, we noted that the method used to obtain the Ki-67 result was not always apparent and varied between articles.

Part 1: articles reporting survival by ENETS grade

Part 1 of this systematic review took into account a total of 4322 patients, of which 2540 patients had a recorded Ki-67 LI staining. Sample size ranged from 45 to 837. The details of the studies are shown in tables 2 (articles used for the meta-analysis) and 3 (articles excluded from the meta-analysis due to incomplete ENETS grading or presenting only Kaplan–Meier curves).

The methodology used to produce the Ki-67 LI can affect the results. For instance, it has been shown that eyeballing Ki-67 LIs are not as accurate as using a formal counting methodology.[9] Therefore, for each article we ascertained which counting methodology was used and whether the count was done on surgically resected specimens or only biopsies. Out of the 10 articles used to produce summative results in part 1, one was shown not to use a formal counting methodology. Of the remaining nine articles, two articles were based on data from registries, obtained using varied counting methods dependent on the organisation involved. The results reported by these authors were not outliers. Due to the small nature of the article not using a formalised counting methodology and the relative size of the two articles using varied methodology, we believe it to be very important to include all articles within the final summative data and not exclude articles where 'eyeballing' might have been used.

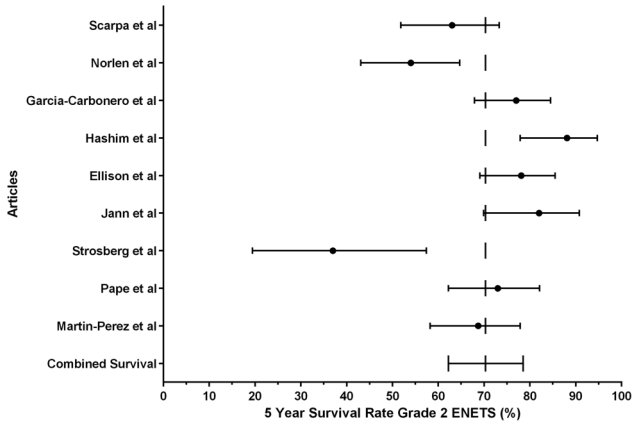

Figure 3 Forest plot depicting survival for patients with grade 2 gastroenteropancreatic neuroendocrine Tumours (Ki-67 3–20%). Dashed line indicates grade 2 combined estimate. ENETS, European Neuroendocrine Tumor Society.

The summation of data is reported in table 4 (based on articles in table 2 only). The 5-year survival rates were as follows: grade 1 89% (95% CI 85% to 92%, m=12 studies, n=977 participants), grade 2 70% (95% CI 62% to 79%, m=9, n=726) and grade 3 25% (95% CI 12% to 38%, m=10, n=208).

Figures 2–4 depict forest plots relating to ENETS grades 1, 2 and 3, respectively. Articles presenting 5-year survival rates for grade 1 are very closely grouped, with survival rates ranging from 80.4% to 100% (figure 2). For grades 2 and 3, there is far more variation between studies: the majority of authors report survival rates between 63% and 88.1%, but Norlen *et al*[22] and Strosberg *et al*[19] report noticeably lower survival rates of 54% and 37%, respectively (figure 3). The latter study had a small

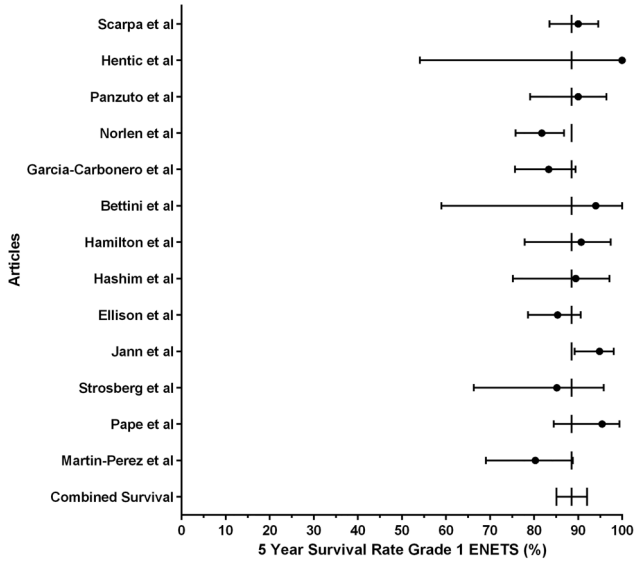

Figure 2 Forest plot depicting survival for patients with grade 1 gastroenteropancreatic neuroendocrine Tumours (Ki-67≤2%). Dashed line indicates grade 1 combined estimate. ENETS, European Neuroendocrine Tumor Society.

