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The tumor markers CA 125 and SCC antigen : their significance in patients with endometrial or cervical carcinoma

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THE TUMOR MARKERS CA 125 AND SCC ANTIGEN

THEIR SIGNIFICANCE IN PATIENTS WITH ENDOMETRIAL OR CERVICAL CARCINOMA

M.J. DUK

THE TUMOR MARKERS CA 125 AND SCC ANTIGEN

Stellingen behorende bij het Proefschrift van M.J. Duk, getiteld:

THE TUMOR MARKERS
CA 125 AND SCC ANTIGEN

THEIR SIGNIFICANCE IN PATIENTS WITH ENDOMETRIAL OR CERVICAL CARCINOMA.

Groningen, 5 september 1990

1. Het meten van de serum-concentratie van tumormerkstoffen is klinisch pas relevant, indien aan de uitslag van de meting(en) consequenties worden verbonden.

2. Een verhoogde serum-concentratie van CA 125 in de aanwezigheid van een zogenaamd "toprecidief" van een adenocarcinoom van het corpus of de cervix uteri dient een extra aanleiding te zijn om afstandsmetastasen uit te sluiten, alvorens tot (in opzet curatieve) behandeling van het recidief over te gaan.

3. Er bestaat behoefte aan studies, die het effect van gecombineerde locale therapie en systeemtherapie onderzoeken bij patienten met een vroeg stadium endometrium- of cervixcarcinoom, die op basis van klinische, histopathologische en biochemische parameters een verhoogd risico hebben om aan de ziekte te overlijden.

4. Er is alles voor te zeggen, dat de arts in opleiding tot gynaecoloog door een ervaren verloskundige wordt onderwezen in de vaardigheden, die horen bij de fysiologische verloskunde.

5. Op een oncologische afdeling moeten de naast betrokkenen van de patient, indien gewenst, in staat worden gesteld te participeren in de verpleegkundige zorg voor die patient.

6. De in het obstetrisch spraakgebruik gangbare kwalificatie "kostbare zwangerschap" impliceert een oneigenlijke meerwaarde, die niet van invloed mag zijn op de zorgvuldigheid van het medisch handelen.

7. Bij verdenking op een longaandoening dient de fysische diagnostiek van de long volledig te worden uitgevoerd.

8. Omdat er onvoldoende argumenten waren om de opleiding tot gynaecoloog van 5 tot 6 jaar te verlengen (Aalders J.G., Proefschrift, Stelling 15, Groningen, 1982), zijn er oneigenlijke argumenten gebruikt (Bekedam D.J., Proefschrift, Stelling 6, Groningen, 1989) om zulks toch te doen.

9. Bij recidiverende vage pijnklachten in de liesstreek bij een overigens gezonde jonge man hoort men een dreigende torsio testis niet over het hoofd te zien.

10. In de oncologische gynaecologie kunnen de meeste recidieven worden opgespoord door gerichte anamnese, lichamelijk onderzoek, cytologische controle en serum-bepaling van de juiste tumormerkstof(fen).

11. Té vroege opsporing van een recidief is een reëel probleem.

12. De vleugels van een Peugeot 404 zijn een metafoor. De huidige windtunnelmodellen verwijzen slechts naar de invloed van de elementen op het rijgedrag.

13. Iemand, die 95 stellingen kan bedenken verdient een plaatsje in de geschiedenisboeken.

14. De verdedigbaarheid van een stelling, die de positie van de barende tot onderwerp heeft, is afhankelijk van de positie van de barende in die stelling.

RIJKSUNIVERSITEIT GRONINGEN

The Tumor Markers CA 125 and SCC Antigen

Their significance in patients with endometrial or cervical carcinoma

PROEFSCHRIFT

ter verkrijging van het doctoraat in de geneeskunde
aan de Rijksuniversiteit Groningen
op gezag van de Rector Magnificus Dr. L.J. Engels
in het openbaar te verdedigen
op woensdag, 5 september 1990, des namiddags te 4.00 uur

door

Marinus Jitze Duk

Geboren op 19 juni 1957 te Hooge- en Lage Zwaluwe

1990

Promotores: Prof. Dr. J.G. Aalders
Prof. Dr. G.J. Fleuren

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Prof. Dr. H. Schraffordt Koops

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Aan mijn vader en moeder
Aan Welmoed

Contents

CHAPTER I

General introduction and aims of the study

General introduction

Malignant gynecologic neoplasms do not represent a single entity. They occur at all sites in the female genital tract, 97% being carcinomas of various epithelial types and the remainder being sarcomas and fetal rest growths.[1] When studying the etiology, pathophysiology or the biologic behavior of malignant tumors of the female genital tract, one should appreciate the variety in origin, structure, topography and function of the cells from which these tumors are derived. However, the choice of therapy and the prognosis depend largely upon the extent of the tumor at diagnosis. It has been well-recognized that a uniform staging system is mandatory in order to present results which are comparable between clinics and between differing modes of therapy. The International Federation of Gynecology and Obstetrics (FIGO) has established a staging system which stratifies gynecologic tumors into four stages.[2] The accurate delineation of the extent of the tumor represents a serious diagnostic problem which remains to be resolved. Particularly the detection of early tumor spread to distant sites is arduous. A tumor mass at the lowest limit of radiographic detection may contain as many as 10^9 cells and be some 1 cm^3 in size.[3-5] Metastatic tumor cells, having successfully passed through a complex sequence of discrete events after separation from the primary tumor, have been shown to differ considerably from the cells populating the parent tumor.[6] This process of progressive variability and selection of tumor cell lines is a dynamic contingency which may occur at an early stage of tumor evolution.[7] Metastases may be detected at the initial diagnosis or may manifest themselves clinically, as 'recurrent disease', during follow-up. Metastatic disease almost invariably confronts the patient and the physician with a formidable obstacle to succesful treatment.

One of the major clinical implications of these fundamental aspects of tumor progression is the presumption that early detection may offer better chances for survival. The application of biochemical techniques aimed at the detection of tumor cell antigens, offers new and more refined opportunities for the staging, treatment monitoring and follow-up of cancer patients. In addition, imaging techniques and immunotherapy using monoclonal antibodies carrying radioactiveisotopes or cytotoxic agents, are being intensively investigated at present.[8] With the discovery of the tumor-associated antigens Cancer Antigen (CA) 125 and Squamous Cell Carcinoma (SCC) antigen gynecologic oncologists have new tools at their disposal for the critical evaluation of tumor staging and treatment results. It should be emphasized, however, that there is no such thing as an ideal tumor marker. An ideal tumor marker would need to have a sensitivity and specificity of one hundred per cent in a situation where it can potentially influence a decision about how the patient should be managed. The fact that such markers do not exist is implicated by the process of tumorigenesis itself in relation to interaction with the host.

Aims of the study

In 1983, Bast et al[9,10] reported circulating antigen levels detected by the monoclonal antibody OC 125 which was raised against a serous ovarian cyst-adenocarcinoma cell line. The antigen was designated Cancer Antigen (CA) 125. Serum antigen concentrations were found to reflect tumor burden and to parallel the clinical course of disease.

In the first immunohistochemical study on the tissue distribution of this antigen by Kabawat et al,[11] it was found that the antigen could be detected in normal and neoplastic epithelial cells of Müllerian origin, e.g. the epithelial glandular lining of the upper female genital tract. In 1984, Niloff et al[12] reported increased serum CA 125 concentrations in patients with advanced endometrial cancer or cervical adenocarcinoma. Until that time, the only serum marker which had been reported to be of clinical significance with these types of female genital tract cancer was carcinoembryonic antigen (CEA).[13-17] CEA was also the only serum marker which had been repeatedly shown to be of clinical importance in the management of patients with squamous cell cancer of the uterine cervix.[13,14,18-20] In 1977, Kato and Torigoe[21] isolated an antigen from a squamous cell carcinoma of the uterine cervix, which they designated TA-4. Subsequent studies indicated that serum concentrations of TA-4 were of potential clinical value for the monito-

ring of patients being treated for squamous cell carcinoma of the uterine cervix.[22-25]

TA-4 consists of at least 14 subfractions which share one common antigenic determinant.[26,27] One of these allotypes, named Squamous Cell Carcinoma (SCC) antigen,[28] has been isolated from a liver metastasis from a cervical squamous cell carcinoma and is used as a standard in a double-antibody radioimmunoassay.[29]

This study is actuated by these observations and focusses on the following central issues:

I. What is the clinical relevance of serum determinations of CA 125, SCC antigen or CEA for the management of patients with carcinoma of the uterine corpus or cervix?

II. Can we define clinical or histologic parameters which may influence the serum level of these substances in order to gain a better understanding of the pathophysiology of these tumor-associated antigens and the biologic behavior of uterine tumors?

Outline of the thesis

In the studies on the significance of CA 125, SCC antigen or CEA in malignant epithelial tumors of the uterus, the results of the serum analyses of 655 patients who had been admitted between 1978 and 1989 were examined in relation to the course of the disease.

In the framework of the research into factors influencing the serum marker levels, the interrelations between serum marker concentrations and tumor-related clinical or histopathologic parameters were investigated. In addition, two prospective studies were carried out using the data of 116 patients with nonmalignant disease. Increase of the serum CA 125 concentration in association with pelvic inflammatory disease had been reported at the time this study was initiated. Inflammatory lesions of the female genital tract in association with increased serum CA 125 levels provide a *clinical* model for studying the alleged role of peritoneal surface cells as a source of CA 125 production. Furthermore, increased serum concentrations of SCC antigen were determined in a patient who was in complete remission after treatment for cancer of the uterine cervix but who was found to have severe psoriasis. This finding led to the study on SCC antigen in patients with benign skin disease, which wascarried out in cooperation with the Department of Dermatology.

The next chapter contains an epitome of the genotypic and phenotypic

aspects of the malignant and ultimately metastatic cancer cell (Chapter 2). A short review is presented on polyclonal and monoclonal antibody techniques with special emphasis on the tumor markers CA 125 and SCC antigen.

Chapter 3 presents the results of a clinical and immunohistochemical study on the significance of CA 125 in patients with endometrial carcinoma.

In a comparative study, the serum levels of CA 125, SCC antigen and CEA in patients with adenocarcinoma or adenosquamous carcinoma of the uterine cervix were analyzed in relation to the clinical course of disease. The results of this study are presented in Chapter 4.

Chapter 5 is a sequel to this study, investigating the prognostic value of the pretreatment serum concentrations of these three antigens in relation to the histopathology and biologic behavior of the tumor with emphasis on early stage disease.

The clinical and theoretical implications of the previous observations that pelvic inflammatory disease can be associated with an increase in the serum CA 125 concentration, are presented in Chapter 6.

The results of a study on the effect of benign skin disease on the specificity of serum SCC antigen determinations, are presented in Chapter 7.

