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Ansa Riaz Date

Ki67-Adjusted Mitotic Score (KAMS): a novel prognostic metric in well-differentiated pancreatic neuroendocrine tumors

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By

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Bachelor of Science
Georgia State University
2014

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An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
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Abstract

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Introduction: The grading of PanNETs presents numerous diagnostic challenges and limits our ability to accurately predict their clinical behavior. The current WHO grading system uses Ki67 index (KI) and/or mitotic count (MC) to obtain a histological grade (G1, G2, G3) for tumors. However, there are numerous ambiguities in this grading system, including different scales of measurement of Ki67 and mitosis, sub-optimal categorical cut-offs and lack of consensus on best counting methodologies. To fully exploit the prognosticating power of both KI and MC, we rationally integrated them and derived a new metric, Ki67-adjusted mitotic score (KAMS), which represents the proportion of mitotic cells amongst cycling Ki67-positive tumor cells.

Methods: Among 97 PanNETs KAMS was calculated by transforming monotonic ordinal MC into % mitotic cells and dividing it by % Ki67. Survival stratification was done via Kaplan-Meier estimator based on KAMS and KI.

Results: Using current established thresholds in PanNET grading, the survival stratification for KI showed significance between high (Grade 3) and low (Grade 1) Ki67 survival percentages ($p=0.02$). However, KAMS was able to stratify patients into two statistically significant survival groups ($p= 0.04$): The "above-threshold KAMS" group had 74% survival while the "below-threshold KAMS" group had a 53% survival. The ideal threshold of KAMS was .0033.

Conclusion: This study underscores the significance of our new metric, KAMS, to provide a more accurate risk prediction in PanNETs. Low KAMS significantly predict poor prognosis in PanNETs and is superior to Ki67 in survival stratification. Although validation of the KAMS score in other larger datasets is warranted, it appears that KAMS could significantly improve PanNETs prognostic risk determination by identifying individuals at higher risk of progressing to metastatic disease.

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Table of Contents

CHAPTER 1: Literature Review and Introduction……1
- **Epidemiology in the United States ……1**
- **Global Burden……4**
- **Clinical Background……8**
- **Treatment Options……9**
- **Grading and Related Issues……10**
- **Research Question ……13**

CHAPTER 2: Manuscript……14
- **Abstract……15**
- **Introduction……16**
- **Methods……18**
- **Results……19**
- **Discussion……24**
- **References……27**

CHAPTER 3: Public Health Implications……28

REFERENCES……30

Chapter 1: Literature Review and Introduction

Pancreatic Neuroendocrine tumors are epithelial tumors that originate from diffuse neuroendocrine cells. They are a clinically rare and heterogeneous disease of the pancreas, which present with varying clinical symptoms. Furthermore, the potential for malignancy varies depending on the tumor site, metastasis and the degree of differentiation present (Uppin, 2017). In the literature review below, the epidemiology including the incidence, prevalence, and mortality further expounds the conclusion that there is an increasing diagnosis of this tumor not only in the United States but globally as well. The clinical background section reinforces the need for continued research in order to find effective therapeutic options and to resolve the debates surrounding classification and grading.

Epidemiology in the United States

The main source of data for incidence and prevalence of pancreatic neuroendocrine tumors in the United States comes from the Surveillance, Epidemiology, and End Results (SEER) Program, which was established in 1973 by the National Cancer Institute. As of 2010, there are 18 locations that report to this registry; Connecticut, Iowa, New Mexico, Utah, Hawaii, Georgia, New Jersey, Louisiana, Kentucky, and California and the metropolitan areas of Detroit and Seattle with the addition of the Alaska Native and Arizona Indians population (National Cancer Institute, 2016). The registry covers approximately 28% of the entire US population (National Cancer Institute, 2016). The image below shows the geographic distribution of the SEER registries.

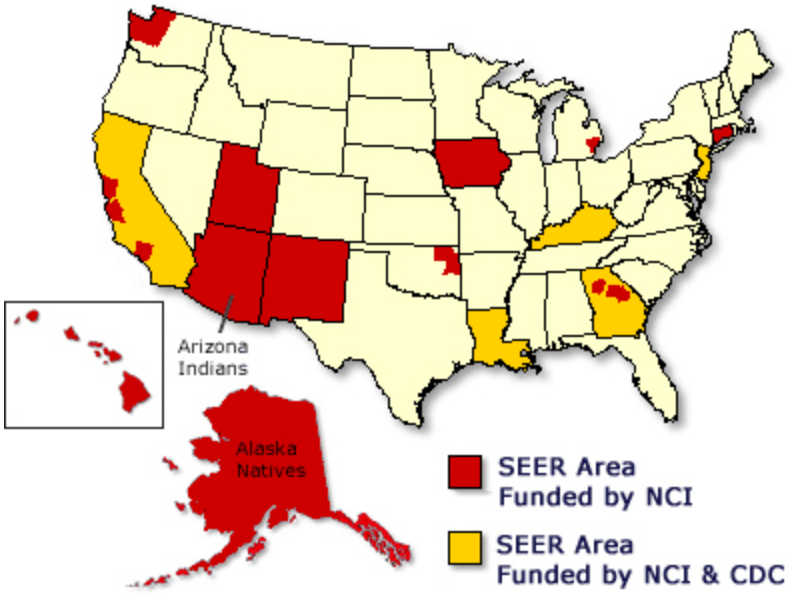

Figure 1: Geographic distribution of the SEER registries, 2017. NCI: National Cancer Institute, CDC: Center for Disease control

Unfortunately, there is no literature available analyzing incidence and prevalence of pancreatic neuroendocrine tumors using data from the SEER 18 registry. However, there is analysis of data from the registries prior to the 18 locations it covers currently.

Using the SEER registry from the years 1988-2001, there were 29,729 cases of pancreatic cancer with confirmed microscopic results. The graph below shows the survival rates for endocrine and exocrine pancreatic cancers. For cancers that arose from the exocrine pancreas the 5-year relative survival rate was approximately 4% whereas cancers arising from the endocrine pancreas had a better prognosis at 42% (Ries, 2007).

