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Original contribution

# Improved histologic and clinicopathologic criteria for prognostic evaluation of pancreatic endocrine tumors☆

**Stefano La Rosa MD[a,*], Catherine Klersy MD[b], Silvia Uccella MD[c], Linda Dainese MD[c], Luca Albarello MD[d], Angelica Sonzogni MD[e], Claudio Doglioni MD[d], Carlo Capella MD[c], Enrico Solcia MD[b,f]**

[a]*Department of Pathology, Ospedale di Circolo, 21100 Varese, Italy*
[b]*Scientific Direction, IRCCS Policlinico San Matteo, 27100 Pavia, Italy*
[c]*Department of Human Morphology, University of Insubria, 21100 Varese, Italy*
[d]*Pathology Unit, San Raffaele Scientific Institute, 20100 Milan, Italy*
[e]*Division of Pathology and Laboratory Medicine, European Institute of Oncology, 20100 Milan, Italy*
[f]*Department of Human and Hereditary Pathology, University of Pavia, 27100 Pavia, Italy*

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**Summary** Currently used histopathologic criteria for the diagnosis of pancreatic endocrine tumors are still under discussion as far as to their capacity to identify prognostically different tumor subsets, which are potentially helpful for patient management. A recently developed TNM staging system and a variety of proposed histologic and clinicopathologic parameters still need to be fully validated. One hundred fifty-five pancreatic endocrine tumors encompassing all the main histologic types and stages, operated with intention to cure and then followed up for a median 126 months, were carefully investigated histologically to identify prognostically informative parameters at univariable, bivariable, and multivariable analysis. Ki67 index, mitotic rate, neuroinvasion with or without vascular, peritumoral or stromal infiltrative patterns, as well as tumor size, and association with endocrine syndromes other than insulinoma proved effective in predicting recurrence and disease-specific death among well-differentiated tumors. Poorly differentiated histologic features, more than 10 mitoses/10 high power fields, and necrosis were helpful in the identification of high-grade cancers with an invariably poor prognosis. The TNM system proved to be highly predictive of patient outcome and easy to combine with histologic and clinicopathologic parameters to classify pancreatic endocrine tumors into groups of increasing malignant potential.


## 1. Introduction

Pancreatic endocrine tumors display considerable prognostic variability, from the essentially benign behavior of some well-differentiated tumors apparently cured by surgery, as shown by prolonged follow-up, to severely malignant,

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* Corresponding author. Servizio di Anatomia Patologica, Ospedale di Circolo, 21100 Varese, Italy.
*E-mail address:* anapat@ospedale.varese.it (S. La Rosa).

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poorly differentiated neoplasms killing the patient in a few months irrespective of surgical or pharmacologic therapy. Staging of operated tumors has been found to predict behavior, at least in the case of gastrinomas and with reference to the dominant influence of liver and distant metastases over local lymph node metastases or direct peripancreatic tissue gross invasion, which are the accepted criteria for diagnosing malignancy [1,2]. However, for tumors confined to the pancreas, only size seems to be informative at a macroscopic level and indeed it formed the main basis for T assessment in the recently proposed TNM classification [3].

To improve prognostic evaluation, a number of histologic parameters have been analyzed and proposed, with some success. First, poorly differentiated endocrine carcinomas of ominous behavior have been separated, in keeping with tumors of other sites [4,5], from well-differentiated tumors based on severe cellular atypia, high-proliferative rate, and focal to extensive necrosis [6-9]. It is more difficult to predict the behavior of well-differentiated endocrine tumors, especially those confined to the pancreas and lacking obvious macroscopic signs of malignancy. Prediction of recurrence of such tumors may be important to define therapeutic and follow-up programs. In addition, the increasing frequency with which unexpected, small, well-demarcated tumors are detected with modern imaging techniques raises the issue of whether an invasive therapeutic approach such as pancreatic surgery is indeed needed. For this purpose, the assessment of vascular, nerve or capsular invasion, proliferative rate, nuclear atypia, focal necrosis, ploidy status as well as the expression of cytokeratin 19, p53 gene protein, or inappropriate (ectopic) hormonal products have been proposed and found to be potential prognostic markers, though sometimes with controversial findings and interpretations [10-17]. As a result, a 4-step classification of pancreatic endocrine tumors has been developed, where tumors with proven malignancy were firstly separated from those lacking actual evidence of it and then low and high-grade cancers were distinguished mainly on the basis of tumor cell atypia, mitotic rate, and necrosis, whereas among tumors lacking evidence of malignancy, those with a lower or higher risk of recurrence and/or subsequent malignant behavior were tentatively separated [6-8,18]. Although widely accepted in principle, this approach is still open to discussion, especially because there is only limited evidence of its effectiveness when applied to a sufficiently large number of cases encompassing all main tumor categories and stages and followed up for a time long enough to obtain a reasonable assessment of tumor behavior [16,17,19,20].

In this investigation, 155 tumors that had undergone potentially curative surgery have been extensively investigated and observed with the aim of answering the following questions:

1. Is it possible to predict, on the basis of histologic evidence, those tumors that are at increased risk of recurrence after curative surgery? And do histologic parameters add information to stage?
2. At which level of histologic differentiation and/or proliferative rate should separation between tumors of well/poor differentiation or low/high-grade malignancy be established? Does an intermediate, moderately differentiated or G2 grade tumor category exist? And can it be ascertained on a survival basis?
3. Does the type of associated functional syndrome influence prognosis independently of stage?
4. Is the TNM staging system recently proposed for pancreatic endocrine tumors effective?

## 2. Materials and methods

### 2.1. Cases

One hundred fifty-five pancreatic endocrine tumors were collected from the files of the Departments of Pathology of the Varese Ospedale di Circolo, Italy; San Matteo Hospital, Pavia, Italy; San Raffaele Hospital, Milan, Italy; and European Institute of Oncology, Milan, Italy, from November 1978 to December 2001. Intention to cure surgery and available follow-up information for each case was a sine qua non requirement for entering the study. Clinical information on age, endocrine and nonendocrine symptoms, presence of metastases at the time of diagnosis, evidence of local invasion at surgery, perioperative mortality, and evidence of local recurrence or distant metastases during follow-up were collected from patient charts and clinical records or by contacting family doctors. For tumor staging, the TNM system recently proposed by Rindi et al [3] was used, with the only change that all locally invasive tumors, including those invading the duodenum and choledochus, were classified as T4. A probably incomplete tumor resection was deduced from pathologic findings on surgical specimens in 5 cases, 4 of whom died of disease during follow-up. The median follow-up was of 126 months (interquartile range [IQR], 84-157). Relevant clinicopathologic data of patients are outlined in Tables 1 and 2; the average age was 51 years (SD, 15 years), and 93 patients (60%) were female.

This study was performed in agreement with the clinical standards laid down in the 1975 Declaration of Helsinki and its revision in 1983 and according to the rules of the Ethics Committee of the Ospedale di Circolo, Varese, Italy.

### 2.2. Morphologic and immunohistochemical study

Samples of primary tumors were fixed in buffered formalin (formaldehyde, 4% wt/vol, and acetate buffer, 0.05 mol/L) and routinely processed to paraffin wax. Serial sections were stained with hematoxylin-eosin and Grimelius' silver impregnation for histopathologic evaluations. In addition, a panel of immunohistochemical tests was applied