Chapter 8 describes the sensitivity and specificity of serum SCC antigen determinations in patients with squamous cell cancer of the uterine cervix.

The results of this study are discussed in Chapter 9.

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CHAPTER II

Perspective of this thesis

Introduction

The concentration of tumor-associated substances can be measured from a variety of body fluids, using different types of assay systems. Most commonly, these assays are used to detect antigen activity in the serum of patients with cancer. Thus, in clinical practice, the physician uses a test and is consequently confronted with the issues of the sensitivity and specificity of the test. These test characteristics depend on the production and shedding of such antigens into body fluids. The expression of substances associated with tumor growth forms part of the genetic changes which result in or coincide with the transformation of a normal cell into a tumor cell. The issue of sensitivity or specificity of tumor markers is therefore better understood when placed within the concept of the intricate interplay of the genotypic and phenotypic manifestations of tumor growth.

The genotypic and phenotypic manifestations of cancer

Protooncogenes

Much of what we know now about the transformation of the normal cell into a cancer cell has been brought to light by pioneering work on tumor viruses. The ability of oncogenic viruses to convert a cell to cancerous growth is ascribed to the activity of so-called *viral oncogenes* (v-onc's) present in the viral genome. A large number of viral oncogenes such as v-myc, v-erbB and v-ras, associated with virtually all forms of neoplasia, have been identified.[1,2] Bishop, Varmus and Stehelin discovered that *cellular oncogenes* (c-onc's) were

the normal cellular homologues of viral transforming genes of RNA tumor viruses (retroviruses).[3-7] These cellular genes, e.g. c-myc, c-erbB and c-ras are called *protooncogenes* or *cellular oncogenes*. In normal cells these (protoonco)genes are expressed in a tightly regulated fashion. This finding forms the basis of the concept that retrovirus oncogenes are copies of normal cellular genes, which these viruses have acquired from the genome of somatic cells at some time in the evolution. The genesis of viral oncogenes from cellular protooncogenes is called *transduction*.

Other investigators then set out to search for tumorigeneic genes which were *not* related to viral oncogenes. Weinberg, Cooper and othershave shown that the *transfection* of genes derived from experimentally induced tumor cell DNA can transform normal cells into cancerous growth.[8-12] These segments such as int-1 and int-2, met, neu and other cellular oncogenes have been isolated from a variety of malignancies[1,2,13] and represent a second class of oncogenes. These oncogenes are thought to be activated by mutagens such as radiation or chemical agents. It became clear from these studies that the development of a malignant tumor is by far not a single step process but rather results from multiple events, probably involving multiple genes.[2,9,14]

Recently, the finding of *anti-oncogene-genes* or *tumor-suppressor-genes* which restrain or confine normal cellular proliferation has received increasing interest. Although data are still scarce, these genes may play an important etiologic role in the tumorigenesis (e.g. retinoblastoma).[2,14]

Cancer cells often show damaged chromosomes. Translocation, deletion or amplification are predominant features of such chromosomes. These damages may affect protooncogenes and change the expression or the biochemical function of that gene product.[7,15,16] Protooncogenes play an important role in the control and regulation of proliferation and differentiation. "Cancer may be a malady of genes"[7] which triggers numerous events resulting in unrestrained growth.[2,7,13,16] For example, activation of oncogenes has been shown to result in both quantitative and qualitative changes in their encoded proteins. Amplification of the protooncogene may lead to the overproduction of the gene product, such as protein kinases, growth factor receptors or signal transducing proteins. Mutation or chromosomal rearrangement following translocation or deletion can generate the expression of abnormal gene products: proteins having altered or overpowering enzymatic activity. Also, structural changes in membrane receptors may render the cell less accessible to modulating signals. Detailed knowledge of the structure and function of these proteins may help to develop new diagnostic and therapeutic approaches to cancer.

Infiltrative growth and metastasis

How do tumor cells end up in tissues to which they do not belong? Neoplastic proliferation may proceed either immediately or some time after a latent period following the carcinogenic event.[2,12,14,17] Tissue compartments are separated from each other by two types of extracellular matrix: basement membranes and interstitial stroma. The invasive process, both at the edge of the primary tumor and in target organs, is thought to be the result of a complex interaction between tumor cells and the interstitial connective tissue matrix.[18-21] Tumor cells produce enzymes, such as Collagenase IV which katalyzes the degradation of Collagen type IV, an important basement membrane component. Barsky and others have shown that benign tumors exhibit intact basement membranes, whereas loss or disorganization of the basement membrane is a common finding in malignant neoplasms.[22,23] Thus, these proteolytic enzymes aid tumor cells in their transgression through connective tissue barriers. Tumor cells also produce other substances such as Tumor Angiogenesis Factor which stimulates the formation of a vascular network, permitting continuous growth.[24,25] The quality of these newly induced vessels is often inferior and is characterized by loose endothelial junctions.[19] In addition, it has been demonstrated that lymphatic capillaries lack a basement membrane containing Collagen type IV and laminin.[19,26] Such conditions provide a "leacky" barrier between tumor and circulation and offer tumor cells the opportunity of migrating through the vessel wall, to invade the vasculary system and to spread to distant sites. Tumor cells can be found circulating in the blood of many cancer patients.[27] The greater portion of the cells which have invaded the lymphatic system or peripheral blood stream are eliminated by metabolic disadvantage, nonimmune defenses or immunologic destruction by natural killer (NK) or lymphokine-activated killer (LAK) cells.[28-30] In a classical experiment, Fidler[31] has elegantly shown that the major portion of circulating tumor cells is destroyed leaving a very small number of cells (an estimated 0.01%) which are able to establish metastases. In addition, it has been shown that the adhesive interaction between tumor cell membranes and the target organ parenchyma determines the preferential site of metastasis: the "seed *versus* soil" hypothesis.[20,21,32] Fidler[33] has concluded quite clearly from all these studies that the process of tumor growth and metastasis is not random: only the cells with the highest metastatic propensity through progressive selection will survive, attach and grow.

The changes and anomalies in the cell genome which prelude the formation of the cancer cell have their phenotypic equivalents. It is generally accepted that in most cases cancer begins in one single cell[28,34-36] and that the clonal evolution of tumor cell populations is a multistep process.[28,34-38] By the time that the tumor becomes clinically detectable the population size is between 10^6 and 10^9 cells, the size of the tumor is 1 cm^3 and its weight is approximately 1 gram.[33] It takes thirty doublings of the tumor to reach this size and it takes another ten doublings to reach the size of a kilo, which, in most cases, is lethal.[39] These figures illustrate that a tumor is *under cover* for almost three-quarter of its biologic existence.

Studies on the progression of neoplastic cells have yielded the important concept of the selective neoplastic proliferation as a result of genetic lability of the tumor cell.[28,33,37,40-42] This genetic instability promotes cytogenetic and phenotypic heterogeneity allowing for the appearance of mutant cells within tumor cell populations which become precursors of new predominant subpopulations. These mutants have acquired a growth advantage over the original tumor cells and are more capable of surviving in a hostile environment. The final stage in tumor progression is the acquisition of metastatic potential.[28]

The concept of progressive tumor heterogeneity during the clonal evolution of primary and metastatic tumors[43] has made a considerable contribution to the explanation of differences within and between tumors with respect to, e.g. cell kinetics, degree of differentiation, hormone receptors, invasive and metastatic capacity, resistance to natural killer cells or cytotoxic drugs.[34,33,38,44-46] Stable or transient heterogeneous antigen expression by tumor cells[47] is another example and a common phenomenon for, e.g. the pathologist: immunohistochemistry often shows distinct regions with antigen positive and negative cells. Moreover, during tumor progression these cells can become entirely negative or display activity of antigens which are not detected in the tissue from which the tumor originates. This also explains why unexpected changes in serum antigen levels can be observed during progression of the disease.[48]

The rationale that early detection of malignant neoplastic growth offers the best chances for succesful treatment, emanates from these fundamental aspects of tumor progression. It is a challenge for the oncologist to preclude the formation of cells which are resistent to any treatment regimen.

The application of polyclonal and monoclonal antibody techniques in clinical gynecologic oncology

Like all cells, tumor cells express a variety of substances which have antigenic activity, most of which are identical to those which are produced by normal cells. However, the changes in the genome of the tumor cell can generate alterations in its antigenic expression. Essentially, it is this feature of neoplastic cells which has prompted researchers to elucidate the possibilities of a different diagnostic and therapeutic approach of cancer. The detection of tumor cell antigens from body fluids or the immunochemical demonstration of these antigens in tumor cells or tissues, can provide important information for the physician. The discovery made by Köhler and Milstein[49] which allows the production of large amounts of antibody with predefined specificity, greatly increased the possibilities for the detection of tumor cell antigens. One of the major advantages of monoclonal antibodies is that they can be raised against antigens which have not (yet) been identified. In the field of oncology this is particularly useful in studies of cell-surface proteins and for identifying new serum markers for different human tumors. The assays which have been developed in the recent years have a high specificity and are capable of detecting very small amounts of antigen. One of the abilities of these tumor cell antigens is that they serve as so-called 'tumor markers'. It has become increasingly clear, however, that most tumor markers are also present in normal cells and that their utility depends largely upon quantitative rather than qualitative differences. This observation is understandable in the light of the transitional means of evolution - although presumably in a discrete *multistep* process - from the normal cell to a cancerous cell.

A number of 'tumor marker assays' are now commercially available which test for the presence or absence of tumor activity. The ideal tumor marker would have to meet various requirements: firstly, it would be ideal if the presence of a particular tumor marker in fluid or cells would be pathognomonic for a specific type of malignant tumor. Such markers do not exist and so they cannot be used to establish a diagnosis. Until now, the most important role of tumor markers in clinical oncology lies in their (serial) serum determination in the blood of patients with a malignant tumor in order to monitor the disease process during treatment and follow-up. A tumor marker must therefore have a high degree of sensitivity and specificity. It is this sensitivity and specificity which determines the fate of a newly proposed tumor marker. An ideal tumor marker would also provide prognostic information. This characteristic refers to the ability of a good tumor marker assay to influence decisions between alternative plans for patient management.

Two nearly perfect tumor markers have firmly been established in the field of gynecologic oncology: the hormones hCG (human chorionic gonadotropin)[50] in gestational trophoblastic disease (GTN), nongestational choriocarcinoma and embryonal carcinoma of the ovary and αFP (alpha-fetoprotein)[51] in endodermal sinus tumor or embryonal carcinoma of the ovary. These tests demonstrate high sensitivity and specificity and the outcome of the test influences the decisions for further management.

The use of CEA, an oncofetal antigen which was initially described as a marker for patients with colon carcinoma,[52] has been widely investigated in gynecologic malignancies as well.[53-61] Although CEA measurements may be of value in selected patients, these studies have mainly reported low sensitivity and specificity rates.