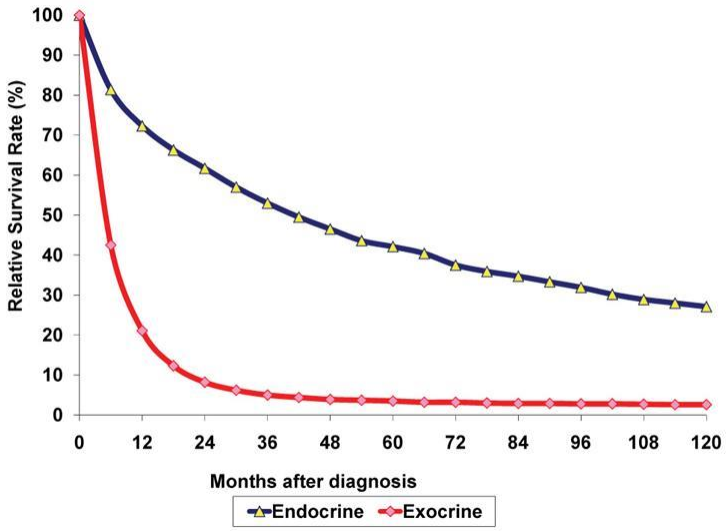

Figure 2: Cancer of the Pancreas: Relative Survival Rates (%) by Histologic Subtype, Ages 20+, 12 SEER Areas, 1988- 2001

A specific type of cancer that arises in the endocrine pancreas is the Neuroendocrine tumor (NET). Using the same SEER registry mentioned above (1988-2001), only 3.3% of all pancreatic cases arise from the endocrine pancreas and among those only 1.4% (411 cases) were specifically classified as pancreatic neuroendocrine tumors (Ries, 2007). Data from the SEER 17 registry shows us a glimpse of the frequency of the most common primary site of gastroenteropancreatic NET (GEP-NET) cases from the year 2000-2007. Pancreatic NETs account for 7% of all GEP-NET, coming after rectum, small intestine and colon (Lawrence, 2011). Data collected from SEER in the years 1973-2007 shows that incidence rate has more than doubled from 0.17 per 100,000 in the early 70's to 0.43 per 100,000 in 2007(Lawrence, 2011). This increase in incidence may partly be due to more clinical awareness and better diagnostic tools and methods used by pathologists to confirm pancreatic cancer. Even though the incidence rate is steadily increasing, this is still a relatively rare tumor and data is limited. In a separate analysis of the SEER database, the estimated 28-year prevalence of pancreatic NETs in the United States was 2,705 cases on January 1, 2003 (Yao, 2007).

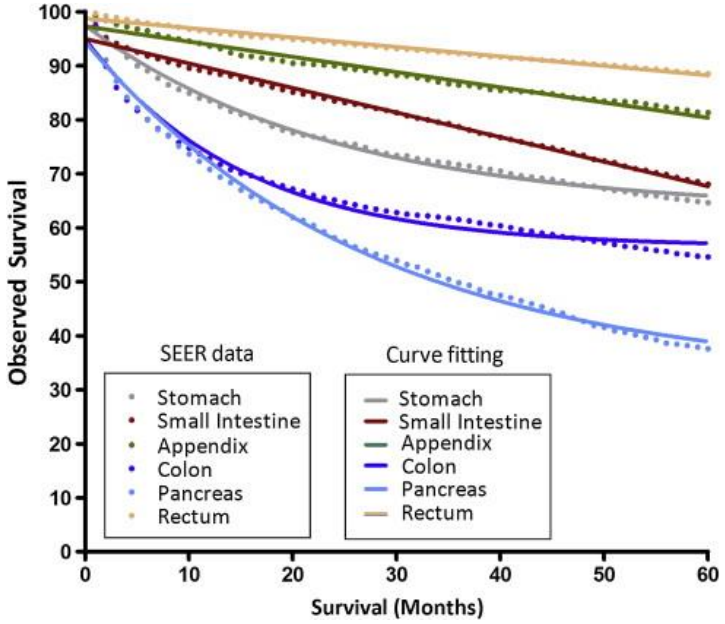

Figure 3: Observed five-year survival rates for GEP-NET primary sites in SEER 17 (1973–2007).

The graph above shows the 5-year survival for primary sites of GEP-NET using the SEER 17 registry from 1973-2007 (Fraenkel, 2012). Even though tumors arising in the endocrine pancreas have an overall better prognosis compared to those arising in the exocrine pancreas, the survival rate for pancreatic NETs is the lowest when compared to other neuroendocrine tumors. The 5-year relative survival rate for pancreatic NETs was 37.6% as compared to rectal NET which was 88.5% (Lawrence, 2011). To further detail this, an analysis conducted over 35,000 NET cases using SEER 17 showed that the median survival duration was 2 years for patients with metastatic pancreatic NET (Yao, 2007). However, an institutional study conducted using 900 NET patients showed that the median disease free survival for pancreatic NETs is 4 years for patients who underwent resection of the primary site (Ter-minassian, 2013). Analyzing the pancreatic NET demographic shows that there is a slight male predominance and majority of the cases, approximately 84% are white and 10% are black. The mean and median age at diagnosis is 58 years (Halfdanarson, 2008 and Yao, 2007).

Global Burden

When conducting a literature review for the global burden of disease, most of the data comes from high income countries or upper middle income countries. The absence of a national cancer surveillance system in low income countries perhaps leads to the lack of population-wide data. The articles mentioned below reveal many nuanced differences like frequency of symptomatic GEP-NETs, rate of distant metastasis, and presence of molecular biomarkers between the global regions. However, I have restricted my comparative analysis to incidence, prevalence, survival data, and the demographics of the population.

Europe:

The German Neuroendocrine Tumor registry collected data from 29 centers across Germany and obtained 1,263 patients in the years between 2004 and 2007. The data shows that the median age at diagnosis was 56 with slight male predominance and the median overall survival was 2.5 years which is relatively the same as the United States at 2 years. However, in Germany the pancreas is the primary site in 31% of cases among all NETs which is significantly different than the 7% reported in the United States (Ploeckinger, 2009). Databases from Spain and Italy have shown similar results where the pancreas is the primary site for NETs. The registries in these countries are not population based, thus there is no incidence or prevalence data to comparatively analyze (Faggiano, 2012 and Garcia-Carbonero, 2010).

One such country that doesn't have a central registry system is France. There is no data available on prevalence and the management protocol of GEP-NETs, however, data on 668 patients was gathered from 87 centers in 2001-2002. It showed that unlike Germany, Spain, and Italy, the pancreas was the second most common primary site after small colon and bowel (Lombard-Bohas, 2009). Additionally, more recent studies from Europe show that there is an upward trend in the incidence rate rising from 0.1 of previous decades to now 0.3 per 100,000. This is the same trend occurring in the United States where the latest incidence rate was 0.43 in 2007 (Fraenkel, 2012). One article states, "Outside the US, five-year survival rates were reported: 45% between 1993 and 2004 in Norway, 61% in Spain, and 63.5% in Tuscany, Italy (1985–2005)" (Fraenkel, 2012). No studies have been conducted to understand why the survival rate differs so much among such geographically close countries.

