Blood measurement of neuroendocrine gene transcripts defines the effectiveness of operative resection and ablation strategies

Irvin M. Modlin, MD, PhD, DSc,[a] Andrea Frilling, MD, PhD,[b] Ronald R. Salem, MD,[c] Daniele Alaimo, MS,[d] Panagiotis Drymousis, MD, PhD,[b] Harpreet S. Wasan, MD, FRCP,[b] Stephen Callahan, MS,[d] Omar Faiz, MBBS, FRCS, MS,[e] Lei Weng, MBBS,[b] Nancy Teixeira, MSN,[d] Lisa Bodei, MD, PhD,[d] Ignat Drozdov, PhD,[d] *and* **Mark Kidd, PhD,[d]** *New Haven and Branford, CT, and London, UK*

Background. *Surgery is the only curative treatment for gastroenteropancreatic neuroendocrine tumors (GEP-NETs), but the prediction of residual disease/recurrence is limited in the absence of optimal biomarkers. We examined whether a blood-based multianalyte neuroendocrine gene transcript assay (NETest) would define tumor cytoreduction and therapeutic efficacy.*

Methods. *The NETest is a polymerase chain reaction–based analysis of 51 genes. Disease activity is scaled 0–100%; minimal <14%, low 14–47%, and high >47%. A total of 35 GEP-NETs in 2 groups were evaluated. I: after surgery (R0,* n *= 15; residual,* n *= 12); II: nonsurgery (*n *= 8: embolization with gel-foam alone [bland:* n *= 3]), transarterial chemoembolization (*n *= 2), and radiofrequency embolization (*n *= 3). Measurement (quantitative real-time-polymerase chain reaction) and chromogranin A (CgA; enzyme-linked immunosorbent assay) were undertaken preoperatively and 1 month after treatment.*

Results. *NETest score was increased in 35 (100%) preoperatively; 14 (40%) had increased CgA ($\chi^2 = 30$,* P *$< 2 \times 10^{-8}$). Resection reduced NETest from 80 ± 5% to 29% ± 5, (*P *< .0001). CgA decrease was insignificant (14.3 ± 1.6U/L to 12.2 ± 1.7U/L). NETest decreases correlated with diminished tumor volume ($R^2 = 0.29$,* P *= .03). Cytoreduction significantly reduced NETest from 82 ± 3% to 41% ± 6,* P *< .0001). CgA was not decreased (21.4 ± 5.5U/L to 18.4 ± 10.1U/L). Four (36%) of 11 R0s with increased NETest at 1 month developed positive imaging (sensitivity 100%, specificity 20%). One hundred percent (ablated group) were transcript- and image-positive.*

Conclusion. *Blood NET transcripts delineate surgical resection/cytoreduction and facilitate identification of residual disease. (Surgery 2016;159:336-47.)*

From Surgery, Emeritus Prof,[a] and Department of Surgery,[c] Yale University School of Medicine, New Haven, CT; Department of Surgery and Cancer,[b] Imperial College London, London, UK; Wren Laboratories,[d] Branford, CT; and Department of Colorectal Surgery,[e] St Mark's Hospital, London, UK

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Reprint requests: Irvin M. Modlin, MD, PhD, DSc, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510. E-mail: imodlin@optonline.net.

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Neuroendocrine tumors (NETs) are diverse tumors considered previously as "carcinoids."[1] The lesions are ubiquitous in location but are especially common within the gastrointestinal tract.[2] There is a general consensus that operative resection is a critical element of therapy and remains the only curative treatment option.[3] Curative surgery, however, often is not feasible because most gastroenteropancreatic (GEP)-NETs exhibit metastatic disease at diagnosis. Additional therapeutic strategies include pharmacotherapeutics, eg, somatostatin analogs, which diminish symptoms that may extend progression-free survival in low-grade disease.[4] Similarly, a variety of targeted agents, including everolimus, sunitinib, and temozolamide, have been used

with variable efficacy. Imaging currently is the mainstay of therapeutic assessment. The more recent introduction of positron emission tomography/computed tomography (PET/CT) with somatostatin analogues, DOTATOC, DOTATATE, and DOTANOC, has improved detection rates, with pooled sensitivities of 93–96% and specificities of 85–100% (area under the receiver operating characteristic curve: 0.96–0.98).[5] ^{68}Ga-PET is able to modify the overall therapeutic strategy in 55–60% of cases.[6] In particular, operative management is modified in 20% of cases; however, standardization and metrics are still considered to have not attained optimal parameters.