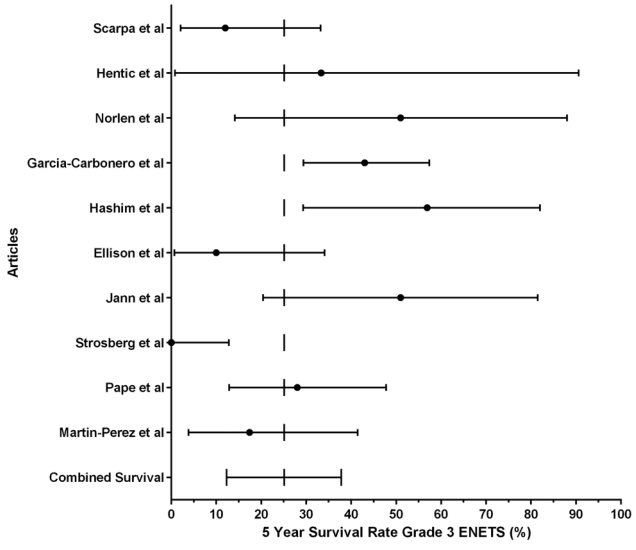

Figure 4 Forest plot depicting survival for patients with grade 3 gastroenteropancreatic neuroendocrine Tumours. Dashed line indicates grade 3 combined estimate. ENETS, European Neuroendocrine Tumor Society.

Table 5 Measure of heterogeneity of studies for each grade

Grading (%)	I^2
≤2	62.6
2–20	85.1
≤5	76.3
5–20	0.0
>20	85.3

population stained for Ki-67 (n=28) and solely investigated patients with metastatic GEP NETs. For grade 3, 5-year survival rates ranged from 0%[19] to 56.9%.[24] All papers presenting ENETS grade 3 5-year survival rates had low population sizes, ranging from 11 patients to 57 patients, which may in part explain the large variation between studies. It seems likely that the intention of the reviewed studies was to focus on patients with grade 1 and 2 disease.

Study heterogeneity was assessed by I^2; this describes the percentage of variability between observed effects that is due to study heterogeneity rather than sampling error. Heterogeneity was observed to be substantial; I^2 was >60% in each case, suggesting large variation across studies (see table 5).

Part 2: articles reporting survival using an alternative cut-off between grades 1 and 2 of 5% Ki-67 LI

Part 2 of this systematic review looked only at articles where the cut-off between grade 1 and grade 2 GEP NETS was made at Ki-67 LI of 5%. Articles reported survival for 900 patients in total, with sample sizes ranging from 41 to 259. Data could be summated from four articles (table 6), while for three additional articles only data relating to patients with a Ki-67 <5% were used (table 7). Table 8 shows that 5-year survival was 89% (95% CI 84% to 94%, m=7 studies, n=654 participants) for patients with Ki-67 ≤5% and 51% (95% CI 44% to 59%, m=4, n=183) for patients with Ki-67 5–20%.

Of the seven articles included in the final summative data in part 2, four were shown to use formal counting methodology, with two articles with ambiguity and one article that used eyeballing method.

Figures 5 and 6 depict forest plots relating to 5-year survival rates for patients with Ki-67 LI <5%, when 5-year survival ranged from 85.7% to 95%, and Ki-67 LI 5–20%, when 5-year survival ranged from 46.3% to 58%.

Study heterogeneity was substantial for the 5% cut-off, but was not apparent for the 5–20% grade (see table 8); the latter result may be due to the small number of studies included in this part of the analysis.

