Plasma Neurokinin A Levels Predict Survival in Well-Differentiated Neuroendocrine Tumors of the Small Bowel

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Objectives: Elevated neurokinin A (NKA) levels are associated with poor prognosis in patients with small bowel neuroendocrine tumors. We hypothesized that patients with NKA levels that remain elevated despite treatment with surgical cytoreduction have a poor prognosis.

Methods: Patients diagnosed with small bowel neuroendocrine tumors who underwent surgical cytoreduction at our institution were identified. Demographics, histopathologic characteristics, and biochemical data were collected. Patients were grouped by the trend of their NKA levels (group 1, continuously normal; group 2, transiently elevated but normalized after therapy; group 3, remained elevated despite therapy). Survival rates were calculated from the date of the patient’s first NKA level.

Results: Serial NKA values after surgical cytoreduction were monitored in 267 patients. Kaplan-Meier 2-year, 5-year, and 10-year survival rates were as follows: group 1 (n = 157), 97%, 89%, and 62%; group 2 (n = 78), 99%, 90%, and 78%; and group 3 (n = 32), 88%, 69%, and 0%. Survival rates were statistically significant between groups 1 and 3 and between groups 2 and 3 (P < 0.01).

Conclusions: Serial monitoring of plasma NKA levels is useful in identifying patients who have a poor prognosis. Elevated NKA levels can indicate the need for immediate therapeutic intervention.

Key Words: neurokinin A, neuroendocrine tumor, cytoreduction, survival, small bowel carcinoid, surgery

Neuroendocrine tumors (NETs) are a heterogeneous group of neoplasms arising from cells of the diffuse neuroendocrine system. Dispersed throughout the entire body, these neuroendocrine cells allow NETs to occur virtually anywhere. The majority of NETs originate in the gastrointestinal tract and vary significantly in their clinical presentation and biological behavior. Neuroendocrine tumors have historically been considered rare neoplasms; however, recent studies show that the incidence of NETs has steadily increased over the last several decades.1,2

Neuroendocrine tumors of the small bowel constitute the majority of NETs originating in the gastrointestinal tract. Small bowel NETs can secrete serotonin, chromogranin A (CgA), and various tachykinins, such as neurokinin A (NKA), neuropeptide K, and substance P. Tachykinins have been shown to have effects on gastrointestinal motility, vasodilation, and flushing.3-5 High levels of these substances in circulation may cause a collection of symptoms referred to as carcinoid syndrome. This syndrome is characterized by flushing, diarrhea, fatigue, and/or bronchospasm. Circulating levels of these substances can be detected by biochemical evaluation. Patients with small bowel NETs display a wide spectrum of clinical presentations and often report nonspecific symptoms for decades before diagnosis. As a result, many patients have widespread metastatic disease at diagnosis.6

In patients with suspected or confirmed NETs, measurement of circulating biomarkers may offer clinicians the ability to (1) establish the diagnosis, (2) evaluate prognosis, and/or (3) detect recurrence or changes in tumor volume over time.7 These relatively low-cost noninvasive tests are easily performed and can be repeated at intervals for monitoring response to treatment or detection of recurrence.7

Serial evaluation of circulating biomarkers provides clinicians with a useful diagnostic and prognostic tool to aid in their decision-making. Several studies have shown the importance of monitoring NKA levels in patients with small bowel NETs.8,9-10 Despite the findings reported in previous studies, few centers have adopted the use of routine serial monitoring of plasma NKA levels in their management protocols. Two sensitive and commercially available NKA assays have been developed, including one assay in the United States (InterScience Institute, Inglewood, Calif) and one assay in Europe (Royal Infirmary, Belfast, North Ireland). In a cross-validation study, these assays provided nearly identical results and support comparisons of outcomes by investigators when studies use either of these assays.11 Multiple studies have established that persistent elevation of NKA levels indicates a poor prognosis in patients with small bowel NETs and should prompt immediate intervention by clinicians.7-10

Many NET specialty groups have developed specific protocols for management and surveillance of patients with small bowel NETs.7,12-17 The New Orleans Louisiana Neuroendocrine Tumor Specialists (NOLANETS) is a multidisciplinary team formed as a collaborative effort between Louisiana State University Health
Sciences Center in New Orleans and Ochsner Medical Center in Kenner, LA. The current NOLANETS protocol for management of well-differentiated small bowel NETs uses serial monitoring of a panel of NET biomarkers, including plasma NKA, plasma serotonin, plasma pancreastatin, and 24-hour urinary or plasma 5-hydroxyindoleacetic acid (5-HIAA) in addition to radiographic and scintigraphic evaluation. In all patients with small bowel NETs, this panel is measured on initial presentation, before surgery or other therapy, and at defined intervals for surveillance.