A large number of other tumor-associated antigens have been evaluated clinically during the past two decades. These substances include proteins such as SP1 (or PsB1G: pregnancy-specific ß-1-glycoprotein),[62] placentaproteins (PP_2-PP_{14}),[63] OCAA[64] and OCA,[65] TAG-72,[66] enzymes such as LDH or placental alkaline phosphatase (P[L]AP),[67] and carbohydrate determinants such as CA 19.9[68] or CA 15.3,[69-71] which were originally prepared for the monitoring of cancer of the digestive tract and breast, respectively. Only a few of these antigens have been introduced into routine clinical practice, because most of them lack sufficient sensitivity and specificity in gynecologic cancer. So far, a limited number of reports on the use of sensitive and specific markers for patients with (corporal or cervical) cancer of the uterus have been published (see below).

A few of the management problems regarding these tumors are highlighted below, because they are the subject of this study.

Although cervical or endometrial tumors are relatively easily accesible to conventional diagnostic techniques, the *accurate staging* remains a controversial issue.[72] It has been repeatedly demonstrated that the clinical, i.e. pretreatment, staging is inaccurate. In cervical cancer for example, a variety of both invasive and noninvasive techniques are being applied in order to determine the actual extent of the tumor burden. These methods have been proved to be insufficiently sensitive and/or non-specific (chest X-rays) or expensive and not mandatory in a routine situation (ultrasonography or CT scans).[73,74] Invasive methods, such as lymphangiography, also show significant false positive and false negative rates.[75] As outlined above, the important differences on a molecular and cellular level should be acknowledged between cells that populate the parent tumor and metastasized cells. During the past decade, it has become increasingly clear that the identification of the patient at risk for metastatic tumor spread is a prerequisite for the optimalization of

the treatment results. Both in endometrial and cervical cancer, clinical and histopathologic tumor features - which are essentially phenotypic manifestations of the disease - have been linked to the risk for metastazised disease and patient outcome.[72] These risk factors include parameters such as histologic type and grade, depth of infiltration, vascular invasion, tumor volume, peritoneal cytology, and, more recently, ploidy patterns.[72,73,76-90]

Furthermore, *the detection of residual or recurrent disease* after treatment or during follow-up is often quite difficult and it is seldom possible to cure patients who clinically present with new tumor lesions.[72,73,91] The early detection of residual or recurrent disease in a situation where additional treatment options are available may result in higher survival rates.*

The advent of any test which can help to solve these fundamental clinical problems would be greatly appreciated.

The tumor-associated antigen CA 125

In 1981, Bast et al[92] published the first report on Cancer Antigen (CA) 125. BALB/c mice were immunized with a human cell line derived from ascitic fluid from a patient with a serous papillary cystadenocarcinoma of the ovary (OVCA433). Spleen cells of these mice were fused with a plasmacytoma cell line (P3/NS-1). Antibodies produced by the hybrid clones were screened for reactivity with OVCA433-cells and a lack of reactivity with an Epstein-Barr virus transformed autologous B lymphocyte line or with normal allogeneic human ovary cells. Using an indirect immunofluorescence technique, the antibody produced by clone 125 reacted with all 6 epithelial ovarian cancers but not with non ovarian cancer cell lines or nonmalignant tissues. The anti-

* The term 'recurrent disease' or 'tumor relapse' usually applies to the reappearance of tumor lesions after a period of complete remission (no evidence of disease). In clinical practice it is of dual significance: it may cover the reappearance of tumor lesions at organ sites distant from the primary tumor site, which is always due to growth of a metastatic clone or clones. It also implies that routine diagnostic procedures have failed to detect this metastatic lesion at the first screening of the patient. Secondly, the term is used to describe the (local) reappearance of the tumor at the primary lesion site after the primary tumor has been eradicated. In this case it may not always be clear whether this new tumor lesion is in fact residual tumor or the resulting growth of cells that have metastasized locally. Although this may seem a trivial issue in a clinical setting, there are major differences between the - nonmetastatic - cells within the parent tumor and the cells which populate a metastatic growth.

body recognizes an epitope on a glycoprotein with a molecular weight in excess of 1000 kD with a lower-molecular weight moiety of 200-400 kD and a carbohydrate component of approximately 24%.[93] The antibody, OC 125, has been incorporated into a radioimmunoassay[94] which became commercially available in 1983.[95]

The antigenic determinant CA 125 recognized by the monoclonal antibody OC 125 has been originally proposed as a tumor marker for ovarian cancer, in particular for serous cystadenocarcinoma.[95] A series of reviews on the biochemical characteristics, function, tissue distribution and clinical relevance of CA 125 have been published.[96-99]

Immunohistochemical methods have shown positivity in a large number of tissues, both normal and pathologic. Fetal coelomic epithelium stains positively when incubated with OC 125.[100] In the adult, CA 125 is present in coelomic derivatives, such as the normal glandular lining epithelium of Müllerian origin in the female reproductive tract (Fallopian tube, endometrium and endocervix).[100-106] During pregnancy, CA 125 has been localized in the decidua and amnion.[107,108] In benign disease, CA 125 is expressed in, e.g. benign ovarian tumor tissues[109-111] and in endometriotic lesions[112,113] In addition, reactive mesothelial cells on the surface of the peritoneum, pleura or pericard, are positive,[100,110,111,114] whereas quiescent mesothelial cells react only weakly or not at all.[110,115] In the original report by Kabawat et al,[116] no reactivity was demonstrable in mucinous tumors or in normal fetal or adult surface epithelium of the ovary, except for papillary excrescences or crypts associated with metaplasia. More recent studies, however, using more sensitive methods, have shown that normal ovary surface epithelial cells and mucinous tumors can express antigen activity as well.[101,103,109-111,115,117] In addition, other malignant tumors arising from the Müllerian epithelium, such as endometrial or endocervical carcinomas exhibit intense positivity.[103-106]

CA 125 is also present in a variety of tissues of noncoelomic origin. Antigen activity - although mostly weak and/or in a low frequency - has been reported in the normal luminal epithelium of the pancreas, kidney, colon, stomach, gall bladder, breast ducts and lung.[101,114] Traces of CA 125 have also been found in a proportion of cancers which originate from these tissues, such as cancer of the breast,[100,117] pancreas,[94,95,101] colon,[100,101,117,118] and bronchus.[100,101,114,117] A small number of tumors of the kidney, gall bladder, testis and thymus also stain positively.[101]

Most of these studies have revealed that the normal lining cells of the female genital tract show a distinct staining pattern disclosing antigen concentrations at the apical (Fallopian tube) and/or luminal border (endometri-

um) or throughout the cytoplasm (endocervix). The staining pattern in cancerous cells is different: heterogeneously distributed areas of negative and membrane-bound positive (mostly luminal) CA 125 reactivity can be found with unevently scattered or vacuolized localization of the antigen throughout the cytoplasm of the cells.

Several studies have made it clear that CA 125 is shed into the cavities which are lined by the tissues which express antigen activity. CA 125 concentrations can be measured in, e.g. cytosol fractions of normal and neoplastic tissues of Müllerian origin,[119] in normal peritoneal fluid,[120] ascitic fluid or pleural effusions,[121-123] benign or malignant ovarian cysts,[109] human milk,[93] seminal plasma,[124] or amniotic fluid.[107,125]

Extraordinarily high CA 125 levels have been measured in tubal or uterine secretions or cervical mucus of healthy premenopausal women.[102,103,126] At the University Hospital of Groningen, levels in excess of 4.000.000 U/ml have been determined (unpublished data). Nanbu et al[126] reported that CA 125 concentrations in cervical mucus were significantly higher during the follicular phase of the menstrual cycle. Bischof et al[127] found higher CA 125 concentrations in medium of cultured endometrial stromal cells during the proliferative or early secretory stage. Recently, bronchial mucus has been shown to contain concentrations of CA 125 comparable to those obtained from cervical mucus.[128] Although these findings suggest that CA 125 may have a physiologic function, there is little or almost no information available on this issue or on factors which influence its expression. To date, only three studies have been published which suggest that CA 125 expression may be regulated by hormonal substances.[128,130,131]

On analogy with the topographic distribution of CA 125 in normal and pathologic tissues, raised serum CA 125 levels have also been noted in a variety of physiologic, benign or malignant conditions which interfere with these tissues.[101,103,109,112,113,115,121,122] Extensive reviews regarding these conditions have been published elsewhere.[96-99]

Since the first publications by Bast and colleagues an impressive number of clinical studies have shown that CA 125 is of great value for the management of ovarian carcinoma patients.[95-99,109,133-139] Serum CA 125 concentrations correlate well with tumor burden and serial determinations run parallel to the clinical course of disease. Elevated serum CA 125 concentrations prior to second-look laparotomy are indicative for the presence of residual tumor nodules.[134,136-138] The significance of serum CA 125 determinations for the discrimination of benign and malignant pelvic masses[140-144] or screening purposes[96,145-149] is presently under intensive investigation.

By the time this study was initiated, almost no data were available on serum CA 125 concentrations in patients with endometrial or endocervical carcinoma. In 1984, Niloff et al[150] reported elevations in 14 of 18 patients with advanced stage or recurrent endometrial adenocarcinoma and in 5 of 6 patients with adenocarcinoma of the uterine cervix.

Squamous Cell Carcinoma (SCC) antigen

In 1977, Kato et al[151] purified an antigen from a human cervical squamous cell carcinoma. The purification required four steps: salt fractionation with a saturated ammonium sulfate solution (TA-1); column chromatography using columns of Sephadex G-200 (TA-2) and DEAE-Sephadex A-50 (TA-3); the last step of purification was performed using preparative polyacrylamide gel electrophoresis (TA-4). Subsequent studies on the nature of this antigen showed that TA-4 consists of at least 14 subfractions, with a molecular weight of 42,000 - 48,000 daltons, sharing one common antigenic determinant.[152,153] Isoelectric focussing revealed that these subfractions can roughly be divided into a basic-neutral and an acidic subfamily with a pI below or above 6.25. Squamous cell carcinomas contain both groups, whereas TA-4 in normal squamous epithelium is mostly neutral.[153,154] Furthermore, in an in vitro study, Aramaki et al[138] showed that the acidic components are released into the culture medium of squamous cancer tissue but the basic-neutral subfractions are not. The main - and most neutral - fraction, designated SCC antigen, has been prepared from a liver metastasis of a squamous cell carcinoma of the cervix.[156] The radioimmunoassay for this antigen recognizes all TA-4 subfractions and the assay value represents the sum of the immunologic activity of all subfractions (i.e. TA-4 activity) including SCC antigen.[153, 154]

Initial reports have suggested that TA-4 activity is mainly restricted to premalignant and cancerous squamous cell lesions of the uterine cervix.[157-163] Immunohistochemical data on the bio-distribution of SCC antigen remain scarce, however. TA-4 (or SCC antigen) determinations from cytosol fractions have shown antigen activity in normal and malignant female genital tract or breast tissues, although usually at much lower intensity.[161,162]

