Criteria for Malignancy in Pancreatic Endocrine Tumors

Carlo Capella, MD, PhD, Stefano La Rosa, MD, and Enrico Solcia, MD, PhD

Unquestionable evidence of malignancy of pancreatic endocrine tumors (PETs) is given by gross invasion of adjacent organs and metastases in the regional lymph nodes or other distant sites [1–4].

The size of the primary tumor is greater in metastatic than in nonmetastatic tumors [1,2,5]. This has been confirmed by more recent studies [6] showing that nonmetastatic PETs (mean maximum diameter 1.7 cm) are significantly smaller in size than metastatic PETs (mean, 6.4 cm). Tumors larger than 6 cm in size should be considered malignant, since 93% of a large series of proven carcinomas were of this size or larger [2]. In addition, it has been shown that, in gastrinomas, the size of the primary tumor correlates significantly with the presence of liver metastases [7]. On the contrary, metastases to lymph node do not depend on tumor size. Liver metastases are significantly more frequent in patients with pancreatic gastrinomas than in patients with duodenal gastrinomas, and this seems to be owing to the larger mean size of pancreatic tumors (3.8 vs 0.8 cm). Survival of patients with liver, but not lymph node metastases, is shortened [7]. Independently of the type of hormonal syndrome, pancreatic endocrine tumors of <4 cm in diameter are associated with significantly ($p < 0.001$) longer survival than those of larger size [8].

There is general agreement among various authors [3,4,9] that, in the majority of PETs, malignancy cannot be predicted on the basis of the sole histological features, as seen in routinely stained sections. Exceptions are some rare poorly differentiated (small- or intermediate-cell) endocrine carcinomas with a high grade of cellular anaplasia, widespread necrosis, and an elevated mitotic index. In well-differentiated PETs, nuclear pleomorphism seems an unreliable criterion of malignancy. Features suggestive for a possible malignant behavior of well-differentiated PETs, though not universally accepted as proof of actual malignancy, are a high mitotic index, tumor necrosis, and definite microinvasion of tumor capsule, blood vessels, lymphatic vessels, and nerves. Invasion of vessels, particularly of veins located in the tumor capsule, has been observed in the majority of malignant cases [2]. Blood vessel invasion should be accepted as true only when tumor thrombi are attached to the vessel wall and/or a definite focus of invasion into the vessel wall is recognized. Because it is often difficult to decide whether an intracapsular nest of tumor cells is located within a vessel or not, immunostains for endothelial cells, such as factor VIII-related antigen and CD31, have to be adopted for a safe estimation of vascular microinvasion within a tumor. In this context, we found that vascular microinvasion, evaluated on sections stained with antibodies directed against factor VIII-related antigen or CD31, was highly sensitive and specific in detecting benign and malignant cases (97.2% of malignant and 92.9% of benign tumors correctly predicted [10]). On the contrary, vascular

Department of Clinical and Biological Sciences, University of Pavia at Varese, Viale Borri 57, 21100 Varese, Italy (CC, SL); Department of Human and Hereditary Pathology, University of Pavia, Viale Forlanini 16, 27100 Pavia, Italy (ES).

Address correspondence to Dr. Carlo Capella, Servizio di Anatomia Patologica, Ospedale Multizonale, Viale Borri 57, 21100 Varese, Italy.

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microinvasion, evaluated on H&E-stained sections, did not distinguish between benign and malignant neoplasms ($p = 0.2$) (unpublished data, 1996). Analogously, perineural microinvasion is better evaluated in protein-S100-immunostained sections than in H&E-stained sections.

Several efforts have been made recently to solve the problem of assessing a prognostic evaluation based only on traditional histopathology. Tumor cell morphometry, ploidy, nucleolar organizers (AgNORs) or mitotic rate, and expression of oncogene proteins, progesterone receptor, α-human chorionic gonadotropin (α-hCG), proliferating cell nuclear antigen (PCNA), and Ki-67 have been investigated. A number of nuclei per mm^2 of 2000 or more and a nucleo-cytoplasmic ratio of 30% or more [6]; aneuploidy with a DNA index (relative DNA content of aneuploid stemline compared with diploid cells) of more than 1.5, especially when coupled with multiploidy [11]; Ha-*ras* oncogene overexpression [12]; presence of hCG or its α-subunit [13]; more than 5 mitoses/10 high-power fields [6]; more than 5% AgNOR-rich cells (6 AgNOR/nucleolus) [14]; and a PCNA and Ki-67 index above 5% [15,16] have all been suggested to be predictive of malignancy.