Plasma NKA level has not previously been evaluated as a prognostic variable for monitoring patients after surgical cytoreduction. This study was undertaken to support the practice of serial monitoring of NKA levels, to further validate NKA as a prognostic biomarker in small bowel NETs, and to evaluate NKA levels as a prognostic indicator after surgical cytoreduction. We hypothesized that patients with NKA levels that remain elevated despite treatment with surgical cytoreduction have a poor prognosis. Based on the findings of the previous NOLANETS study, we also hypothesized that patients with elevated NKA levels that normalize after therapeutic intervention have similar survival rates to patients with consistently normal NKA levels.

MATERIALS AND METHODS

Institutional review board approvals from both Louisiana State University Health Sciences Center, New Orleans and Ochsner Medical Center, were obtained as part of a larger study evaluating multiple NET biomarkers. Clinical data from all patients evaluated by the NOLANETS team were entered into a web-based patient database (VELOS Inc, Fremont, Calif). Data were entered retrospectively before December 2007 and prospectively after December 2007. The database was queried for patients diagnosed with NETs of the ileum, jejunum, or small intestine, not otherwise specified. Only patients who underwent surgical cytoreduction at our institution were included.

The medical records of 516 patients who met entry-level criteria were reviewed. Patient demographics, tumor characteristics, and biochemical data were collected from our database and supplemented by medical records. Serial plasma NKA levels were available in 267 patients (267/516, 52%). Neurakin A levels for all patients were analyzed by a single laboratory (InterScience Institute; Inglewood, Calif) using a reference range of less than or equal to 40 pg/mL as normal. All NKA values before June 1, 2017 (end date of the study) were included. The NKA assay developed by InterScience Institute was previously validated against the NOLANETS assay performed at the Royal Infirmary in Belfast, Northern Ireland.11

Survival was first calculated for our entire cohort from the date of initial diagnosis to either the date of the patient’s death or the end of the study (June 1, 2017). The date of diagnosis was defined as the date that the patient was histologically confirmed to have a NET, even if the primary site was unknown. The date of death was confirmed using obituaries, clinic notification by family members, or family records. Follow-up time was measured from the date of diagnosis to the date of the patient’s most recent NKA level.

To evaluate the prognostic value of serial monitoring of NKA levels, survival time was calculated from the initial date that NKA level monitoring was initiated (the date of the patient’s first NKA level) to either the date of death or the end of the study (June 1, 2017). Patients were sorted into 3 groups based on the trend of their NKA level after surgical cytoreduction. Group 1 included patients whose NKA levels were continuously less than or equal to 40 pg/mL. Group 2 included patients whose NKA values increased transiently to greater than 40 pg/mL but returned to less than or equal to 40 pg/mL after treatment. Group 3 included patients who had an elevated NKA level of greater than 40 pg/mL at their most recent visit or immediately before date of death.

Statistical analyses were performed using MedCalc Statistical Software version 17.9.7 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2017). Patient data were entered into a Microsoft Office Excel spreadsheet (Microsoft, Redmond, Wash) and were transferred into MedCalc for survival analyses. Death due to all causes was used as the outcome parameter. Patients who were alive at the end of the study (June 1, 2017) were treated as censored data points. Censored survival curves were generated using MedCalc. Survival rates were calculated using the Kaplan-Meier method.19,20 Median survival times are expressed with a 95% confidence interval (CI). Comparison of survival rates between groups was assessed by the log-rank test using a P value less than 0.01 to determine statistical significance.

RESULTS

Demographics

Our study cohort included 267 patients with small bowel NETs who underwent surgical cytoreduction at our institution from October 2003 to January 2016. Median age at diagnosis was 56 years with a range of 20 to 88 years. Demographic features of our entire cohort are shown in Table 1.

Presenting symptoms were available for 250 of the 267 patients included in our study. At initial presentation to our clinic, 63% of patients (157/250, 63%) displayed symptoms consistent with carcinoid syndrome and 22% (58/267, 22%) of patients reported regular use of PPIs for symptom control. Before surgical cytoreduction, somatostatin analog (SSA) therapy was initiated in 87% of patients (233/267, 87%). These patients included 157 patients with carcinoid syndrome who were treated for symptom management and 76 patients who were asymptomatic and treated prophylactically owing to the known antitumor effects of SSAs.19,20 Histopathologic characteristics for our entire cohort are shown in Table 2.