Hoshina et al[157] showed that normal cervical squamous epithelium stains preferentially in the intermediate layer. Cells of the basal layer were negative as were glandular cells distal from the squamocolumnar junction. Towards the surface of the epithelium the intensity of TA-4 staining decreased and a shift was noticeable of TA-4 activity from nucleus to cytoplasm. Preneo-

plastic lesions of the cervix showed abundant TA-4 activity. Positive staining could also be observed in the superficial keratotic layer. Keratinizing and large cell non-keratinizing carcinomas showed strong positivity. Again, TA-4 initially appeared in the nucleus and then in the cytoplasma. Small cell carcinomas and cervical adenocarcinomas were negative for TA-4. Positivity was observed in stratified cells of two cases of adenosquamous cancer. Recently, Crombach et al[162] measured concentrations of SCC antigen in the cytosol of nonmalignant and malignant squamous epithelia of the female genital tract. The SCC antigen concentration in the cytosol of normal squamous epithelia of the cervix was significantly higher than in the cytosol of cervical squamous cell carcinomas, whereas the vast majority of normal subjects showed normal serum SCC antigen values in contrast to the patients with invasive cervical squamous cell carcinoma. The finding that SCC antigen concentrations in the cytosol of normal breast skin was comparable to the SCC antigen concentration in the cytosol of cervical cancer patients, stresses the fact that the production of this antigen is not restricted to the epithelium of the female genital tract. So far, no study has yet addressed the distribution of SCC antigen in different body fluids. Preliminary observations at the University Hospital of Groningen have shown that SCC antigen can be detected from the urine of healthy (male and female) subjects, from the fluid present in lymph cysts or from serous lymphoid drainage fluid obtained after radical surgery for gynecologic cancer (unpublished data). Kudo et al[163] provided evidence that the skin can be a source of SCC antigen as well: they found high concentrations of SCC antigen in the blister fluid of patients with pemphigus. Hussa et al[164] demonstrated that TA-4 is produced and readily released into the culture medium by CaSki cells, a cell line derived from an epidermoid carcinoma of the uterine cervix. Using the same cell line, Maruo et al[165] reported on the enhancing effect of Epidermal Growth Factor on the expression and release of TA-4, whereas no effect was observed on cell proliferation.

Pilot studies, mainly from Japan, have shown that the serum determination of TA-4 is of clinical significance for the management of cervical cancer patients. These studies have indicated that the incidence of elevated pretreatment serum SCC antigen levels and the absolute pretreatment serum SCC antigen value correlate with the extent of the disease and serial determinations follow the course of the disease.[151,158,166-169] In a study on 198 patients, 17 patients experienced recurrent disease.[168] Eleven of these patients (65%) showed abnormal serum SCC antigen determinations before any other clinical sign had indicated renewed tumor activity. The results of preliminary studies from Europe and the United States presented in 1987 at

the "Proceedings of the Abbott SCC marker meeting" in Nice, France, endorsed these observations.[170] Increased serum SCC antigen concentrations have also been reported in squamous cell carcinomas of the lung,[170-171] esophagus[170,171] and head and neck region,[170,171,173,174] the skin,[175] and the vulva.[170,176] In addition, it has been reported that liver or severe renal function abnormalities affect the specificity of the serum SCC-antigen assay: Fischbach et al[173] noted elevation of the serum SCC antigen concentration in 7% and 53% of such patients, respectively.

Patients and methods

This study was based on the clinical and histopathologic profiles of 771 patients who were admitted to the Department of Obstetrics and Gynecology and the Department of Dermatology of the University Hospital of Groningen, The Netherlands, between 1978 and 1989. Serial serum samples from these patients were stored at the Serum Bank of the Department of Obstetrics and Gynecology and analyzed for the presence of CA 125, SCC antigen or CEA. An outline of the study is presented in Chapter 1. Except for the study on the role of CA 125 in endometrial carcinoma (Chapter 3), which was performed in 1985, all studies have been carried out between March 1987 and July 1989.

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CHAPTER III

CA 125: A Useful Marker In Endometrial Carcinoma

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SUMMARY

In a retrospective study 121 patients with endometrial cancer were examined. In addition, 20 primary endometrial adenocarcinomas were tested immunohistochemically for CA 125. All tumor tissues were demonstrated to contain CA 125. However, only 25% of 110 patients had elevated CA 125 levels in serum before treatment. The incidence of elevated CA 125 serum levels increased with higher tumor staging up to 55% and 86% in surgical Stages III and IV, respectively. In Stage I and II disease (International Federation of Gynecology and Obstetrics) elevated serum levels before treatment correlated with the presence of tumor tissues outside the uterine body or outside the uterus, respectively, as was determined histopathologically after operation. In addition, a close correlation between elevated levels and vessel invasion of tumor cells was revealed. Serum levels of CA 125 paralleled the clinical course of disease. Tumor recurrence in the abdomen can be preceded by an increase of serum CA 125 levels.
Key words: CA 125, endometrial cancer, risk factors, tumor marker

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Introduction

Endometrial carcinoma is generally accepted as a disease with a good prognosis, because most patients have Stage I disease without metastasis at diagnosis. However, the prognosis is bad when the tumor is no longer confined to the uterine body.[1-3] Therefore, in clinical staging procedures, it is of the utmost importance to select patients who are at risk of extracorporeal tumor spread because of its implications for the choice of therapy.[4]

Until now, the indication for additional therapy has been based on findings at operation and after careful examination of the operative specimen. Moderately and poorly differentiated tumors, deep infiltration of the myometrium, and the presence of tumor cells in endothelium-lined spaces (vessel invasion) are related to a substantial risk of extrauterine tumor spread and a worse prognosis.[5-7]

Elevated serum levels of the cancer antigen CA 125 are found in patients with epithelial ovarian cancer, carcinoma of the pancreas, breast cancer, and carcinoma of the fallopian tube.[8-9]

Niloff et al[9] reported serum levels of CA 125 exceeding the level of 35 U/ml in 14 of 18 patients with Stage IV endometrial carcinoma or recurrent disease but in none of 11 patients with Stage I or II tumors at presentation. This study was undertaken in order to clarify the signifance of serum CA 125 levels in the clinical staging and follow-up of patients with endometrial carcinoma.

Patients and methods

Between 1978 and 1985, 121 patients attended the University Hospital of Groningen, The Netherlands, with newly diagnosed, persistent, or recurrent endometrial carcinoma. Serial serum samples of these patients were stored at the Serum Bank of the Department of Obstetrics and Gynecology at -70°C and used for this study. Endometrial carcinomas were mainly classified and graded according to Kurman and Norris.[10] In addition, adenocarcinomas with squamous differentation were divided into adenocarcinomas with benign squamous epithelium (adenoacanthomas) and those with malignant squamous epithelium (adenosquamous carcinomas). Histologically, 111 patients were found to have adenocarcinoma of the endometrium, including 17 adenocanthomas and 20 adenosquamous carcinomas; six patients had clear cell carcinoma, and four patients had undifferentiated carcinoma of the uterus. Pretreatment sera were available in 110 cases.

Charts from all patients were reviewed by two independent observers before the serum samples were analyzed. Diagnosis and judgment of regression, stability, or progression of disease were based on clinical examinations (as allowed by international agreement in the staging and follow-up of patients with carcinoma of the uterus), on intraoperative observations, and on histopathologic study of the extirpated or biopsy specimen. Before treatment, clinical staging was determined according to the International Federation of Gynecology and Obstetrics (FIGO) criteria (Table I).

• TABLE I. Study population described to tumor stage

		No. of patients	
Stage	*Classification*	*FIGO Stage*	*Surgical Stage*
I	Carcinoma confined to the uterine body	70	71
II	Extension to the cervix	30	18
III	Extrauterine pelvic extension	5	12
IV	Extension outside the true pelvis and/or perforation of bladder or rectum	5	9
Total		110	110

By using intraoperative observations and after histopathologic examination of the extirpated specimen a surgical stage was established. In addition, the histologic type of the tumor, the degree of differentation, the depth of myometrial infiltration, and the presence of tumor cells in uterine vessels were determined.

In a bi-variate statistical analysis mutual correlations between these four histopathologic prognostic risk factors were investigated. Subsequently, correlations between these histopathologic risk factors and elevated CA 125 serum levels were determined. The correlation between pretreatment serum levels of CA 125 and tumor extension was revealed by dividing 62 patients with Stage I disease into one of two groups after operation and histopathologic examination of the extirpated specimen. One group consisted of patients

with tumor confined to the uterine body and the other group consisted of patients with tumor extension outside the uterine body (extracorporeal tumor extension). Thirty patients with Stage II disease were divided into one of two groups on the basis of extrauterine tumor extension.

The clinical course of disease was recorded in all of the patients and serial serum samples were analyzed for CA 125 during a period from two to 72 months. Regression of tumor was defined as a reduction in demonstrable tumor tissues of more than 50% within a period of three months after treatment. Stable disease existed when there were no alterations in serial observations of the clinical status of the patient who was known to have tumor present. Progression of disease required distinct changes in the clinical status of the patient who was known to have tumor present or the appearance of new lesions, if possible confirmed by histopathologic reports.

For statistical analysis of the results the X^2test, Fisher's exact test, and the Mann-Whitney U test were used.

Immunoradiometric assay of CA 125.

Serum samples were coded to ensure that the investigators who performed the assay were not aware of the source of the serum samples. Levels of CA 125 in these serum samples were assayed by means of a simultaneous sandwich immunoradiometric assay (Abbott Laboratories, Chicago, Il, USA), as described in detail by Bast et al.[8] All values up to a level of 35 U/ml were regarded as normal.[8] The coefficient of variation was 5%, as determined by repeated analysis of a control sample (106.6 ± 5.3 U/ml, $N = 34$).

Immunoperoxidase staining of CA 125.