The determination of a patient's survival after surgery reflects the primary site, the tumor grade, disease stage, and location of metastatic disease, as well as the magnitude of postresection tumor burden. Despite apparent complete resection of hepatic metastases, early detection of covert residual disease represents a major clinical problem and is a key determinant in defining the timing of further therapeutic intervention and determination of long-term prognosis. For both operative interventions as well as ablation approaches, strategies for early detection of disease recurrence remain relatively limited in their sensitivity and specificity.[1]

Operative resection is associated with improved and prolonged disease control. Retrospective studies, despite limitations, demonstrate enhanced outcomes compared with individuals who did not undergo surgical resection.[7] Although tumors with metastatic spread have overall poorer outcomes, surgery often is undertaken to obviate local mechanical events such as bleeding, bowel obstruction, or vascular encasement. R0 and R1 resections are the norm, and outcomes are predicated on a number of factors, including residual tumor burden.[8]

NET recurrence usually is identified by a combination of biochemical as well as radiologic and nuclear medicine techniques. Imagery strategies used are both anatomical and functional; however, all have significant limitations in their capacity of tumor resolution: 2 millimeters for computed tomography/magnetic resonance imaging, 4–6 mm for positron emission tomography (PET including ^{68}Ga-PET) and ~10 mm for Somatostatin Receptor Scintigraphy.[9] Similarly, current biomarkers (eg, chromogranin A [CgA], pancreastatin, neurokinin A) used for the detection of NET have substantial limitations in terms of sensitivity, specificity, and reproducibility.[10]

We have reported previously the utility of a PCR-based tool to quantitate (score) the circulating GEP-NET molecular signature with high sensitivity and specificity (>95%).[11,12] This multianalyte-derived signature encompassing 51 genes identifies all GEP-NETs and significantly out-performs monoanalyte-based assays for the detection of NET.[11,12] Gene expression is captured in a 0–8 score derived from 4 different prediction algorithms that can be mathematically scaled to disease activity (0–100%) by the use of expression of transcripts that capture the hallmarks of neoplasia.[13] Disease activity scores of 0–14% are associated with minimal activity, 14–47% low activity and >47%, high activity.[14] Activity levels correlate with clinical status, eg, stable or progressive disease.[14]

Currently, alteration in tumor size generally is regarded as indicative of disease progression or regression. The clinical difficulty, however, is the absence of sensitive or specific enough imaging to define this change. An alternative strategy would therefore entail the development of blood-based measurements of tumor function. We hypothesized that alteration in the NET circulating blood signature would reflect operative resection or ablation of liver metastases. Our aims were to evaluate the effect of surgery and ablation/chemoembolization on the NET signature and specifically examine (1) whether tumor resection decreased the blood NET signature, (2) whether this decrease reflected the extent of resection, (3) whether R0 resection reduced circulating NET transcript levels to normal, and (4) whether increased blood NET transcript levels after R0 resection predicted clinical recurrence.

PATIENTS AND METHODS

Patients with GEP-NET (n = 35) [M/F 14:21; median age: 58 years, range: 33–80; stomach n = 1, pancreas n = 8, gall bladder: n = 1, small intestine: n = 21, appendix n = 2, rectum n = 2; G1 = 27, G2 = 7, G3 = 1] were included (Table I). Surgery was performed in 27 (1) to remove primary tumor, including loco-regional lymph nodes (n = 21); (2) for debulking (n = 4); and (3) for suspicion of NET (small intestine: n = 1; appendix: n = 1). Tumor volume pre- and postsurgery was assessed with imaging, and operative measurement and pathological data were used to quantitate tumor volumes. Nonoperative strategies were undertaken in 8 subjects and included embolization with gel-foam alone (bland: n = 3), trans-arterial chemoembolization (n = 2), and radiofrequency ablation (n = 3) for hepatic metastases. Ablation/embolization was applied to liver lesions.