<table>
<thead>
<tr>
<th>Feature</th>
<th>n (%)</th>
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<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>141 (53)</td>
</tr>
<tr>
<td>Male</td>
<td>126 (47)</td>
</tr>
<tr>
<td>Age group at diagnosis, y</td>
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<tr>
<td>0–40</td>
<td>22 (8)</td>
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<tr>
<td>41–50</td>
<td>55 (21)</td>
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<tr>
<td>61–70</td>
<td>70 (26)</td>
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<tr>
<td>71 and over</td>
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<tr>
<td>Race/ethnicity</td>
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<tr>
<td>White</td>
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</tr>
<tr>
<td>African American</td>
<td>33 (12)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>4 (1)</td>
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<tr>
<td>Unknown</td>
<td>2 (1)</td>
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</tbody>
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Data are presented as number of patients with each feature with percentages in parentheses.

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The initial NKA level was assessed within 12 months of diagnosis in 64% of our patients (172/267, 64%). Serial monitoring of NKA levels included consecutive evaluation of 7 or more samples in 86 patients (86/267, 32%) and 4 to 6 samples in 138 patients (138/267, 52%). Only 43 patients (43/267, 16%) had 3 or less consecutive NKA samples available. Median follow-up time for patients with serial NKA monitoring was 65 months (5.4 years).

**Survival Analysis**

A total of 52 patients (52/267, 12%) expired during our study. Mean (standard deviation) overall survival from date of diagnosis was 252 (32) months (21 [2.6] years). Median overall survival was 215 months (18 years; 95% CI, 181–250 months). The Kaplan-Meier 5-year, 10-year, and 20-year survival rates for all patients were 91%, 74%, and 47%, respectively.

Survival from date of first NKA level was calculated, and patients were grouped based on NKA level trend. Group 1 included 157 patients (157/267, 59%) who had NKA levels that were continuously less than or equal to 40 pg/mL. Group 2 included 78 patients (78/267, 29%) who had NKA values that increased transiently to greater than 40 pg/mL but returned to less than or equal to 40 pg/mL in response to treatment and remained normal at their most recent visit or date of death. Group 3 included 32 patients (32/267, 12%) who had elevated NKA levels of greater than 40 pg/mL that remained elevated despite therapeutic intervention.

Of the 157 patients in Group 1, 21 patients (21/157, 13%) expired during our study. Mean (SD) survival was 112 (5) months (9 [0.4] years). Their median survival was not reached. The Kaplan-Meier 2-year, 5-year, and 10-year survival rates for group 1 were 97%, 89%, and 62%, respectively.

Of the 78 patients in group 2, 14 patients (14/78, 18%) expired during this study. Mean (SD) survival was 120 (4) months (10 [0.3] years). Their median survival was not reached. The Kaplan-Meier 2-year, 5-year, and 10-year survival rates for group 2 were 99%, 90%, and 78%, respectively.

More than half of the patients in group 3 (17/32, 53%) expired during this study, including 10 patients (10/32, 31%) who expired within 12 months of the date of their most recent elevated NKA level. Mean (SD) survival for group 3 was 77 (7) months (6 [0.6] years). Median survival was 82 months (6.8 years; 95% CI, 69–103 months). The Kaplan-Meier 2-year, 5-year, and 10-year survival rates were 88%, 69%, and 0%, respectively.

Kaplan-Meier survival results for all groups are shown in Table 3. Survival curves are shown in Figure 1. Differences in survival rates between groups 1 and 3 and between groups 2 and 3 were statistically significant.

**DISCUSSION**

Based on previous work by the NOLANETS and other investigators, we support the use of serial determination of NKA levels to identify patients with well-differentiated small bowel NETs that have a poor prognosis. Although current literature offers an abundance of studies on biomarkers in small bowel NETs, only a few have evaluated NKA as a prognostic biomarker. The paucity of studies on NKA has led to skepticism and disagreement among NET specialists who question the importance of the utility and clinical relevance of serial monitoring of NKA for management of small bowel NETs. Some NET specialists and community physicians may be unfamiliar with NKA and its role in small bowel NETs or may be unaware of the availability of a commercial NKA assay. Limited experience in interpreting NKA levels may also contribute to hesitation among clinicians in monitoring NKA levels. We conducted this study to further validate the use of NKA as a prognostic biomarker in small bowel NETs.