East Asia:

A study conducted in Japan, the first of its kind, in 2005 surprisingly reported epidemiological data because it was a nationwide survey. The incidence rate for functioning and non-functioning pancreatic NETs was 1.01 and the prevalence rate was 2.23 per 100,000 which is more than double the amount reported within United States at 0.43 per 100,000 (Ito, 2010). Additionally, US epidemiological data shows that this cancer is more common among Caucasians and the incidence rate among Asian Americans was 0.25 per 100,000, which is glaringly different in Japan (Ito, 2010). The variations in these findings may be due to dietary and environmental differences or more rigorous diagnosing methods in Japan. While the mean age of onset is the same as the US and other European countries, 62% of the cases were from females which contrasts with the usual even split between males and females or in some countries with slightly male predominance. Additionally, there was equal distribution of functioning and non-functioning pancreatic NETs, 49% and 47% respectively whereas, the United States tends to have more nonfunctional tumors. Functioning neuroendocrine tumors are those that secrete extra amount of hormone, such as gastrin, insulin, and glucagon, which in turn cause signs and symptoms (Ito, 2010).

Most countries in Europe, except for France, show that the pancreas is the most common primary site for NETs however in East Asia, South Korea and China, both show that the most common primary site was the rectum followed by the pancreas. The difference for this is unclear but it may be due to racial or ethnic differences. There is very limited data available in these countries however, a study conducted in South Korea from 2000-2009

provides some insights. A continuous increase of GEP-NETs has occurred where the incidence in 2009 was nine times that of the incidence in 2000 (Cho, 2012).

Unfortunately, the analysis wasn't taken further into specific types of NETs so, it isn't possible to see the trend for pancreatic NETs. In Western China, the median age at diagnosis was 52 years, which is several years younger than the age at diagnosis in most European countries and in the United States which is at 58 years. Additionally, there is a steady increase in prevalence of GEP-NETs in females, from 24% of the cases analyzed in 2009 to 58% in 2013 (Guo, 2016).

Middle East:

A patient database from 2001-2012 was used to obtain information on NETs in Lebanon. The primary site of GEP-NETs was the pancreas and most cases were detected early on in grade 1. The table below shows a breakdown of GEP-NETs comparing Lebanon to other European countries(Kourie, 2016).

	Lebanon	Austria	Germany	Spain	France	Poland	China
Years	2001-2012	2004-2005	1999-2010	2000-2009	2001-2002	2002-2011	2001-2013
Reference	Kourie et al. 2015	Niederle et al. 2010	Begum et al. 2010	Galvan et al. 2014	Lombard-Bohas . 2009	Lewkowicz et al . 2015	Jiao et al. 2015
Number of patients	89	285	2009	110	668	122	154
Pancreas	24.7%	11.9%	41.8%	20.0%	37.4%	18.9%	40.90%
Stomach	20.8%	23.5%	7.9%	16.4%	5.9%	17.2%	22.10%
Duodenum	18.2%	5.8%	5.8%	25.5%	51.1%	20.5%	4.50%
Small intestine	11.7%	15.9%	31.6%				
Colon	9.1%	7.2%	8.4%	20.9%		9.0%	27.90%
Rectum	2.6%	14.4%			2.1%	18.9%	
Appendix	13.0%	21.3%	4.5%	17.3%	3.5%	15.5%	

Table 1: Comparison of the Distribution of GEP-NET According to their Primary Site in Different Countries

The mean age was 58.7 which is similar to the 56 years in other European countries like France, China, Germany, and Spain (Kourie, 2016). No further information was available.

Clinical Background

The data provided in the previous section has established that Pancreatic NETS are a rare tumor not exclusive to the United States but also in other countries across the globe. In terms of the clinical origins, there are neuroendocrine tumors that originate in other organs of the GI system, however experts agree that pancreatic NETs should be treated and investigated separately as they have distinct biological differences which causes them to respond differently to drug therapy (Kulke, 2011).

Pancreatic NETs are typically randomly occurring and can arise most commonly in the pancreatic head but can also occur in other parts of the pancreas (Reid, 2014). It is also noted in literature that pancreatic NETs "may also arise in a background of familial syndromes including von Hippel–Lindau (VHL) syndrome, tuberous sclerosis complex (TSC), neurofibromatosis type 1 (NF1), and multiple endocrine neoplasia type 1 (MEN1) " (Reid, 2014). Those patients with MEN-1 syndrome are 80% likely to develop multiple pancreatic tumors (Reid, 2014).

The tumors originate in the islet cells and may either be functional (produce hormones) or non-functional. The tumor is named according to the hormone or vasoactive peptide it secretes. Some examples of functional tumors are Gastrinoma, Insulinoma, Glucagonoma, Somatostatinoma, VIPoma. Up to 50% of gastrinomas are malignant and clinically present with hypersecretion of gastrin which is associated with Zollinger–Ellison syndrome (ZES). Consequently, patients present with diarrhea and gastric hyperacidity and are more likely to have distant metastasis to the liver (McKenna, 2014). Additionally, 15-35% of patients with gastrinomas are also likely to be associated with MEN-1 syndrome. In contrast to gastrinomas, only 10% of insulinomas are malignant because they are detected

very early due to the obvious symptoms like hypoglycemia that occur due to overproduction of insulin ("Pancreatic Neuroendocrine", 2015). In even further contrast to the benign insulinomas, glucagonomas are diagnosed much later, thus they are malignant in 75% of the cases. Compared to other pancreatic NETs, glucagonomas are much larger in size and are easily visible on a CT scan however, the physical symptoms are subtle and lab results must show a very large fluctuation in serum glucagon level to confirm a diagnosis, thus leading to the resulting delay.

About 15% of pancreatic NETS are found to be non-functional, however there is good reason to believe that this number has risen over the recent years due to the improvements in diagnostic modalities (Reid, 2014). These are also diagnosed in later stages as they secrete inactive peptides Some examples of non-functional tumors are neurotensin, alpha-hcg, neuron-specific enolase, pancreatic polypeptide, and Chromogranin A.

One flaw in this classification system is that research has found that most tumors secrete multiple hormones or the hormone productivity changes over time, thus rendering the belief that tumor functionality is linked to prognosis ineffective (Reid, 2014).

Treatment Options

Surgery is the most common and only curative treatment modality employed by oncologists across the United States and globally ("Pancreatic Neuroendocrine", 2015). Surgery effectively eliminates the hypersecretion of hormones from the tumor rsulting in symptom relief. However, surgery is not recommended for patients with MEN-1 syndrome as those patients have multiple lesions and extensive pancreatic resections are not curative.

