Applied Methods in Oncology

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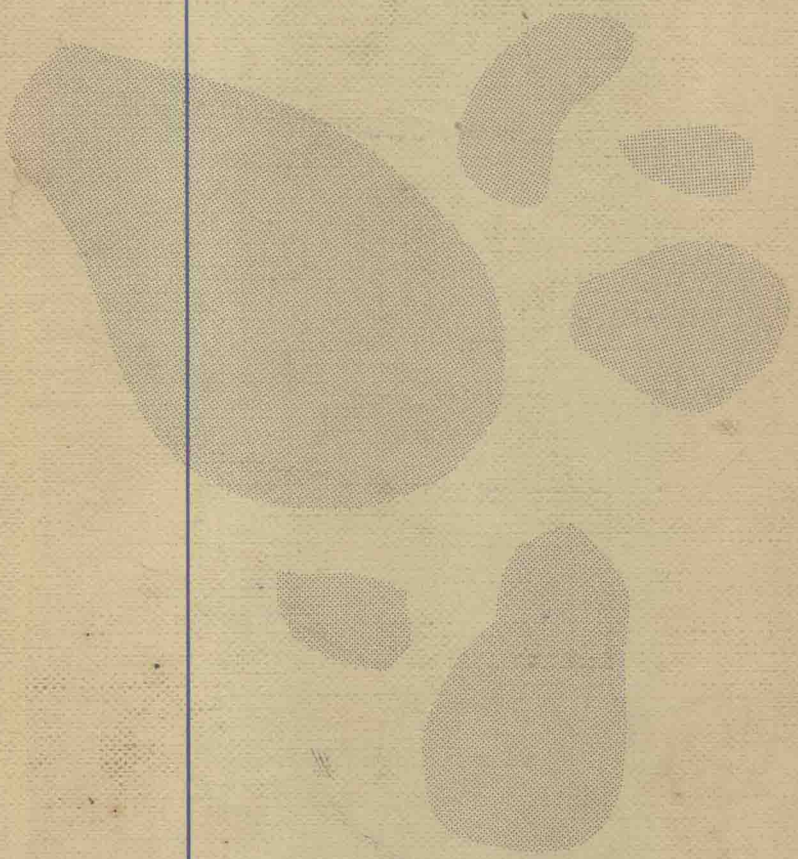
Tumour Markers: Impact and Prospects

E. Boelsma and Ph. Rümke Editors



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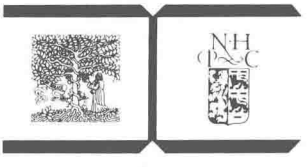
TUMOUR MARKERS: IMPACT AND PROSPECTS

Workshop on Tumour Markers: Fundamental Aspects and Clinical Evaluation held in Lunteren, The Netherlands on December 12-14, 1978.
Organized under the auspices of the Scientific Council of The Netherlands Cancer Society (Koningin Wilhelmina Fonds).

Editors
E. Boelsma
Ph. Rümke



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Volume 2

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INTRODUCTION

The workshop on "tumour markers" was organised under the auspices of the Dutch Scientific Council on Cancer Research and is the third in a consecutive series on methods applied in oncology. The purpose of the workshops is to get together leading scientists from the international scene with investigators in the field from our own country. The topics which are dealt with are in research areas upon which the attention of many researchers is currently focussed. Indeed, research on tumour markers may be called fashionable.
A major goal of cancer research is to find new tools to improve cancer diagnosis and treatment. Since carcinoembryonic antigen was first isolated from colon tumours in 1965, expectations were high that biological markers in body fluids would generate practical screens for cancer. Now that the initial optimism has faded, it becomes necessary to establish the true value of tumour markers. If not useful as specific tools in early diagnosis tumour markers may be, and in some cases have indeed proved to be, suitable for monitoring the disease during and after treatment. Markers can also be useful in predicting recurrences.

In principle compounds can be considered as possible tumour markers when derived from the synthesis or degradation processes in tumour cells, and when differing either in quantity or in quality from normal-cell products. Hormones, enzymes, membrane constituents and other tumour cell derivatives can, when discharged into the circulation, be measured in plasma or urine with sensitive methods, usually immunological.