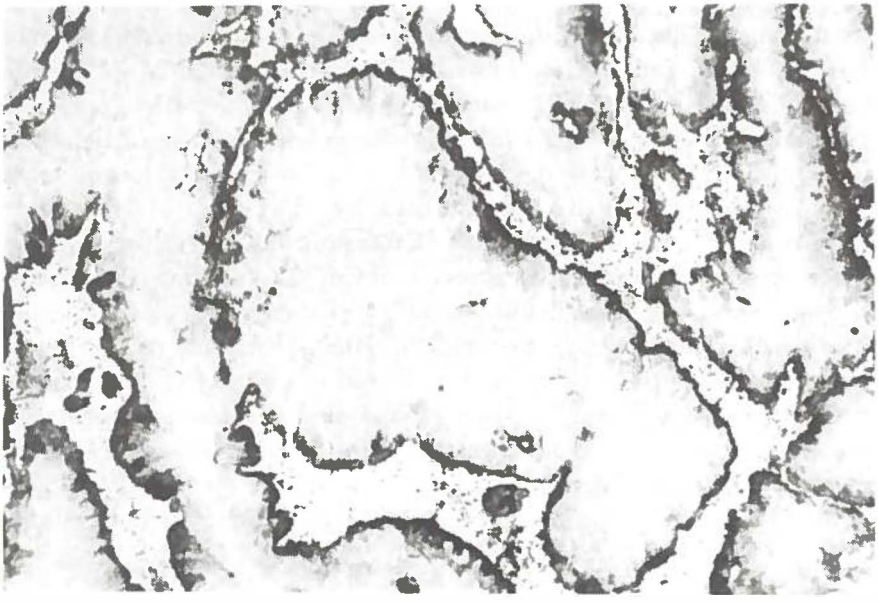
The presence of CA 125 was determined by means of the indirect immunoperoxidase method in snap-frozen tissue blocks.[11] Tissue sections of 4 µm were fixed in acetone for four minutes and washed in 0.01 mol/l phosphate-buffered saline, pH = 7.4. The sections were incubated for one hour with monoclonal antibody OC 125 (ORIS Lapam, Saint Quentin, France) at room temperature in a moist chamber. The sections were washed three times for 10 minutes with phosphate-buffered saline and subsequently incubated for 30 minutes with horseradish peroxidase-conjugated rabbit antimouse IgG (Dakopatts, Copenhagen, Denmark). Peroxidase activity was developed by adding a freshly prepared solution of 3-amino-9-ethylcarbazole in 0.1 mol/l acetate buffer, pH = 5.0 containing 0.015% hydrogen peroxide, resulting in a red reaction product. The sections were counterstained with hematoxylin for two minutes and mounted in Kaiser's gelatin glycerin (Merck, Darmstadt, W-Germany).

Results

Positive immunohistochemical staining for CA 125 was detected in all off eight tissue blocks of normal endometrium obtained at different stages of the menstrual cycle. The positive reaction was present in a sharp-staining pattern in the luminal cell membranes of the glandular structures. Focally, sharp staining of the basal cell membranes was also observed.

Tissue blocks of 20 primary endometrial carcinomas were tested immunohistochemically. The histologic differentation ranged from grade 1 to grade 3. The presence of CA 125 could be demonstrated in all the tissue sections. Both positive and negative glandular structures were observed. In eosin and hematoxylin-stained sections positive and negative glandular structures appeared identical. Positive glandular structures were mainly present in periodic acid-Schiff positive and diastase-resistant tumor areas. Solid tumor areas were mostly negative for CA 125 (Fig. 1). Before treatment serum levels of CA 125 in excess of 35 U/ml were found in 27 of 110 patients (25%, range 36 - 981 U/ml) (Table II). All four patients with undifferentiated carcinoma of the uterus had low levels of CA 125 at any stage of the disease. This and the immunohistologic findings regarding solid tumor areas were reason to exlude these patients from further analysis (including tables and figures).

• FIG. 1. A grade II endometrial adenocarcinoma stained for CA 125. The surface of the glandular structures shows strong staining; the solid areas are negative for staining. (Hematoxylin counterstain. x 130.)



• TABLE II. Incidence of elevated serum levels of CA 125 in patients with endometrial adenocarcinoma before treatment

FIGO Stage	N	Patients with elevated CA 125 levels n	(%)
I	70	12	(17)
II	30	10	(33)
III	4	3	(75)
IV	3	2	(67)
Total	107*	27	(25)

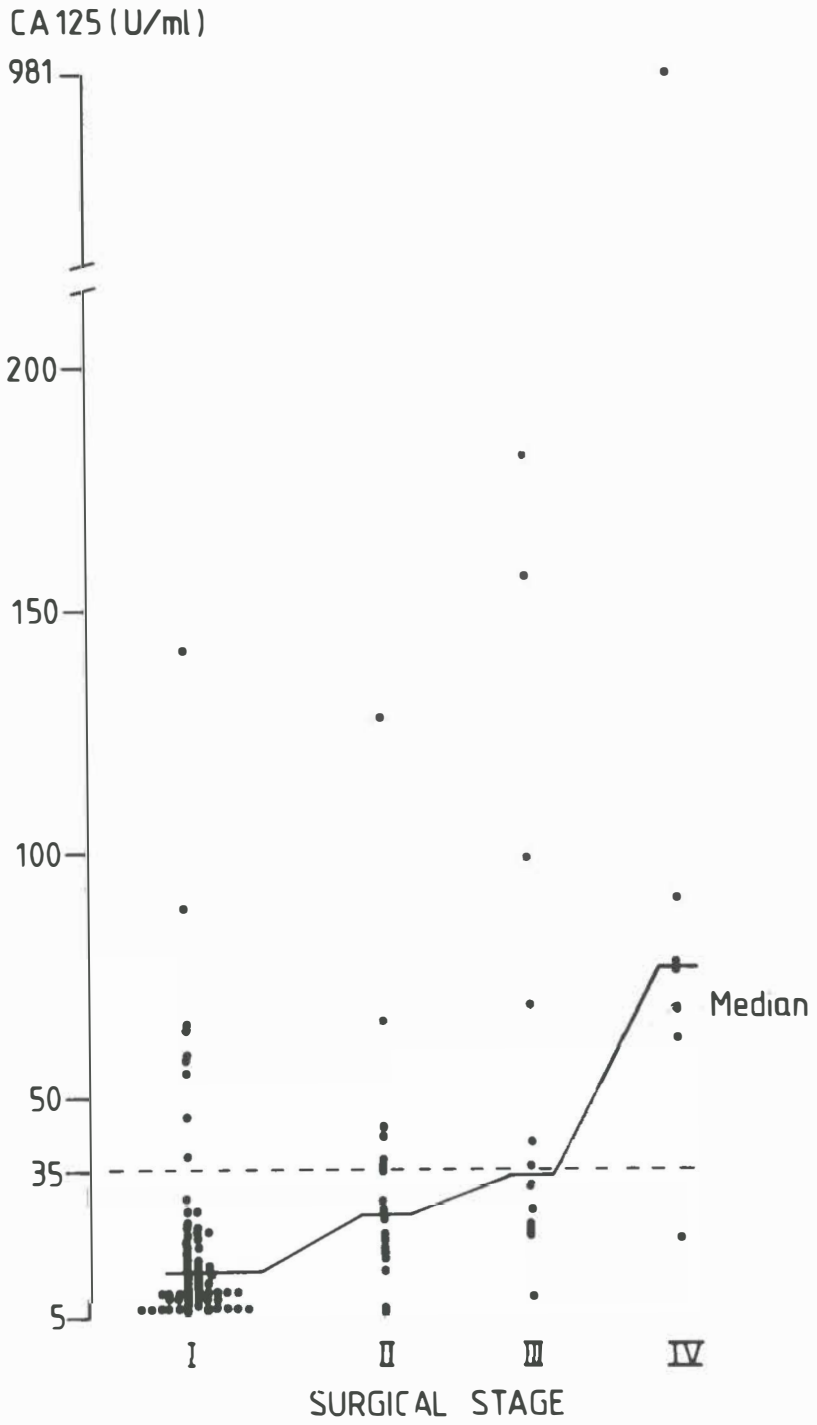
* Patients with undifferentiated carcinoma of the uterus were excluded

Serum CA 125 Levels in Relation to Tumor Stage. Before treatment, there was an increasing incidence of elevated levels of CA 125 both in relation to a higher tumor stage, according to FIGO criteria, and in relation to a higher surgical stage (Table II; Fig. 2). In surgical Stages I, II, III and IV an incidence of elevated serum levels of CA 125 of 13% (nine of 71), 33% (six of 18), 55% (six of 11), and 86% (six of 7), respectively, were found.

Serum CA 125 Levels in Relation to Risk Factors. The risk factors studied included the histologic type of the tumor, the depth of myometrial infiltration, the degree of histologic differentation, and the invasion of tumor cells in vessels. Only a close correlation between elevated CA 125 serum levels and vessel invasion was found (Table III). Although a close correlation between vessel invasion and a loss of tumor differentation was determined ($X^2 = 14.15$, $P < 0.0025$), there was no correlation between a loss of tumor differentation and elevated serum levels of CA 125.

Serum CA 125 Levels and Tumor Extension. Table IV shows the correlation between pretreatment serum levels of CA 125 and tumor extension in patients with Stage I and II disease (FIGO). Patients with elevated serum levels mainly had extensive metastasis to lymph nodes only *(N = 4)* or to lymph nodes and other tissues such as bowels or ovaries *(N = 7)*. Patients with normal serum levels of CA 125 and extracorporeal or extrauterine tumor extension had a solitary metastasis to the left or right ovary *(N = 4)* or metastasis to lymph nodes *(N = 3)*.

In eight of the 30 patients who had a Stage II tumor at presentation,



• FIG. 2. Endometrial adenocarcinoma pretreatment serum CA 125 levels in relation to surgical stage

• TABLE III. Endometrial adenocarcinoma: Elevated serum CA 125 levels in relation to histologic parameters in patients with surgical Stage I or II disease *(N = 62)*

Parameter	X^2	*P*
Vessel invasion	6.55	< 0.025
Histologic type	3.08	NS
Degree of differentiation (G1 *vs* G2/G3)	1.07	NS
Depth of myometrial infiltration ($<^1/_3$ *vs* $>^1/_3$)	0.75	NS

NS = Not significant

• TABLE IV. Predictive value of pretreatment serum CA 125 values in patients with FIGO Stage I and II endometrial adenocarcinoma

Stage		*Pretreatment serum CA 125 level (U/ml)*	*Total No. of patients*	*n*	*(%)*	*P*
I:	Extracorporeal tumor	> 35	11	3	(27)	<0.05
		≤ 35	51	3	(6)	
II:	Extrauterine tumor	> 35	10	88	(80)	= 0.0025
		≤ 35	20	4	(20)	

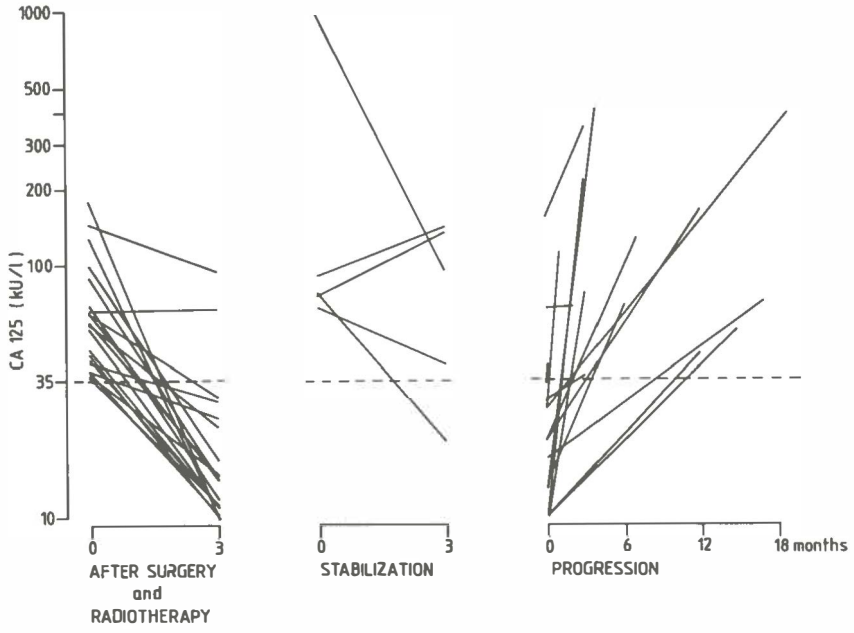
definite involvement of the cervix could not be confirmed after operation. All of these eight patients had low levels of serum CA 125 ($X^2 = 10.80$, $P < 0.005$).