Within the medical community, chemotherapy has been an area of debate as pancreatic NETs are slow-growing, thus they don't respond effectively to the established cytotoxic drugs used in chemotherapy. Research is now more focused on developing targeted therapies like the drug, everolimus, which is currently in phase IV of clinical trials. Other drugs like Cabozantinib (a tyrosine kinase inhibitor) and sunitinib (a vascular endothelial growth factor inhibitor) are also being heavily researched (Reid, 2014).

Grading and Related Issues

The most widely used classification system is the 2010 World Health Organization Classification system. The system divides tumors into 2 categories: well-differentiated tumors (Grade 1 and 2) and poorly differentiated (grade 3) carcinomas. Two parameters are used to determine the grade: mitotic count and ki-67 index. Below is the scale that is used (Reid, 2014).

Differentiation	Grade	Mitoses[a]/10 |HPF	Ki-67[b] proliferation |index (%)
Well-differentiated PanNET	Grade 1	<2	<3
	Grade 2	2-20	3-20
Poorly-differentiated PanNEC	Grade 3	>20	>20

Table 2: Classification system for pancreatic neuroendocrine tumors. Modified from WHO 2010.

To determine the number of cells undergoing mitosis, the WHO guidelines suggest pathologists view the slide in 50 high power fields and to determine the Ki-67 count at least 500 cells must be counted. In cases, where these two numbers fall in different grade categories, the higher grade level must be reported (Reid, 2014). Morphology is not incorporated into the grading scheme as it is with, for example, breast cancer.

There are many issues with this grading system, starting with the concern that the Ki-67 index covers the entire cell cycle (G1, S, G2, and M) so, it is almost always higher then the mitoses number. So, essentially the mitotic number is irrelevant in this system. Furthermore, different hospitals adopt different practices when it comes to counting Ki-67 positive cells. A method previously widely used was the "eye-balling" method wherein pathologists approximate the number of ki-67 cells seen on a slide. This has been proven to not be reproducible from person to person (Tang, 2012). Other methods that are used are automated counting systems, manual counting under a microscope without a grid, and manually counting camera captured and printed pictures. The reproducibility varies between each of these methodologies but it has been shown that the most reliable and cost-effective method is the manual counting of cells from camera captured-printed pictures. However, it is largely left to the discretion of the hospitals as to which method to use or in some cases it even varies from pathologists to pathologists (Reid, 2016). Even with all of these potential variations in prognostic indicators, Ki-67 has still been shown to be correlated with clinical outcome (Reid, 2014).

Novel tumor-grade-metastasis system

Researchers in China have tried to incorporate morphological parameters into the current staging system. They combined the protocol from the AJCC 2010 staging manual and the WHO 2010 grading classification (Yang, 2016). Staging consists of three parameters TNM (tumor size, node metastasis, and metastasis to other organs) however in this study tumor-grade-metastasis was evaluated. Researchers removed the parameter of lymph node metastasis and instead incorporated G_a (collectively combined G1 and G2) and G_b (G3 cases). "The new TGM staging system was determined as follows: stage I was

defined as T1–2, *Ga*, M0; stage II as T3, *Ga*, M0 or as T1–3, *Gb*, M0; stage III as T4, *Ga–b*, M0 and stage IV as any T, M1" (Yang, 2016). Survival analysis was conducted on data obtained from 120 patients from one institution. Stage I patients showed better survival than those in stage II, and stage II showed better survival when compared to stage III and IV. However the differences between stage III and IV were negligible.

There are a few glaring flaws within this study, for example most of the cases were functional insulinomas. This would influence the staging as some pancreatic NETs are more metastatic and larger in size then others due to late diagnosis. Also, as discussed in the previous section there are problems inherent to the WHO grading system and incorporating WHO grading into another schematic is not going to resolve the issues that are present may pose problems in reproducibility of these results (Yang, 2016).

Research Question:

In sum, the correct grade, among other factors, helps doctors develop a strong treatment plan and determines the outcome and course of the cancer. The grading of pancreatic NETs presents numerous challenges and limits our ability to accurately predict their clinical behavior. Some aggressive pancreatic NETs may appear deceptively bland while others with overt "malignant" cytology may exhibit indolent behavior. The current WHO grading system uses Ki67 index (KI) and/or mitotic count (MC) to independently grade (Grade 1, 2, 3) tumors. However, there are numerous ambiguities and gaps in this grading system, including different scales of measurement of Ki67 and mitosis, sub-optimal categorical cut-offs, numerous counting methodologies, and lack of consensus on best counting methodologies. Additionally, KI is almost always higher than MC suggesting that the latter may be an unnecessary step in tumor grading.

To fully exploit the prognosticating power of both KI and MC, we propose to rationally integrate them and derive a new metric, Ki67 adjusted mitotic score (KAMS) which represents the proportion of mitotic cells amongst cycling Ki67 positive tumor cells. We hypothesize that the proportion of mitotic cells among the Ki67 positive cells decreases as one moves from Grade 1 to Grade 2 to Grade 3. Pathologists consider Ki-67-positive cells as “actively dividing”, leading to the mistaken view that an increase in KI reflects an increased proportion of mitotic cells. For a tumor to progress, there is a clear switch from cell proliferation to migration thus, we postulate that low-grade tumors undergo rapid mitotic turnover and will have a high KAMS while high-grade tumors focus more on “metastasis” and will have a lower KAMS.

$$\text{KAMS} = \frac{\%\ \text{mitotic cells}}{\%\ \text{Ki67 positive cells}}$$

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Abstract

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Methods: Among 97 PanNETs KAMS was calculated by transforming monotonic ordinal MC into % mitotic cells and dividing it by % Ki67. Survival stratification was done via Kaplan-Meier estimator based on KAMS and KI.

Results: Using current established thresholds in PanNET grading, the survival stratification for KI showed significance between high (Grade 3) and low (Grade 1) Ki67 survival percentages ($p=0.02$). However KAMS was able to stratify patients into two statistically significant survival groups ($p= 0.04$): The "above-threshold KAMS" group had 74% survival while the "below-threshold KAMS" group had a 53% survival. The ideal threshold of KAMS was .0033.

Conclusion: This study underscores the significance of our new metric, KAMS, to provide a more accurate risk prediction in PanNETs. Low KAMS significantly predict poor prognosis in PanNETs and is superior to Ki67 in survival stratification. Although validation of the KAMS score in other larger datasets is warranted, it appears that KAMS could significantly improve PanNETs prognostic risk determination by identifying individuals at higher risk of progressing to metastatic disease.