Plasma CgA was once widely considered the standard biomarker for NETs. Many institutions rely on serial determination of CgA levels for evaluating response to treatment and monitoring for tumor progression. However, the high rate of false positives associated with CgA limits the utility and clinical relevance of CgA as a prognostic biomarker. Jensen et al previously assessed the utility of CgA and 5-HIAA as indicators of outcome after surgical cytoreduction. They found that a greater than or equal to 80% reduction in CgA level after surgical cytoreduction was highly predictive of partial or complete resolution of symptoms and control of disease progression. Although a greater than or equal to 80% reduction in 5-HIAA level was also predictive of symptom relief,
this finding did not correlate with control of disease progression. Because CgA and 5-HIAA levels can be elevated in other conditions and are easily influenced by various dietary habits and medications, these biomarkers have a limited relevance in determining prognosis after surgical cytoreduction.\(^6,23,25\)

The North Ireland Neuroendocrine Tumor Group (Victoria Hospital, Belfast, United Kingdom) was the first to report the prognostic importance of monitoring NKA levels in patients with well-differentiated small bowel NETs. In 2006, the North Ireland NET Group evaluated 139 patients with midgut NETs using serial monitoring of NKA.\(^10\) Their study established plasma NKA level as a prognostic marker using a patient’s most recent NKA level as the most important predictor of outcome. In addition, they reported that patients whose NKA level continued to rise despite treatment with SSA therapy had a significantly worse 1-year survival compared with patients whose NKA levels normalized after treatment (40% vs 87%). In their most recent study, Ardill et al\(^9\) from the North Ireland NET Group evaluated 86 patients with small bowel NETs and reported significantly lower median survival in patients whose NKA levels remained elevated compared with patients whose NKA levels returned to normal (11.1 months vs 72.4 months).

The NOLANETS previously evaluated NKA as a prognostic biomarker in 180 patients with stage IV well-differentiated small bowel NETs and found elevated NKA level to be associated with poor short-term prognosis.\(^8\) Patients whose NKA levels remained greater than 50 pg/mL had a 2-year survival rate of 48% compared with 93% in patients with consistently normal NKA levels. The previous study prompted our group to be more diligent in monitoring NKA levels in small bowel NET patients. However, for easier application, the NOLANETS protocol used the upper limit of normal of the reference range for InterScience Institute (≤ 40 pg/mL) to assess fluctuations in NKA levels.

Our findings in the current study support serial monitoring of NKA levels in patients with small bowel NETs. Patients with continuously normal NKA levels (group 1) and patients who had transiently elevated NKA levels that normalized after treatment (group 2) had significantly improved survival compared with patients whose NKA levels remained elevated despite therapeutic intervention (group 3). The 5-year survival rates for group 1 and

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**FIGURE 1.** Kaplan-Meier survival rates stratified by NKA level trend (N = 267). Survival was measured from the date of the patient’s first NKA level to the date of death or the study end date (June 1, 2017). Median follow-up time for the entire cohort was 65 months (5.4 years). Group 1 includes patients (n = 157) with consistently normal NKA levels. Group 2 includes patients (n = 78) with transiently elevated NKA levels that returned to normal after therapeutic intervention. Group 3 includes patients (n = 32) with persistently elevated NKA levels. A, Survival rates for all groups. B, Comparison of survival rates between group 1 and group 2 was not statistically significant. C, Comparison of survival rates between group 1 and group 3 was statistically significant (P < 0.01). D, Comparison of survival rates between group 2 and group 3 was statistically significant (P < 0.01).
group 2 patients were 89% and 90%, respectively, versus 69% in group 3. Comparison of 5-year survival rates between groups also revealed no significant differences in survival rates between group 1 and group 2 patients. During our study, 53% of patients in group 3 expired, including 37% who expired within 12 months of their most recent elevated NKA level. These findings suggest that elevated NKA can be useful to alert physicians to possible changes in tumor behavior and indicate the immediate need for aggressive therapeutic intervention.

Based on the current study, serial measurement of NKA levels can assist physicians in identifying patients who have a poor postoperative prognosis and indicate the need for adjuvant therapy. Despite previous evidence of the importance of NKA and other biomarkers in monitoring small bowel NETs, current guidelines from the National Comprehensive Cancer Network only recommend monitoring CgA and 5-HIAA but do not suggest specific intervals for follow-up. Other organizations who issue guidelines for NETs, including the European Neuroendocrine Tumor Society and the North American Neuroendocrine Tumor Society, also recommend serum CgA and urinary 5-HIAA for diagnosis and in follow-up for monitoring tumor recurrence and progression. The guidelines from these organizations do not recommend measurement of NKA levels and suggest that new markers need to be further validated. The guidelines issued by the United Kingdom and Ireland Neuroendocrine Tumor Society have recommended monitoring CgA, urinary 5-HIAA, and NKA in patients with small bowel NETs since 2012 as does the recently published eighth edition of the American Joint Committee on Cancer Cancer Staging Manual.