DISCUSSION

Ki-67 LI is an important prognostic marker in GEP NETs. Grading systems have provided a platform in which patient care may be stratified, both medically and surgically.[5–7] The ENETS/WHO classification systems have very helpfully provided a consistent structure to which NET research and treatment based on Ki-67 can occur.[5 6] Due to the rarity of this condition, there is a lack of large prospective studies, but a consistent approach has made it possible to systematically review and produce summary data.[1] This has allowed the accumulation of a large series of patients that would otherwise not be possible.

There has been much debate regarding the most appropriate Ki-67 cut-off values for the grading of NETs. Some authors have suggested cut-offs of 5% (G1) and 10% (G2) provide more discriminative prognostic information than the currently recommended 2% cut-off value.[28] Grading by the alternative 5% Ki-67 cut-off value appears in our review to provide more numerically distinct prognostic categories than the 2% Ki-67 cut-off, but we have no evidence that the groupings represent distinct clinical entities or that the 5% cut-off is more clinically relevant. Although we hesitate to draw wide-ranging conclusions, the results presented suggest that those in the 2–5% range for Ki-67 LI are more similar to those <2% than those between 5% and 20%, with regard to 5-year survival. It seems likely that in fact Ki-67 is linearly related to worse prognosis, with higher values representing increasingly poor prognosis.[30] More research must be undertaken to fully understand the implications of the Ki-67 value when planning treatment, for example, radical surgery.[31]

There are important limitations to this analysis. First, the data used were from retrospective studies, meaning that reporting and publication bias may be an issue. The articles also included contained a large variation in sample size, tumour subtypes, stage and treatments. For example, for the purposes of this review we have combined pancreatic and non-pancreatic GEP NETs. These diseases are often regarded as distinct entities. We

Table 6 Articles reporting survival by Ki-67 using a cut-off of 5% and 20%

Author	Patient demographic	Ki-67 notes		
		Ki-67 labelling index (%)	5-Year survival (%)	No.
Martin-Perez *et al*[28]	481 consecutive patients, (184 stained), pancreatic neuroendocrine tumours (NETs) and peripancreatic NETs. Surgical resection and biopsy	≤5 >5–20 >20	85.7 46.3 17.4	118 46 20
Bertani *et al*[29]	110 consecutive patients (41 patients stained) primary NET with unresectable liver metastases. Surgical resection only	≤5 >5–20 >20	90 58 20	10 21 10
Scarpa *et al*[20]	274 consecutive patients (237 patients stained) Pancreatic endocrine tumours operated on. Surgical resection and biopsy	≤5 >5–20 >20	91 52 12	166 93 30
Hentic *et al*[21]	45 consecutive patients, metastatic digestive endocrine carcinoma. All with liver metastases. Surgical resection and biopsy	≤5 >5–20 >20	95 52 33	19 23 3

Table 7 Articles where only patients with Ki-67 <5% were included in the meta-analysis

Author	Patient demographic	Ki-67 notes		
		Ki-67 labelling index (%)	5-Year survival (%)	No.
Begestuen *et al*[17]	258 consecutive patients (130 stained), small intestinal neuroendocrine tumours. Surgical resection and biopsy	<5	76	101
		≥5	61	29
Pape *et al*[18]	399 consecutive patients (259 stained), gastroenteropancreatic neuroendocrine tumours . Surgical resection and biopsy	≤5	96	133
		>5–10	80	67
		>10	35	59
Bettini *et al*[15]	180 consecutive patients (49 patients stained), non-functioning pancreatic tumours. Surgical resection only	≤5	88	107
		>5	33	45

Table 8 Estimated summary data for survival by Ki-67 using a cut-off of 5% and 20%

Ki-67 labelling index (%)	5-Year survival (%)	95% CI (%)	No.
<5	89.1	84.1 to 94.0	654
5–20	51.3	44.0 to 58.5	183

have also included series where the main treatment was resection. Combining all these series means that substantial study heterogeneity was observed, and any comparisons between articles must be made with the clinical features of included cases in mind.