Introduction

Neuroendocrine tumors originate in many organs of the Gastrointestinal system, however, experts agree that pancreatic NETs should be treated and investigated separately as they have distinct biological differences which causes them to respond differently to drug therapy (Kulke, 2011). Pancreatic NETs are typically sporadically occurring and can arise most commonly in the pancreatic head but can also occur in other parts of the pancreas (Reid, 2014). If the tumor originating in the islet cells displays symptoms of hormone hypersecretion, then it is termed as a functional tumor as it has the potential to secrete endocrine hormones such as gastrin, insulin, and glucagon. If the tumor doesn't display any physical symptoms, it is termed asymptomatic.

The grading of pancreatic NETs presents numerous diagnostic challenges and limits our ability to accurately predict their clinical behavior. Some aggressive Pancreatic NETs may appear deceptively bland while others with overt "malignant" cytology may exhibit indolent behavior. The current WHO grading system uses Ki67 index (KI) and/or mitotic count (MC) to independently obtain a histological grade (G1, G2, G3) for tumors as shown in the table below (Reid, 2014) .

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determine the Ki-67 count at least 500 cells must be counted. In cases, where these two numbers fall in different grade categories, the higher grade level must be reported (Reid, 2014). This is problematic as the Ki-67 index covers the entire cell cycle (G1,S,G2, and M), so it is almost always higher then the mitotic count leaving the mitotic number irrelevant in this system.

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To fully exploit the prognosticating power of both Ki-67 and mitotic count, we propose to rationally integrate them and derive a new metric, Ki67 adjusted mitotic score (KAMS) which represents the proportion of mitotic cells amongst cycling Ki67 positive tumor cells. We hypothesize that the proportion of mitotic cells among the Ki67 positive cells decreases as one moves from Grade 1 to Grade 2 to Grade 3. Pathologists consider Ki-67-positive cells as "actively dividing", leading to the mistaken view that an increase in KI reflects an increased proportion of mitotic cells. For a tumor to progress, there is a clear switch from cell proliferation to migration thus, we postulate that low-grade tumors undergo rapid mitotic turnover and will have a high KAMS while high-grade tumors focus more on "metastasis" and will have a lower KAMS. Thus, the aim of this study is to:

a) confirm that mitotic count has a meaningful place within the grading system and b) confirm that KAMS is a better prognosticator then Ki-67 alone.

$$\text{KAMS} = \frac{\%\ \text{mitotic cells}}{\%\ \text{Ki67 positive cells}}$$

Patients and Methods

Retrieval of cases

This retrospective study was carried out on all cases of pancreatic NETs (97 cases) diagnosed at the Pathology Department at Emory University Hospital, Atlanta, GA during the period from March 1997 to May 2013. Patients were not excluded based on the type of pancreatic neuroendocrine tumor diagnosed. The following data was extracted from pathology reports retrieved from electronic medical records: age, sex, race, tumor grade, tumor size, tumor stage, ki-67, mitotic count, and presence of lymph vascular invasion, perineural invasion and nodal metastasis.

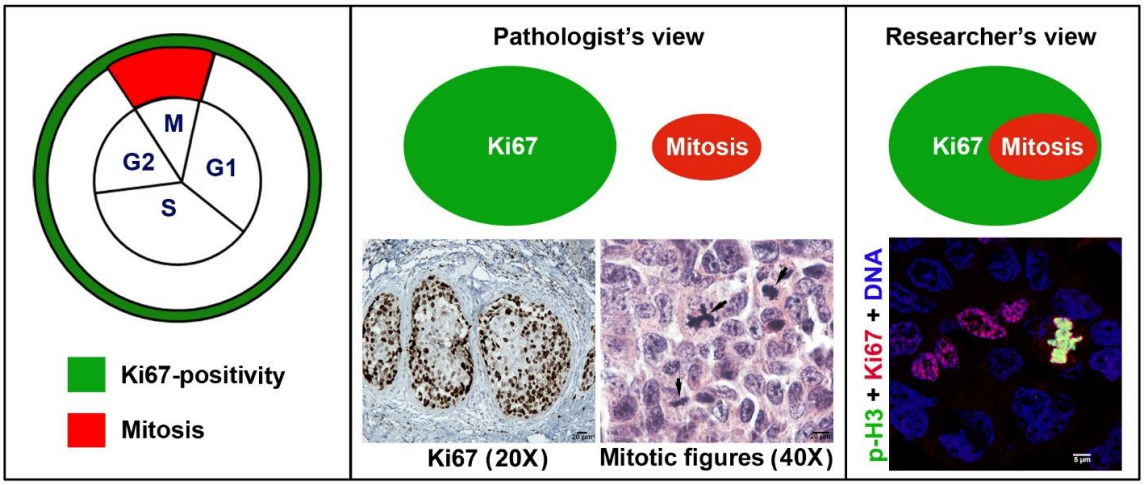
Statistical Analysis

Data was analyzed using SAS Version 9.4. Numerical data were expressed as mean and standard deviation or median and range as appropriate. For quantitative data, comparison between the two groups was done using either Student's *t* test or Analysis of Variance (ANOVA) as appropriate. KAMS was calculated by transforming monotonic ordinal mitotic count into % mitotic cells and dividing it by % Ki67. Survival stratification was done via Kaplan-Meier curves based on KAMS. To identify the ideal threshold cutoff of KAMS for stratifying patients based on survival, we identified the value, which gave the optimal log rank between groups. ANOVA was used to compare mean KAMS values between grades. A p value <0.05 was considered significant.

Results

The proportion of mitotic cells amongst the proliferative population within a tumor provides a measure of the risk associated with the tumor due to erroneous mitoses. This "dangerous" fraction of proliferating cells could potentially be quantitated with a high degree of accuracy by simultaneous visualization of both mitotic and Ki67-positive cells in the same field. Figure 1 below shows a schematic representing divergent perspective of a pathologist and a researcher regarding an actively dividing cell.

Figure 1: Pathologists view Ki67-positivity and mitosis as two mutually exclusive events in cell cycle, whereas a researcher views mitosis as a subset of the full cycle of a proliferating or Ki67-positive cell.