The current NOLANETS protocol for small bowel NETs includes monitoring plasma NKA, plasma serotonin, plasma pancreastatin, and 24-hour urinary plasma or plasma 5-HIAA. In all patients with small bowel NETs, this panel of biomarkers is measured on presentation, before surgery or other therapy, and at defined intervals for surveillance. Although studies assessing the utility of monitoring multiple biomarkers to detect tumor recurrence and progression are limited, other institutions have developed protocols similar to that of the NOLANETS group for management of patients with small bowel NETs. The University of Iowa group reports that their protocol includes preoperative measurements of serum serotonin, CgA, pancreastatin, and less frequently NKA, which is repeated at each follow-up visit. Sherman et al from the University of Iowa evaluated their panel of biomarkers (CgA, serotonin, pancreastatin, and NKA) in 98 patients with small bowel NETs who underwent surgery at their institution. In 52 patients with small bowel NETs, no difference was observed in preoperative and postoperative NKA levels and no correlation was shown between preoperative NKA levels and survival. However, their study included only 2 observations of NKA levels: preoperative and postoperative. Their study does not investigate the efficacy of serial monitoring of NKA and its effect on prognosis over time.

In our study, we found the most recent NKA level to be the most accurate predictor of outcome. This suggests that a patient’s prognosis may vary with NKA levels over time. For patients who initially present with normal NKA levels, the NOLANETS monitor NKA levels every 3 months for 2 years after diagnosis and then decrease sampling frequency to every 6 months. Patients who present with an elevated NKA level at any point are monitored with serial NKA levels every 3 months. Typically, at least 3 abnormal values must be detected on separate consecutive occasions for clinicians to consider patients to have established an abnormal biomarker trend. However, a single value in the abnormal range for NKA levels should prompt physicians to be diligent in measuring serial NKA levels. In an effort to reduce circulating NKA levels, all available therapeutic modalities are used as appropriate by the NOLANETS. These therapies may include SSA therapy, cytoreductive surgery, chemotherapy, biologic response modifiers, radioembolization using yttrium-90 microspheres, bland embolization, transarterial chemoembolization, peptide receptor radionuclide therapy, and radioactive iodine-131-metaiodobenzylguanidine therapy. Because of the unpredictable nature and the variable timeline of tumor progression in NETs, close monitoring and frequent assessment of patients provide the best chance for detecting changes in prognosis. The “wait and see” practice of the past no longer has merit in patients with small bowel NETs. Especially for patients who wish to take a proactive approach in monitoring and surveillance, serial biomarkers are useful tests that are low cost, easy to perform, and may provide substantial information of the patient’s disease state. Minute changes in tumor volume as well as changes in tumor behavior may be detectable on biochemical evaluation months before observation of these changes by standard imaging modalities, symptom intensity, or histopathologic evaluation. Because the effectiveness of certain therapies depends on the timing of initiation of the therapy, early diagnosis and detection of recurrence are paramount to improving survival and outcomes in patients with small bowel NETs. Interestingly, 13% of group 1 patients with consistently normal NKA levels and 18% of group 2 patients whose NKA levels normalized after treatment expired during our study. Because death due to any cause was used as the endpoint in this study, additional investigations of these patients are warranted to identify other useful prognostic factors.

We recognize that limitations exist within our study. Our multidisciplinary NETs specialty group conducted a retrospective study at a single-institutional tertiary referral center. Therefore, our data could not be randomized and entry-level bias could not be avoided. In an attempt to homogenize our cohort, we only included patients with small bowel NETs who underwent surgical cytoreduction at our institution. Biochemical data were standardized by including serial measurements of plasma NKA levels from a single laboratory. Patients who were lost to follow-up or did not have adequate records were excluded from this study. As our study includes one of the largest series of patients studied by a single institution in the United States, we argue that the size of our cohort strengthens our findings. Future prospective studies are needed to definitively validate plasma NKA as a prognostic marker in small bowel NETs.

In conclusion, our study supports the use of plasma NKA as a prognostic biomarker for monitoring patients with small bowel NETs. Despite the limitations and criticisms of other biomarkers for small bowel NETs, serial monitoring of NKA levels is useful to identify patients who have a worse prognosis after surgical cytoreduction and can indicate to physicians that immediate therapeutic intervention is warranted. Future investigations are also warranted to evaluate other biomarkers as prognostic indicators in NETs of the small bowel.

REFERENCES