The initial search has been for compounds that were thought to be exclusively related to a particular cancer. The state of this art is reviewed in part I of this book. Not included are for instance the haemoglobins, angiogenesis factors, ferritins and even the iso-ferritins. Adrenocorticotropic hormone (ACTH), human chorionic gonadotropin (hCG), calcitonin and sialyl transferase are not the subjects of individual papers. Milk proteins and kappa-casein, acute phase proteins, hydroxyprolin, tryptophan have been neglected, and possibly, rightly so.
Some tumour-related products have already been abandoned as tumour indicators because of lack of specificity. One must also bear in mind that marker tests as any clinical test, should be highly reliable and easily performed and interpreted. And, they should offer as little discomfort and inconvenience to the patien as possible. Do these prerequisites apply to the multiparametric approach? Do it make sense to use whole batteries of marker tests? Or to apply a highly sophticated single tumour marker test in the clinic?

It may well be, that no tumour specific products exist and that the so-called specific materials are expressed by normal cells depending on their stage of development or differentiation. In practice, this would not impair their usefulness as tumour markers. This hypothesis, and other fundamental aspects are described in part II. As concerns the molecular hybridisation technique, the application of determining sequences in messenger RNA's in clinical specimens may seem futuristic. However, this fundamental research may provide for a series of different messengers specific for tumour cells or in abundance in tumour cells. Such markers might be in fact more sensitive than the morphological scanning of cells, as it may apply to virtually each cell under investigation. The fundamentals of cell biology are likely to give a better understanding of the process of metastasis, although cell locomotion already proved not to be a useful marker.

The clinical implications of the determination of tumour related products are dealt with in part III. Even if not truly specific, certain markers are useful as tools in the clinic. The pioneer of tumour markers, the carcinoembryonic antigen, appeared to be of value in predicting the course of colorectal but also of other cancers.

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We thank Drs. Munro Neville, P. Emmelot, J.P. Mach, H. Bloemendal, R. Baldwin, Ph. Rümke and T. Chu for chairing the sessions. The very skillful chairmanship of Dr. D. Goldenberg during the general discussion should be acknowledged.
We thank Dr. Munro Neville in particular for his general resume of the meeting.

E. Boelsma
Applied Methods in Oncology,
editor

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PART I: REVIEW OF MARKERS

Tumour Markers: Impact and Prospects E. Boelsma and Ph. Rümke, editors

ECTOPIC HORMONES

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ABSTRACT

The clinical value of ectopic hormone measurements in the routine management of common forms of malignancy is unproven. Recent work indicates that the synthesis of many peptide hormones by tumours, particularly lung cancer, occurs frequently but circulating levels are poorly related to tumour mass. The recognition of hormonal forms relatively specific for neoplasia and definition of factors controlling their secretion are important tasks for the future. In addition, further improvements in assay specificity and sensitivity are required. The detection of hormones and related products in tumour tissue and the circulation, may, however, have some value in assessing prognosis in selecting suitable forms of therapy, and in the functional classification of tumours.

INTRODUCTION

Endocrine abnormalities occur commonly in association with malignancy. The most frequent changes represent the endocrine response of the host to the presence of tumour (tumour-associated changes) while less frequently they are due to secretion by tumour cells of hormones into the body fluids (tumour-derived changes). Tumour-associated hormone changes are of considerable interest in elucidating the pathophysiological and clinical features of malignancy and in assessing prognosis, but they are not specific enough to be reliable as tumour markers in diagnosis and management of individual patients with neoplasia.

The synthesis and secretion of hormones by tumours has important biological implications and increasing clinical relevance. Hormones and related products represent some of the best characterised examples of tumour products. The particular clinical importance of tumour-derived hormones is threefold:

i) their secretion may cause recognisable clinical syndromes
ii) the structure of most hormones is established and allows comparison of tumour hormones with their normal counterparts
iii) sensitive and specific assays are available for the detection of most hormones in tissues and body fluids.