A total of 97 cases were used in this retrospective analysis with a fairly even split between males and females at an average age of 52 years. Table 1 below highlights the clinicopathological features of the dataset. In order to have more cases in each level, the stages were divided into four levels as opposed to the more detailed sublevels of the AJCC staging system. Specifically, there was only one case that was categorized as stage three and this may have had implications in the survival analysis. To conduct survival analysis, the current status of patients was collected with less than one fifth of the sample not alive. The dataset had 70 cases whose Ki-67 and mitotic count were obtained by adhering to the

gold-standard of ensuring that a minimum of 2000 cells were counted. The Ki-67 and mitotic count for the remaining 27 cases were taken from pathology reports in order to increase sample size.

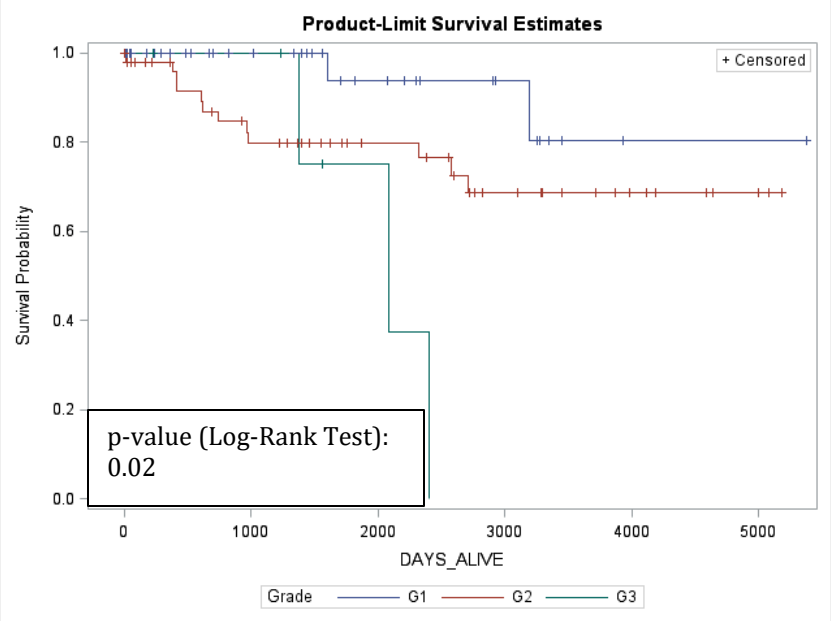
Table 1. Demographic of patients and clinicopathological features of pancreatic NETs

Characteristics	N	Mean or No.	SD or %
Gender, female	97	53	54.6%
Age, yrs	97	52.31	14.02
Clinical Stage	96		
1		48	50 %
2		29	30.2 %
3		1	1 %
4		18	18.8 %
Grade by WHO	97		
1		38	39.2 %
2		53	54.6 %
3		6	6.2 %
Status	97		
Dead		17	17.5 %
Alive		80	82.5 %
Metastasis	97		
Yes		18	18.6%
No		79	81.4%

A survival analysis of patients stratified by the WHO grading system was conducted. Figure 2 below shows that overall survival (OS) varied considerably based on tumor grade, with an OS rate of 80% for low-grade tumors, 69% for intermediate-grade tumors, and 0% for high-grade tumors. The statistical significance is only between Grade 1 and Grade 3 (p=.02). This significance likely occurred due to the disproportionate number of cases in grade 3 (6 cases) as compared to grade 1 (38 cases). There was no prognostic significance between grade 1 and 2 (p=0.36) or grade 2 and 3 (p=0.99) due either to very similar survival or due to the small sample size. Additionally, there was no statistical difference between the grading levels when analysis was conducted on the 70 “gold-standard” cases.

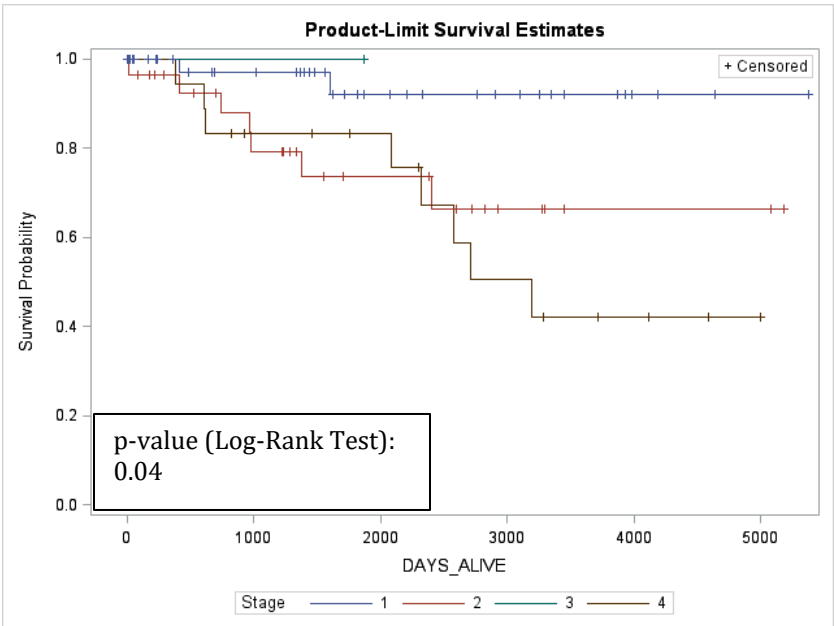
To determine if the AJCC staging system is a better indicator of survival, a second survival analysis was conducted. Figure 3 shows that there is a significant difference between

Figure 2: Kaplan- Meier survival curves of patients stratified via the current WHO pancreatic grading system. Only significant difference is between G1 and G3. (OS: 80 v 69 v 0, Log-rank p= .02)