Patients with Stage I or II disease and metastasis exclusively to the lymph nodes were frequently found to have elevation of serum CA 125 levels. A mean value of 55 U/ml was obtained in these patients *(N = 5)* compared with a mean value of 23 U/ml in the group of patients without lymph node metastasis *(N = 13)*. However, these numbers were too small to calculate any statistical difference (Mann-Whitney U test, $P > 0.2$).

Serum CA 125 Levels and the Clinical Course of Disease. No sign of disease was present three months after primary therapy in 20 patients who initially had elevated serum levels of CA 125 before treatment. In 18 of these

20 patients, CA 125 levels fell below 35 U/ml. One patient still had elevated serum CA 125 levels three months after radical therapy. Four months after treatment she was admitted to the hospital with abdominal tumor recurrence. The only other patient with elevated serum levels of CA 125 had high values during the six years of follow-up. The level stabilized at 90 U/ml without evidence of disease; thus this was the only false positive result in the study (Fig. 3). In four of five patients in whom primary therapy could not be radical and disease remained stable, CA 125 serum levels were still elevated above 35 U/ml three months afterward (Fig. 3).

Fig. 3 also gives serum marker trends of all patients with progressive disease. For the sake of clarity, the profiles were reduced to two-point graphs. All 15 patients demonstrated an increase of serum CA 125 pending tumor progression. In all eight patients with abdominal tumor sites, serum CA 125 levels exceeded 35 U/ml before clinical detection of disease progression (range 0 - 9 months, median lead time 2.8 months). In all seven patients



• FIG. 3. Serum CA 125 Profiles in relation to the clinical course of disease. Patients had elevated serum CA 125 levels before treatment and either no evidence of disease 3 months after radical primary therapy *(left)*, stabilization of disease 3 months after nonradical primary therapy *(middle)*, or progressive disease *(right)*.

with tumor sites in the lungs or in the vaginal vault, clinical detection of the disease progession preceded a rise of serum CA 125 (range 0 - 11 months, median lead time 3.8 months).

Serum CA 125 Levels and Early Detection of Recurrent Disease. Patients who had experienced a complete remission after radical initial treatment were studied. Twelve patients were found to have tumor recurrence and five of these 12 patients (42%) had elevated serum levels of CA 125 before or at the time of tumor relapse (range 0 - 9 months, median lead time 1.8 months). In only one of the eight patients (13%) with local tumor recurrence in the vaginal vault and in all of the four patients with abdominal tumor recurrence, serum levels of CA 125 exceeded 35 U/ml. In all serial observations made in patients without tumor recurrence only once was a slightly elevated serum level of CA 125 measured (39 U/ml).

Serum CA 125 Levels and Survival Rate. In this study all 16 patients who died of endometrial adenocarcinoma had elevated serum levels of CA 125 before death. Eleven of these 16 patients had Stage I or II tumors at presentation. Six of these 11 patients (55%) had elevated serum levels of CA 125 before treatment.

Of the 94 patients with Stage I or II tumors being studied, 21 had elevated serum levels of CA 125 before treatment. Six of these 21 patients (29%) eventually died of disease, whereas mortality was lower in the group with normal pretreatment CA 125 serum levels; five of 73 patients (7%) died of endometrial adenocarcinoma ($X^2 = 7.13$, $P < 0.005$).

Comment

This study demonstrates that the tumor marker CA 125 is of clinical value in the staging and follow-up of patients with endometrial adenocarcinoma. A major problem in endometrial adenocarcinoma has always been the detection of patients with early disease who are at risk of extrauterine tumor extension. Several large studies have made clear that a loss of differentiation, unfavorable histologic type, and deep myometrial infiltration of the tumor and vessel invasion of tumor cells are related to a substantial risk of tumor metastasis.[4-7]

In this study all tumor tissues tested immunohistochemically were demonstrated to contain CA 125. Positive staining was present in glandular structures, whereas solid tumor areas were mostly negative for CA 125. Elevated levels of CA 125 in serum were found in only 25% of all the patients. It appears that elevation of CA 125 in serum occurs only in certain circumstances. In this study we found that elevation of CA 125 serum levels

was highly correlated with the presence of tumor cells in uterine blood vessels or lymphatic vessels but not with the histologic type of the tumor, deep myometrial infiltration, or a loss of tumor differentiation. In this respect the lack of a correlation between elevated serum levels and a loss of tumor differentiation is particularly noteworthy, because a very close correlation was revealed between vessel invasion and a loss of tumor differentiation. The absence of this correlation is probably due to the absence of CA 125 in solid tumor areas, which predominate in moderately and poorly differentiated tumors. We detected CA 125 in normal endometrial tissue, and earlier we measured high levels of CA 125 (> 10,000 U/ml) in serous fluid from the uterine cavity[12] and in cervical mucus.[13] In healthy women CA 125 does not enter the circulation; serum levels below 35 U/ml were found.

These findings suggest that elevation of serum CA 125 levels in patients with endometrial adenocarcinoma occurs after destruction of natural barriers, causing a direct contact between antigen-shedding tumor cells and the peripheral circulation.

Niloff et al[9] found no elevations of CA 125 serum levels in patients with Stage I or II tumors at presentation. This study demonstrates that elevated serum levels of CA 125 are also found in patients with Stage I or II tumors. In fact elevation of pretreatment serum levels of CA 125 in patients with these tumors correlated with extracorporeal tumor extension in Stage I or extrauterine tumor extension in Stage II tumors (FIGO). Although this correlation is less obvious in Stage I tumors, it is more apparent in Stage II disease. In addition, a high incidence of elevated serum CA 125 levels was found in Stages III and IV (55% to 86%) and in intraabdominal tumor recurrence (all four patiens). This incidence is comparable to the observations made in ovarian and pancreatic cancer.[8] These findings indicate that CA 125 serum levels are particularly elevated if the antigen-shedding tumor affects the peritoneum.

Although CA 125 was demonstrated in reactive mesothelial cells,[14] it is feasible from immunohistochemical studies that tumor cells are the main source of elevated serum CA 125 levels in patients with endometrial adenocarcinoma. Elevation of serum CA 125 levels in patients with Stage I or II endometrial adenocarcinoma should initiate a careful restaging of the tumor. The question arises if patients with Stage II tumors and elevated pretreatment CA 125 serum levels should be treated with a systemic regimen, because of the high incidence of metastasis outside the uterus found in these patients. A similar problem occurs in patients with tumor recurrence in the vaginal vault associated with elevated CA 125 serum levels. In these patients careful clinical examination is indicated to exclude intraabdominal tumor sites.

In conclusion the following observations are noteworthy:

1. CA 125 was present in all tumor tissues of endometrial adenocarcinomas tested, with a preference for glandular tumor areas.
2. Elevated levels of serum CA 125 in patients with endometrial adenocarcinoma, measured before treatment, were found in 25% of the patients. The incidence increased from 13% in surgical Stage I to 86% in surgical Stage IV. Elevated levels were correlated with invasion of tumor cells in uterine vessels and with extracorporeal tumor extension in Stage I and extrauterine tumor extension in Stage II (FIGO). A finding of elevated levels should initiate a careful restaging in patients with Stage I or II disease (FIGO).
3. Levels of serum CA 125 followed the clinical course of the disease. Progressive and recurrent disease can be preceded by an increase of serum CA 125 levels, in particular in patients with intraperitoneal tumor sites.
4. Fifty-five percent of the patients with Stage I or II tumors at presentation (FIGO) and who eventually died of endometrial adenocarcinoma had raised values of serum CA 125 before treatment. The determination of CA 125 levels before treatment provides a valuable biochemical prognostic risk factor in endometrial adenocarcinoma.

Acknowledgments. We are indebted to Dr. K.H. Groenier for the statistical analyses of the results. We thank Mr. H. Kooi, Mr. J. Pleiter, Mr. M. Krans, Mr. L.J.C. van den Broek, and Mrs. G.H.H.M. van Leeuwen-Herberts for their technical assistance.

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CHAPTER IV

Tumor Markers CA 125, Squamous Cell Carcinoma Antigen, And Carcinoembryonic Antigen In Patients With Adenocarcinoma Of The Uterine Cervix.

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PRÉCIS

The clinical stage and course of cervical adenocarcinoma and adenosquamous carcinoma is biochemically reflected by the serum profiles of CA 125, SCC antigen and CEA.

SUMMARY

Between 1978 and 1987, 439 patients with primary cervical carcinoma were admitted to our department. Seventy-seven patients (17.5%) had cervical adenocarcinoma and are reviewed in this retrospective study. Serial serum samples of these 77 patients were analyzed for Cancer Antigen (CA) 125,

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Squamous Cell Carcinoma (SCC) antigen, and Carcinoembryonic antigen (CEA). Before treatment, only elevated serum CA 125 levels varied directly with the clinical stage of disease. In Stages IB and II disease (International Federation of Gynecology and Obstetrics [FIGO]), the incidence of elevated serum CA 125 levels was highest in patients with adenosquamous tumor. Serum marker levels, measured three months after therapy, concurred with the treatment results. At that time, 17 of the 23 cases (74%) with at least one elevated serum marker level either had residual disease ($N = 9$) or developed recurrent disease during follow-up ($N = 8$), compared with six of the 40 cases (15%) with normal serum marker levels ($P < 0.001$). Increasing serum marker levels during follow-up coincided with or preceded the clinical detection of recurrent disease. Tumor relapse, clinically located in the vaginal vault, occured concommitant with a rise of at least one serum marker level in six of the seven cases (86%). All 15 patients with abdominal recurrence showed elevation of CA 125 (100%). In progressive disease very high serum CA 125, SCC antigen and CEA levels were determined in patients with adenosquamous tumors, whereas patients with adenocarcinoma demonstrated only high CA 125 levels. We conclude that all three markers are important for monitoring patients with cervical adenocarcinoma.