CLASSIFICATION OF TUMOUR HORMONES

Tumour hormones may be appropriate to the tissue from which the tumour has arisen (i.e. eutopic) or inappropriate (i.e. ectopic). The historical definition of an ectopic hormone requires that all the normal sites of synthesis are known (1), information which is seldom available. Operationally, an ectopic hormone is defined as one which is synthesised by a tumour arising from a tissue other than the normal gland of origin of that hormone. Thus, ACTH is ectopic when it is secreted by a non-pituitary tumour.

Measurements of eutopic hormones have long been used as tumour markers in diagnosis and management of endocrine tumours. In general, the levels of circulating hormones are related to the number of viable tumour cells and therefore show a wide variation in plasma concentration. Diagnosis of small endocrine tumours is often possible biochemically by employing stimulation and suppression tests to reveal altered control mechanisms (e.g. oral whisky stimulation of calcitonin (CT) from medullary carcinoma of thyroid when basal CT levels are normal; glucose suppression of growth hormone (GH) in diagnosis of GH-secreting pituitary adenomas; TRH and metoclopramide stimulation in the diagnosis of prolactin (PRL)-secreting pituitary microadenomas; dexamethasone suppression in the diagnosis and differential diagnosis of Cushing's syndrome).

However, the use of circulating eutopic hormones as markers in malignant disease is limited, since endocrine tumours are relatively rare and their biological behaviour often benign. Until recently, it was also considered that the ectopic production of hormones was rare and therefore of more biological than clinical interest. During the last decade the ectopic secretion of almost all the recognised peptide and protein (but not steroid or thyroid) hormones has been described (2) and with more refined methods, there is evidence that the phenomenon may be a universal concomitant of neoplasia (3). The measurement of circulating ectopic hormones and related products has thus assumed potential clinical significance in diagnosis and management. In addition, the applications of immunochemical methods to tissue hormones, particularly the immunoperoxidase technique, has provided a novel approach to the functional classification and prognosis of tumours and a means by which the histopathologist can predict which (ectopic) index substance(s) to use to monitor the subsequent course of a tumour in a particular patient (4). The application of hormone radioimmunoassay techniques to tumour tissue and biological fluids, together with immunocytochemical methods to tumour specimens seem likely to provide information relevant to early diagnosis, prognosis and management of some of the commoner forms of cancer in the future.

However, the rational clinical use of ectopic hormones and related products as tumour markers first requires detailed information on the prevalence of ectopic hormones in relation to histological type and to stage of disease, and development of simple detection systems for molecular forms of hormones which may be relatively tumour specific.

PREVALENCE OF ECTOPIC HORMONE PRODUCTION

The criteria for assessment of synthesis of ectopic hormones has been summarised by Rees and Ratcliffe, 1974 (5). Many reported cases depend on inferential clinical observations or demonstration of elevated circulating hormone levels, or fall in levels after effective tumour therapy, in a patient with known malignancy. These criteria at best grossly underestimate the true prevalence and at worst may be misleading or erroneous. More critical criteria are the demonstration of an arteriovenous gradient in hormone levels across the tumour, hormone synthesis in vitro, incorporation of labelled amino acids into a specifically characterised hormone in vitro, or demonstration of hormone in tumour tissue by biochemical or immunocytochemical methods. The present discussion is based largely on cases in which hormones have been demonstrated in significant concentrations in tumour tissue.

Lung Cancer

1. Oat cell and carcinoid tumours ("endocrine" tumours, "apudomas") (Fig. 1)

It is now clear that oat (small) cell carcinoma of lung and bronchial carcinoids are tumours derived from cells with endocrine characteristics normally present in the lung (6). Our evidence suggests that virtually all such tumours contain amounts of ACTH and lipotrophin (LPH), in concentrations which are frequently within the ranges associated with ectopic ACTH syndrome (7). There is an excellent correlation of ACTH and LPH concentration ($r = 0.947$, $p < 0.001$) in these tumours suggesting that, as with the normal pituitary, both are expressed together. The suggestion that all oat cell and carcinoid lung tumours synthesise ACTH (8), should therefore be expanded to include LPH. ACTH content correlates well with the presence of secretory granules in "apudomas" (8).