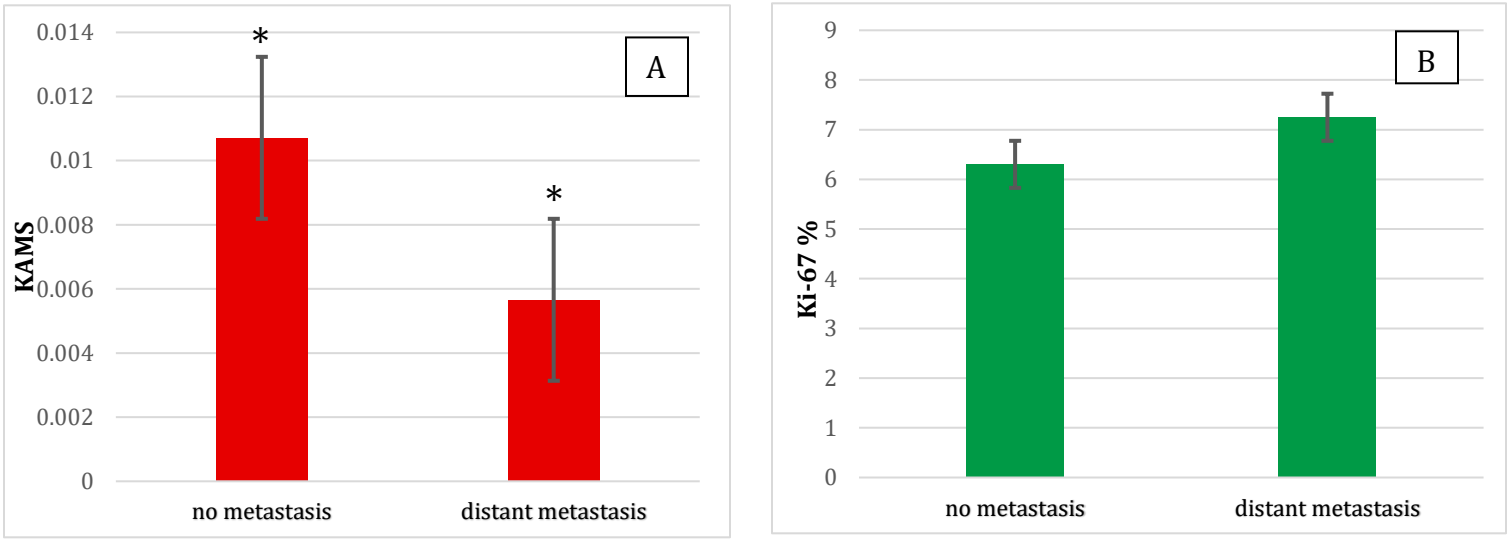
survivals when stratified by stage. The OS rates for AJCC classification stages I, II, and IV were 92%, 66%, and 42%, respectively (*P* = .04). Stage 3 overall survival was disregarded, as there was only one case. It can be concluded that stage is a better indicator of survival then grade in the case of pancreatic NETs.

Figure 3: Kaplan- Meier survival curves of patients stratified via the current AJCC pancreatic staging system. (92% v 66% v 42%, Log-rank p= .04)



Patients with disease progression to the liver or spleen were considered distant metastasis. Lower mitotic frequency was associated with distant metastasis (Figure 4a, p=.003) as there was a significant difference between KAMS values of patients that were grouped based on reoccurrence status, either distant (n=18) or disease free (n=79). This is further proven as Figure 4b shows that there is no significance in the mean Ki-67% values for cases with distant metastasis and without metastasis (n=93, p=0.66). Figure 2 has already confirmed that the Ki-67 based grading system isn't a strong indicator for overall survival and additionally, there was no meaningful or statistical difference between ki-67% among the AJCC stages (n= 93, p=0.98).

Figure 4: A. Bar graph representing mean KAMS for patients with (.0056 ± .0038) and without metastasis (.0107 ±.0116, p=.003) **B**. Bar graph representing mean Ki-67% for patients with (7.25 ± 6.4) and without distant metastasis (6.3 ±12.3, p= 0.66) * denotes significance at p <.05



When stratifying patients based on the calculated KAMS threshold, a significant prognostic benefit is seen as patients who had a below threshold KAMS (OS =53%, n=22) had significantly poorer survival than those patients who had an above threshold KAMS (OS=74%, n=71). A threshold of 0.0033 was determined for KAMS. In order to further

analyze the power of KAMS without categorization, it was graphed against the AJCC staging system. As mentioned previously, there are many problems surrounding the various methodologies in counting Ki-67. Seventy cases of this dataset were graded according to the gold-standard of counting Ki-67 among 2000 cells by one pathologist. Thus, a separate analysis was conducted on these 70 cases in order to glean information in an ideal situation. Figure 6 shows a meaningful and statistical significance as KAMS decreases across stage (p=.03).

Figure 5: Kaplan- Meier survival curves of patients stratified via the KAMS selected threshold of 0.0033. The difference between patients above and below was significantly different. (OS 74 v 53, Log-rank p=0.04)

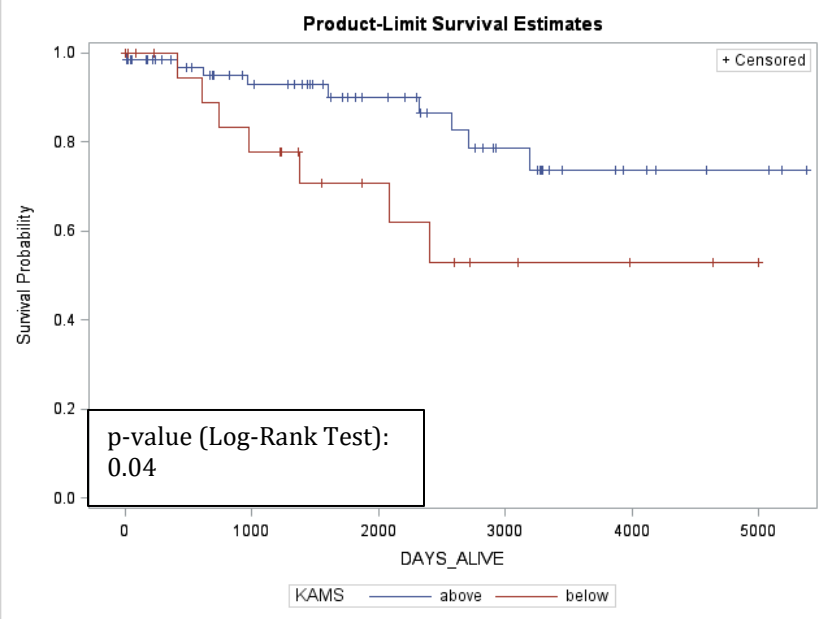
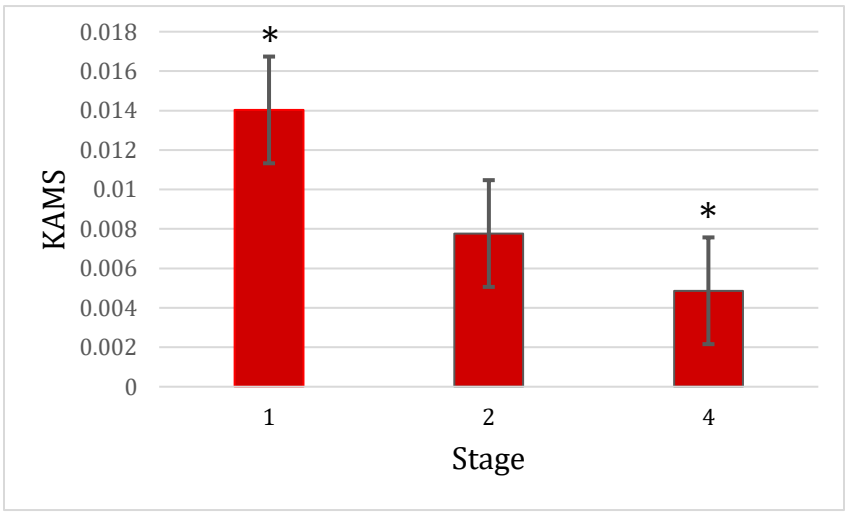