However, the practical usefulness of these findings is limited by several drawbacks. In fact, many metastatic tumors showed 0–2 mitoses/10 high-power fields in their primary sites; Ha-*ras* was also overexpressed in nonmetastatic tumors, although less frequently than in metastatic ones [12]; 36% of malignant PETs expressed the progesterone receptor and 28% of benign tumors failed to express it [17]; 36% of malignant tumors showed <5% AgNOR-rich cells [14]; and, in 30–40% of malignant tumors (especially insulinomas), no hCG or its subunits could be identified [13], whereas up to 18% benign tumors showed it [14,18].

The effectiveness of high DNA index aneuploidy in differentiating benign from malignant PETs has not been assessed in a sufficiently large series of cases [11,19]. In the study of Alanen et al., apparently benign insulinomas were also aneuploid, although with a DNA index <1.5 [11]. Donow and coworkers [19] found that 60% of their malignant and 41% of benign pancreatic endocrine tumors showed an aneuploid pattern. In addition, in various studies, the associated clinical syndrome (instead of tumor cell immunostaining) has not been sufficiently taken into account. For example, only benign insulinomas were sufficiently represented in the PCNA study [15], and all benign functioning tumors were insulinomas, whereas all gastrinomas were malignant in the morphometric study [6]. In this context, it is important to recall that clinical syndromes are *per se* predictive of tumor behavior, with metastatic rates ranging from about 10% for asymptomatic nonfunctioning incidental tumors and insulinomas to 60–90% for glucagonomas, somatostatinomas, VIPomas, gastrinomas, tumors associated with "ectopic" syndromes, and symptomatic nonfunctioning tumors [4,7,8,20,21].

Among proliferative rate indices, Ki-67 index (>5%) proved to be more reliable than PCNA (>5%) in predicting survival of patients with PETs and resulted to be the sole independent predictor of prognosis in a multivariate analysis study in which several other parameters in addition to Ki-67 and PCNA indices were considered, including mitotic index >0.3%, tumor diameter >3 cm, absence of progesterone receptor, nonfunctioning type of tumor, advanced stage, and immunoreactivity for hormones other than insulin [16]. However, Ki-67 remains to be tested for its capacity to differentiate benign (or low-risk) from malignant tumors within separate groups of functioning insulinomas,

glucagonomas, somatostatinomas, VIPomas, and so forth, i.e., independent of the clinical syndrome. The amount of AgNOR-rich cells correlates well with prognosis in insulinoma (all malignant cases and 90% benign cases correctly identified); this is less reliable for gastrinomas (70% malignant and all benign cases correctly identified) and other, mostly nonfunctioning cases (61% malignant and all benign cases identified) [14].

In our experience, a simple multiparametric approach, including such parameters as size (≥4 cm), vascular and/or perineural microinvasion, mitoses (≥2), capsular penetration, Ki-67 index (>2%), nuclear atypia, lack of progesterone receptors, and presence of calcitonin, is useful for identification among well-differentiated tumors of neoplasms at higher risk of recurrence and metastases. In a recent study on 61 (5 poorly differentiated and 56 well-differentiated) cases of nonfunctioning PETs [10], all the aforementioned eight morphologic and immunohistochemical parameters, proved, when analyzed by logistic regression model, to correlate significantly with malignancy of well-differentiated PETs, proven by metastases and/or gross local invasion. Among these eight variables, Ki-67 proliferative index >2% and vascular and/or perineural microinvasion resulted in the most sensitive and specific. The absence or presence of one or both parameters has been taken as the basis for classification of the well-differentiated, nonmetastatic, nonlocally invasive tumors. The tumors showing none of the two variables were classified as lower-risk tumors and were separated from higher-risk tumors showing one or both parameters. These two prognostic groups were separated from well-differentiated carcinomas, showing gross local invasion or metastases and poorly differentiated carcinomas of a high grade of malignancy. These four prognostic groups (lower-risk tumors, higher-risk tumors, well-differentiated carcinomas, and poorly differentiated carcinomas) of nonfunctioning PETs showed distinct survival curves, which were significantly affected by vascular microinvasion, Ki-67 proliferative index, and distant metastases. This rather simple approach to prognostic classification of PETs, which substantiates the classification guidelines for PETs given in a recent paper [22], has to be verified in a large series of PETs causing different hormonal syndromes, such as insulinomas, glucagonomas, somatostatinomas, gastrinomas, and so on.

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