As with ACTH and LPH there is accumulating evidence that oat cell tumours commonly synthesise antidiuretic hormone (ADH), whether or not they are associated with the syndrome of inappropriate ADH secretion (SIADH) (9) and calcitonin (CT) which is not associated with a characteristic clinical syndrome. Recently we demonstrated CT immunoactivity in 92% of 12 bronchial "apudomas", confirming a previous report of 89% in such tumours (10). We have also found GH-like immunoactivity in 46% of 13 bronchial "apudomas" and low levels of PRL-immunoactivity less frequently. However, the proportion of PRL-positivity is greatly dependent on the hormone level assumed to be significant. In

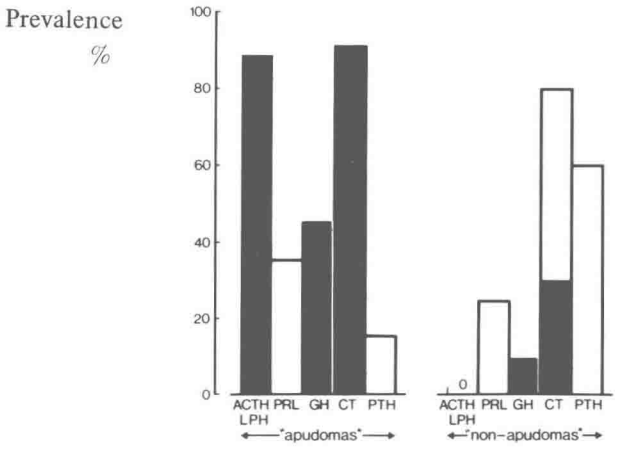

Fig. 1 Adrenocorticotrophin/lipotrophin, prolactin, growth hormone, calcitonin and parathyroid hormone prevalence in lung tumours not associated with an ectopic humoral syndrome.
Left panel – oat cell and carcinoid tumours
Right panel – adenocarcinomas and epidermoid tumours
Open blocks – positivity taken to represent tumour levels sufficient to yield full dose response curves.
Solid blocks – positivity represents tumour levels at least ten fold greater than upper limit of normal circulating levels.

contrast, parathyroid hormone (PTH) is rarely found in measurable amounts in these tumours.

2. Epidermoid tumours and adenocarcinomas ("non-apudomas") (Fig. 1)

The ability of adenocarcinomas and epidermoid (squamous cell) tumours to synthesise ACTH, LPH and ADH is less clear. We have been unable to demonstrate ACTH or LPH in any of 14 such tumours, confirming previous findings with ACTH (8).

Our data contrast with reports that lung tumours of all histological types may synthesise these peptides (3,11). However, the latter reports fail to specify tumour histology in detail and where available (Fig. 2) the apparent levels in "non-apudomas" are low. So called adenocarcinomas and epidermoid tumours with ACTH levels greater than 10 ng/g may represent combination tumours with carcinoid elements (8). Moreover, it is not clear whether very low levels (~ 1 ng/g) indicate ACTH production, retained circulating ACTH or non-specific effects in the assay. It may be relevant that Odell and colleagues, 1977 (3), detected ACTH and βLPH-like activity in normal human tissues, a finding not confirmed in our studies.

As far as we are aware, there are no proven examples of SIADH associated with bronchial "non-apudomas". Morton and colleagues, 1978 (9), failed to show ADH-like activity in any of six "non-apudomas" and elevated circulating ADH levels in patients with epidermoid tumours may be due to a mechanism other than tumour hormone

synthesis (12).

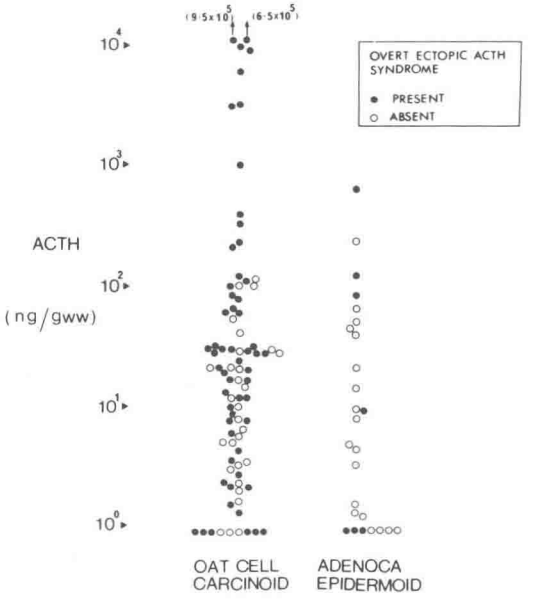

Fig. 2. ACTH levels in lung tumours of defined histological type, with (●) and (○) without ectopic ACTH syndrome. Data taken from the literature up to 1978 (111 cases).