Figure 6: Bar graph representing mean KAMS for patients across Stage 1 (.014 ± .014) Stage 2 (.0078 ± .008) and4(.0049 ± .0033) respectively. (n=66, p=.03) * denotes significance at p <.05



Discussion:

Only 3.3% of all pancreatic cases arise from the endocrine pancreas and among those only 1.4% were specifically classified as pancreatic neuroendocrine tumors (Ries, 2007). Data from the SEER 17 registry shows us that pancreatic NETs account for 7% of all GEP-NET, coming after rectum, small intestine and colon (Lawrence, 2011). Data collected from SEER in the years 1973-2007 shows that incidence rate has more than doubled from 0.17 per 100,000 in the early 70's to 0.43 per 100,000 in 2007(Lawrence, 2011). This increase in incidence may partly be due to more clinical awareness and better diagnostic tools and methods used by pathologists to confirm pancreatic cancer. Even though the incidence rate is steadily increasing, this is still a relatively rare tumor and data is limited.

In this study we analyzed survival outcomes for 97 patients at Emory Hospital, Atlanta. All cases were neuroendocrine tumors however data detailing the specific type of tumor (insulinoma, glucagonoma,etc.) was not available. Strosberg, et al. showed that there was a significant overall survival difference when cases were stratified via low, intermediate, and high grade. However, this categorization involved other histological parameters (necrosis, pleomorphism,etc.) as opposed to solely grading based on Ki-67 values. The results from this dataset show that there is no valuable difference obtained when stratifying on the Ki-67 based grading system between Grade 1 and Grade 2. This is problematic as most cases fall within this category and a robust prognosticator is needed to differentiate between the two grades. Additionally, the sub-optimal cutoffs were made more apparent when there was no significance obtained between the mean Ki-67 values in cases with and without metastatic progression. The new metric, KAMS, was stratified into two levels as the sample size limited further stratification. To avoid losing the full power of

the new metric by making it a categorical variable, it was further evaluated on a continuous scale. There is a significant difference of mean KAMS values among cases with and without distant metastasis. Low KAMS can significantly predict poor prognosis in pancreatic NETS. Studies have shown that high Ki-67 is an indicator for malignancy, and there is trend that shows mitotic count but the rate at which it increases and the proportion gives valuable information (Pelosi, 1996). This shows that the proportion of mitotic cells among the Ki67 positive cells decreases as one moves from Grade 1 to Grade 2 to Grade 3.

Previous studies have shown that the 2010 AJCC staging system is prognostic for overall survival (Strosberg, 2011). Thus, various variables were correlated to a second metric, AJCC Staging as the dataset had too few deaths to conclusively make strong conclusions. When comparing Ki-67 across the four stages, there was no statistical or meaningful significance. Alternatively, there was a statistical significance showing that KAMS decreases as stage increases, which can be reasonably understood to mean that mitotic count decreases as cancer progresses.

The limitations in this study are mainly due to the small sample size and power. Because of the small sample size, there were few deaths in the cohort to allow for strong survival analysis. The next phase of this study is to obtain blocks for these pancreatic NETS from Emory hospital and perform 2-color immunohistochemistry (IHC). Slides stained using the immunofluorescence technique were previously conducted by Dr. Aneja's research group and it proved unsuccessful and unpractical as it involved confocal microscopy. This process was not only time consuming, but microscope is not readily available in all hospitals especially not in low resource settings. IHC is more practical and visualizing both KI and MI within the same slide will allow for a more accurate estimation

of the proportion of mitotic cells among the proliferating cells. Staining and re-reviewing the pathological data will allow us to control more variables such as using the same methodology of counting KI-67 and MI and using only one pathologist to consistently count cells across all the cases.

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Public Health Implications

There are a few fundamental predictors of outcome that are reported in pathology reports: stage, grade, and other factors such as age and general health. In a study using the Delphic consensus process, 20 pathologists and experts within the field of oncology widely agreed that grade should be included in pathology reports, as it is a predictor of biologic aggressiveness and metastatic spread (Klimstra, 2010). The ability to categorize tumors as well differentiated or poorly differentiated gives doctors an estimate of the likelihood of metastasis. A unanimous agreement was reached when asked if mitotic count should be reported in the pathology reports. This is indicative of a desire for the grading system to be revamped to be all inclusive with both Ki-67 and mitotic count. All of these variables predict the prognosis of the patient and are considered when developing a treatment plan.

Tumor grading , specifically is important because it can help guide both treatment and the prognostic for a patient when doctors are planning the next course of action for treatment and follow-up. For example, if a patient has a prognosis of 5 months, the patient will not be recommended surgical resection as a treatment option but instead the medical team would arrange for end of life care including living arrangements and other palliative services.

Tumor grading is also important because it can help guide research. So if a researcher is looking at treating grade 1, 2, 3, etc. they will categorize tumors and the tumors that they model will fall into these categories. Therefore, if a researcher has a grade 1 tumor, the researcher will not use the same treatment for a grade 4 tumor. Not only is grade important when developing treatments, but it also has an impact on the global health implications.

What happens if a “gold-standard” is not established? Since these indicators estimate how fast the cancer is progressing, it can lead to misdiagnosis and potentially even lead to a significant increase in morbidity and mortality. The United States population as a whole is more mobile then other nations. This translates into how treatment is transferred from one hospital to another. The new receiving hospital only has the patients medical record which includes pathology reports to develop or continue the treatment plan as the previous hospital doesn’t provide the original stained slides that were used to obtain ki-67% and mitotic count. Thus, the new medical team would consider the severity of the cancer just based on the parameters reported in the pathology reports. Thus, it is important to obtain optimal cutoffs for these parameters in order for transfer of care to occur smoothly. Additionally, one of the benefits of the new parameter, KAMS, is that it can easily be implemented in low resource settings. The only equipment required is a standard microscope, which is readily available in most facilities and staff trained in 2-color IHC. The WHO grading system requires the same two components, therefore this will be easy to implement upon further stronger scientific evidence.

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