Introduction

Historically, tumor markers have aided in the diagnosis and monitoring of genital tract cancer. The determination of serum tumor markers in gynecologic oncology is of particular value for monitoring the clinical course of the disease. In cervical cancer, Carcinoembryonic antigen is one of the most carefully studied antigens. A good correlation between tumor burden and initial plasma values has been reported. Serial determinations during follow-up provide early detection of tumor recurrence, both in squamous cell carcinoma and in adenocarcinoma of the cervix.[1-4]

In 1977, Kato and Torigoe[5] prepared a heterologous antiserum for human cervical squamous cell carcinoma. Using this antiserum, a tumor antigen was purified from human cervical squamous cell carcinoma tissue. A radioimmunoassay was developed, and antigen activity could be detected in the serum of patients with cervical squamous cell carcinoma. The antigen was termed TA-4, and the purified fraction was designated Squamous Cell Carcinoma antigen.[6] Positive staining was observed mainly in keratinizing and large cell non-keratinizing carcinomas of the ectocervix.[6-11] Detailed studies have made it clear that the serum determination of this antigen is a valuable dia-

gnostic tool in the staging, monitoring of therapy, and follow-up of patients with squamous cell carcinoma of the cervix.[8,12-14]

In 1983, Bast et al[15] reported circulating antigen levels in the serum of 82% of the patients with epithelial ovarian cancer. The antigen, referred to as CA 125, is detected by the monoclonal antibody OC 125, which was raised against an ovarian cancer cell line. Immunohistochemistry has demonstrated the antigen in the fetal coelomic epithelium.[16] In the adult, CA 125 can be detected in almost all normal or benign and malignant neoplastic tissues derived from the coelomic epithelium, such as normal and malignant glandular epithelium of the endocervix.[16-19] Extremely high levels of CA 125 with a median concentration of 68,000 U/ml, have been observed in the cervical mucus and uterine secretions of apparently healthy women in the presence of normal serum CA 125 levels.[18,19] Serum CA 125 determinations have proved to be of benefit in the staging and follow-up of ovarian, endometrial, and tubal cancer.[15,17,19-21]

It has been suggested that the incidence of cervical adenocarcinomas appears to be increasing.[22] This observation stresses the need for studies on epidemiologic factors and the biologic behavior of cervical adenocarcinoma. The main objective of this study was to review our material and evaluate the clinical significance of CA 125, Squamous Cell Carcinoma antigen, and Carcinoembryonic antigen in patients with cervical adenocarcinoma.

Patients and Methods

Between July 1978 and October 1987, 439 patients were admitted to the Gynecologic Oncology Service of the University Hospital of Groningen, The Netherlands, with newly diagnosed, persistent, or recurrent carcinoma of the uterine cervix. Seventy-seven patients, representing 17.5% of the population, were diagnosed as having cervical adenocarcinoma. Serum samples were collected from these patients longitudinally on one to 19 occasions and stored at -70°C at the Serum Bank of the Department of Obstetrics and Gynecology. Pretreatment sera from 68 patients were available. The nine remaining patients had received primary treatment at other hospitals before admission to our department ($N = 4$) or were admitted because of tumor recurrence ($N = 5$). We reviewed patients' records prior to serum analysis. Clinical staging was carried out in accordance with the International Federation of Obstetrics and Gynecology (FIGO) staging system and the histopathologic diagnosis was according to Ferenczy and Winkler[23] (Table I). The diagnosis was made on initial biopsy material and after careful examination of the extirpated spe-

• TABLE I. Classification of the study group

Stage	N	(%)	*Adenocarcinoma*				*Adenosquamous*
			Endocervical	*Papillary*	*Clear cell*	*Endometrioid*	
IA	4	(5)	2	-	1	-	1
IB	41	(53)	22	6	4	1	8
IIA	9	(12)	2	3	1	-	3
IIB	10	(13)	1	2	2	1	4
III	9	(12)	6	1	1	-	1
IV	4	(5)	3	-	-	-	1
Total	77	(100)	36	12	9	2	18
(%)			(47)	(16)	(12)	(3)	(23)

cimen for the major part of the study population (mainly with Stages IB or II disease). In patients who were treated by radiotherapy alone (Stages III and IV) the histopathologic diagnosis was made on biopsy material. If there were any doubts about the primary site of the tumor (e.g. endometrium or endocervix), the patient was eliminated from the study.

Patients with Stages IB and II tumors had been treated mainly by radical hysterectomy, bilateral salpingo-oophorectomy, and pelvic lymphadenectomy, with or wihtout radiotherapy. Higher stages had been treated using radiotherapy alone. The clinical course of the disease had been recorded for all patients. Complete remission (no evidence of disease) was defined as the absence of all tumor lesions three months after treatment. Partial remission required a 50% or more reduction in size of the tumor lesions, without the appearance of new lesions after therapy. All patients with stable or progressive disease had histopathologically verified tumor lesions. Stable disease was defined as no alterations in the clinical status of a patient who was known to have tumor. Progression of disease was defined as a distinct deterioration in the clinical status of a patient who was known to have tumor, or the appearance of new lesions. Twenty-nine such patients were evaluated. In 14 of these patients, the diagnosis of progression of disease was based on the increasing size of a pelvic mass whether or not in combination with signs of bowel and/or urinary tract obstruction, vaginal hemorrhage, or severe lymphedema. Nine other patients with a pelvic mass demonstrated new lesions in the lungs, bone, liver, central nervous system, or lymph nodes. In the remai-

ning six patients, known to have multiple metastases, a deterioration of the clinical performance status ultimately leading to death was considered progressive disease. Recurrent disease was defined as reappearance of disease in patients who had experienced complete remission.

Statistical analyses were performed on the data from 56 of the 60 patients with Stages IB and II tumors using the X^2test and Fisher's exact test.

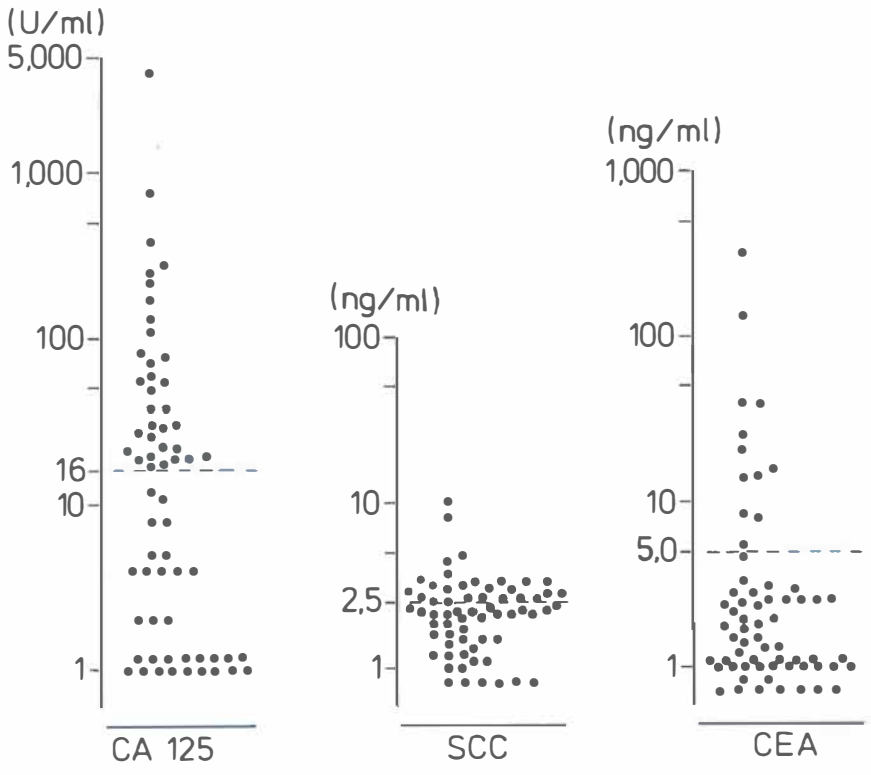
Serum CA 125 levels were measured using a solid-phase enzyme immunoassay (Abbott Laboratories, Chicago, USA) (Shaw et al. Development of a solid-phase enzyme immunoassay for the measurement of CA 125. Abstract of the XIVth Annual Meeting of the International Society for Oncodevelopmental Biology and Medicine. Helsinki, Finland, 1986:175). Variation coefficients of 10.2 and 8.9 percent were found at the level of 20 and 166 U/ml, respectively ($N = 34$). All serum levels of 16 U/ml or less were considered normal. This cutoff value represents the 95th percentile in a population of healthy premenopausal women and is comparable to the level of 30 U/ml when measurements are performed using an immunoradiometric assay.[24] After radical hysterectomy and/or external radiotherapy a serum CA 125 value of 10 U/ml was taken as the upper limit of normal; this is the 95th percentile in a control group of 199 patients in complete remission for a period of at least 1 year after treatment for Stages I or II endometrial or cervical cancer.

For the measurement of serum Squamous Cell Carcinoma antigen levels, we used a double antibody radioimmunoassay (Dainabot Co. Ltd., Tokyo, Japan), developed from a subfraction of a TA-4 preparation isolated from a liver metastasis of a cervical squamous cell carcinoma.[5] In 20 different assays, the variation coefficients were 12.8% at a low level (1.5 ng/ml) and 5.9% at a high level (13.3 ng/ml). A treshold value of 2.5 ng/ml was used as the upper limit of the normal range. This value is based on the 95% specificity in normal subjects.[14]

We measured Carcinoembryonic antigen using a one-step enzyme immunoassay kit (CEA-Enzelsa; Compagnie Oris Industrie, Bagnols sûr Cèze, France). The assay involves two monoclonal antibodies directed against two unique CEA epitopes and not reacting with CEA-cross reacting antigens. The assay has a sensitivity of 0.3 ng/ml and an interassay variation coefficient of 6.4 - 8.8%. An upper limit of 5.0 ng/ml was regarded as normal; this is the 99.1 percentile in a healthy population (smokers and non-smokers) (Sertour et al. Evaluation of a one step monoclonal enzyme immunoassay for the direct measurement of Carcinoembryonic antigen. Abstract of the XIVth Annual Meeting of the International Society for Oncodevelopmental Biology and Medicine. Helsinki, Finland, 1986:25).

•TABLE II. Elevation of serum tumor markers before treatment in patients with cervical adenocarcinoma

Stage	*N*	*CA 125 (>16 U/ml)*	*SCC antigen (>2.5 ng/ml)*	*CEA (>5.0 ng/ml)*
IB	35	12 (34%)	14 (40%)	5 (14%)
II	17	13 (76%)	5 (29%)	2 (12%)
III	8	4 (50%)	4 (50%)	4 (50%)
IV	4	4 (100%)	1 (25%)	1 (25%)
Total	64	33 (52%)	24 (38%)	12 (19%)



•FIG. 1. Serum levels of CA 125 (left), Squamous Cell Carcinoma antigen (SCC;middle), and Carcinoembryonic antigen (CEA;right) before treatment in 64 patients with Stage IB - IV (FIGO) cervical adenocarcinoma.