Additionally, authors were not contacted regarding unpublished work or original datasets. The main effect of this was to limit inclusion of some large series, for example, Khan *et al*.[8] There were differences in the methodology used by different authors to calculate the Ki-67 LI. There were clear differences in the method in which authors presented survival data. However, to our knowledge, no other meta-analysis has been attempted in this area, and we believe that these data provide clinically important data to aid in the provision of care for patients with GEP NETs.

CONCLUSION

In a tumour type in which large prospective trials are notably lacking, this systematic review and summary data provide a substantial body of evidence relating to the use of Ki-67 LI as a prognostic marker in GEP NETs. It has provided us with estimates of 5-year survival rates for both ENETS and 5% cut-off grading systems. These results have clinical relevance, providing an extra tool to guide a clinician's judgement regarding prognosis. However, it is only one aspect of the overall management of patients with GEP NETS.

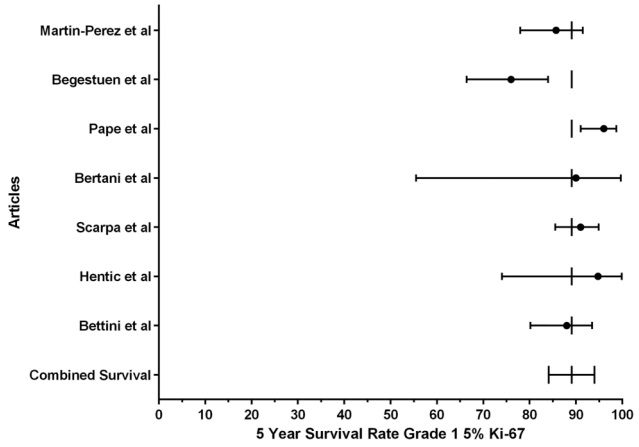

Figure 5 Forest plot depicting survival for patients with Ki-67 <5%. Dashed line indicates grade 1 combined estimate.

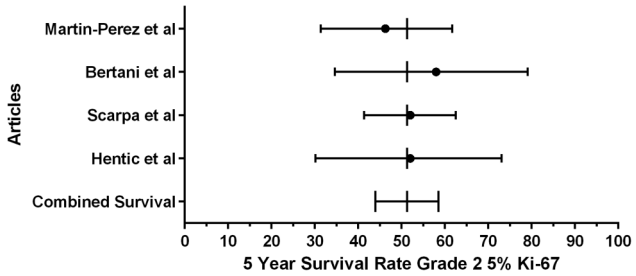

Figure 6 Forest plot depicting survival for patients Ki-67 5–20%. Dashed line indicates grade 2 combined estimate.

Take home messages

- Proliferation rate as assessed by Ki-67 labelling index (LI) has been shown to be strongly associated with prognosis in neuroendocrine tumours and is included in a wide range of neuroendocrine tumour (NET) classification systems.
- The 2007 European Neuroendocrine Tumor Society grading system describes the use of ≤2% (grade 1), 3–20% (grade 2) and >20% (grade 3). There is, however, some debate regarding the most suitable cut-off values. Other authors have suggested cut-off values of 5% or 10% may provide more discriminative prognostic information.
- A meta-analysis of survival data was performed. Heterogeneity was substantial. 5-Year survival for patients with grade 1 and 2 gastroenteropancreatic NETs were estimated to be 89% (95% CI 85% to 92%, m=12 studies, n=977 participants) and 70% (95% CI 62% to 79%, m=9, n=726), respectively.
- Using an alternative grade 1/2 boundary of 5%, 5-year survival rates for Ki-67≤5% and 5–20% were estimated as 89% (95% CI 84% to 94%, m=7, n=654) and 51% (95% CI 44% to 59%, m=4, n=183), respectively. It seems likely that Ki-67 is linearly related to survival, with those in the 2–5% range for Ki-67 LI more similar to those <2% than those between 5% and 20%. We support keeping the Ki-67 cut-off for grading unchanged because consistency aids research in this rare tumour type.

Handling editor Cheok Soon Lee

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Contributors The statistical analysis was performed by SME. Selection of papers and extraction of data was performed by SR-T and JC. All authors contributed to the wording of the manuscript and clinical interpretation of the data.

Competing interests None declared.

Ethics approval NREC.

Provenance and peer review Not commissioned; externally peer reviewed.

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