It does appear however, that significant CT-like immunoactivity occurs in "non-apudomas". Thus CT-like activity was reported in 45% of such tumours (10) and we have found a similar prevalence. In vitro secretion of CT by an epidermoid lung carcinoma (13) and of chorionic gonadotrophin by a large cell tumour (14) confirms the ability of these tumours to elaborate hormone products. We have also detected PRL- and GH-like immunoactivity in "non-apudomas", although concentrations are generally low. Similarly, low levels of PTH-like material are frequently found. The interpretation of low hormone immunoactivity must be treated with caution however, and does not necessarily indicate tumour hormone synthesis.

Gastrointestinal cancer (Fig. 3).

There have been few extensive studies of peptide hormones in gastrointestinal cancer. The demonstration of GH-like activity in 5 of 8 gastric adenocarcinomas suggests that inappropriate hormone production may be common in these tumours (15). However, we have been unable to demonstrate ACTH, LPH or PTH in any of eight gastric tumours (6 adenocarcinomas, 2 carcinoids). Low levels of GH and PRL independently were found in only one of six cases. This

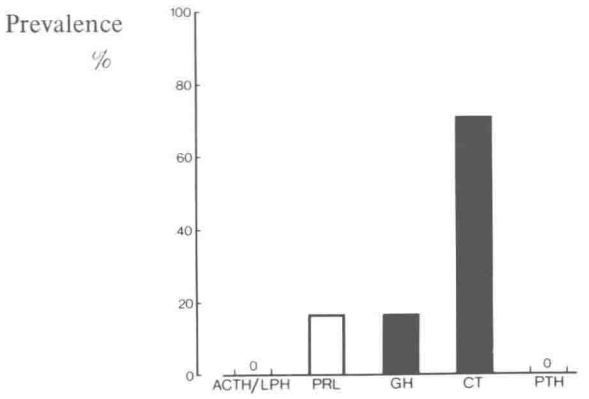

Fig. 3. Hormone prevalences in gastric tumours not associated with an ectopic humoral syndrome. Details of open and closed blocks as in Fig. 1.

suggests that the prevalence of PTH and also of pituitary peptide hormones in gastric adenocarcinoma is rare. The only proven example of the association of a gastric tumour with ectopic ACTH syndrome involves a carcinoid tumour (16). However, we were able to demonstrate CT-like activity in 3 of 5 adenocarcinomas and in both carcinoid tumours studied. This confirms the report of CT in a gastric carcinoma (10).

The presence of human chorionic gonadotrophin (hCG)-like material appears to be relatively common in tumours of stomach, liver and pancreas (17). Indeed, all tumours of stomach, colon and pancreas have been reported to contain hCG (18) while hCG or its subunits were found in all of four malignant islet cell tumours and 63% of sera from such patients but not in tumour or sera from patients with benign lesions (19). Nevertheless, the ectopic status of hCG production by these tumours remains obscure particularly as hCG-like material is found in normal liver and colon tissue (18), pituitary tissue, and urine of normal individuals (20). Similarly, gastrointestinal diseases associated with inflammation and repair are associated with raised circulating hCG levels (17). Consequently, hCG production may be common in certain non-malignant tissues and may therefore be eutopic.

Breast cancer (Fig. 4).

In contrast to lung cancer breast cancer is rarely, if ever, associated with clinically recognised ectopic humoral syndromes. However, we have found significant levels of ACTH and LPH in over 70%, PRL in 69%, GH in 75% and CT in 50% of breast tumours although PRL and CT levels were generally low. Significant amounts of CT have also been reported by Abe and colleagues in three