Results

Before treatment, 33 of the 64 patients (52%) with Stages IB - IV tumors had elevated serum CA 125 levels (range 18 - 4100 U/ml), 24 (38%) had elevated SCC antigen levels (range 2.6 - 10.5 ng/ml), and 12 (19%) had elevated CEA levels (range 5.5 - 322 ng/ml) (Table II; Fig. 1).

In one of the four patients with Stage IA tumor, we observed only a slight elevation of the SCC antigen serum level. These four patients will not be discussed further. Of the three markers tested, only elevated serum CA 125 levels correlated with the clinical stage of the tumor ($P < 0.01$). In particular, the pretreatment serum SCC antigen level appeared to be unrelated to the stage of the disease and ranged mainly between 0.7 and 5 ng/ml; only two patients had levels higher than 5.0 ng/ml. The highest level measured was 10.5 ng/ml in a patient with Stage IB clear-cell carcinoma*.

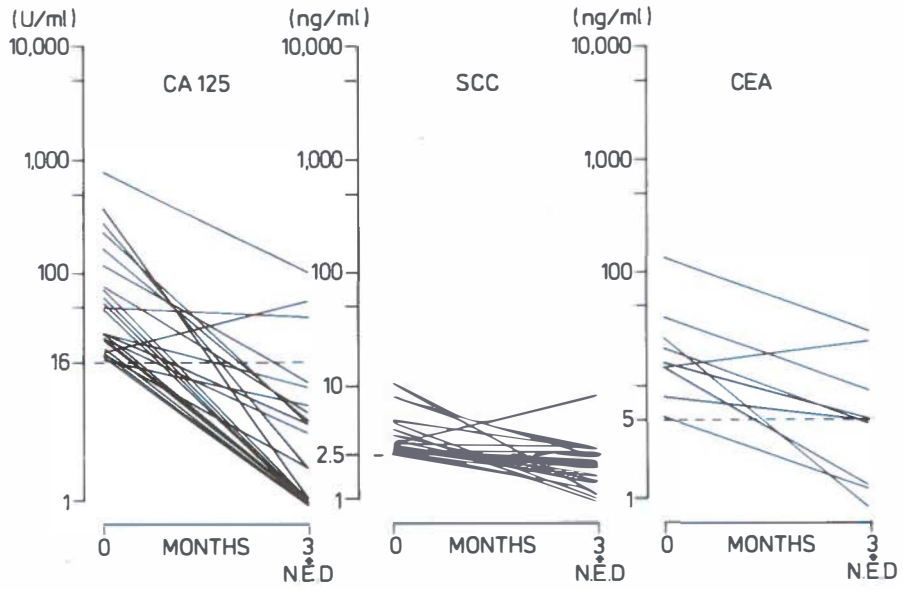
For Stages IB and II, no statistical differences were found in the serum levels of CA 125, SCC antigen, or CEA among adenocarcinomas, adenocarcinomas with a papillary growth pattern, clear-cell carcinomas or endometrioid carcinomas. However, statistically significant differences were discovered between these tumor types and adenosquamous tumors. Therefore, we decided to compare adenosquamous tumors with the total group of other adenocarcinoma subpatterns. The serum CA 125 level correlated with the histologic tumor type, as shown in Table III. Before treatment, serum CA 125

•TABLE III. Relation between histologic tumor type and serum tumor marker levels before treatment in Stages IB and II cervical adenocarcinoma

	Adenosquamous carcinoma (N = 14)	*Adenocarcinoma (N = 38)*	*P*
CA 125 > 16 U/ml	11 (79%)	14 (37%)	<0.025
SCC > 2.5 ng/ml	8 (57%)	11 (29%)	<0.1*
CEA > 5.0 ng/ml	3 (21%)	4 (11%)	NS

SCC = Squamous Cell Carcinoma antigen; CEA = Carcinoembryonic antigen; * = Not significant; NS = Not significant

* After this study was published we discovered that psoriasis can be associated with a rise in the serum SCC antigen level (Chapter 7). All the serum samples taken from this patient, who experienced recurrent disease 7 months after treatment, showed elevated serum SCC antigen levels detected from all samples. She was also recognized as having severe psoriasis.



•FIG. 2. Serum profiles in patients who had elevated levels of CA 125 (left), Squamous Cell Carcinoma antigen (*SCC;middle*), or Carcinoembryonic antigen (*CEA;right*) before treatment and no clinical evidence of disease (N.E.D.), three months after completing primary treatment.

levels were elevated more commonly in patients with adenosquamous tumors than in patients without squamous cell lesions. In addition, the incidence of elevated SCC antigen levels in patients with adenosquamous disease was higher than in patients with adenocarcinoma, but this difference was not significant at the 5% level ($P < 0.1$). Serum CEA levels prior to treatment were not related to the histologic type of the tumor.

We investigated the serum marker profiles in relation to the clinical course of disease. Figure 2 shows the serum marker trends of CA 125, SCC antigen, and CEA in patients who had elevated serum marker levels before primary treatment and no evidence of disease afterward. Effective cytoreductive therapy resulted in a significant drop in serum marker concentrations (Fig. 2). However, elevated serum marker levels after primary therapy were related to treatment failure, judged clinically by the finding of residual disease (partial remission or stable disease) or the reappearance of tumor during follow-up (recurrent disease) (Table IV). This finding did not depend on the pretreatment serum marker level or on the histologic tumor type. In addition, 30 patients with residual ($N = 11$) or recurrent disease ($N = 19$) were studied. Only one patient, who experienced recurrent disease in the vaginal vault, is without evidence of disease four years after additional treatment. Of the 29 remaining patients, 26 eventually died of their disease, and three are alive

•TABLE IV. Serum CA 125, SCC antigen and CEA levels three months after primary therapy in relation to treatment failure

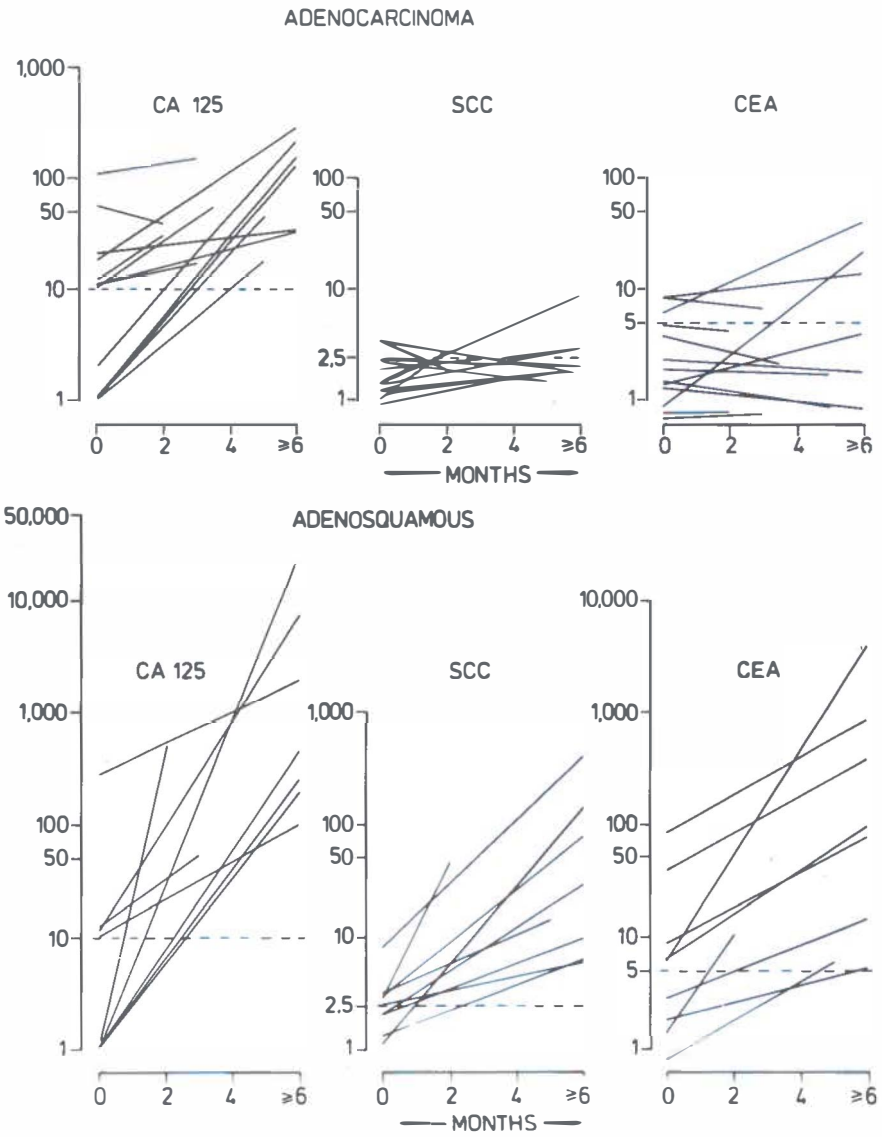
Serum markers	*N*	*Residual disease*	*Recurrent disease**	*Total*	*(%)*	*P*
One or more elevated	23	9	8	17	(74)	<0.001
Normal	40	2	4	6	(15)	
No serum available	7	-	7	7		
Total	70	11	19	30		

* Length of follow-up for patients in complete remission was at least 9 months.

with tumor and are receiving palliative therapy. Twenty-seven of these 29 patients were evaluated after the clinical detection of residual or recurrent disease. Stable disease was diagnosed clinically on 16 occasions in these patients. At that time, CA 125, SCC antigen, and CEA serum levels were elevated in 14, seven, and eight cases, respectively. Figure 3 presents the serum marker profiles in patients with progressive disease. During tumor progression, all 27 patients demonstrated rising serum CA 125 levels. Both the SCC antigen and CEA serum levels were elevated in 15 patients (56%). In the ten patients with adenosquamous tumor very high levels for all three markers were measured. In patients with adenocarcinoma, however, only CA 125 levels were high.

Nineteen patients had recurrent disease. The duration of complete remission before the clinical appearance of new tumor lesions varied from 4 - 56 months, with a median length of 20 months. Clinically, seven of the 19 patients had recurrent disease in the vaginal vault without any evidence of distant metastasis (central recurrence). Twelve patients experienced tumor recurrence at various sites within the abdominal cavity (abdominal recurrence). Three patients with vaginal vault recurrence experienced abdominal recurrence after a period of complete remission varying from 25 to 36 months after adjunctive therapy. Seven cases of central recurrence and 15 of abdominal tumor relapse were thus observed in 19 patients who experienced recurrent disease. All recurrences were confirmed histopathologically.

Table V presents the relation between elevated serum marker levels and the site of the tumor relapse. Recurrence was preceded by increasing serum levels of at least one of the three tumor markers in six of the seven patients



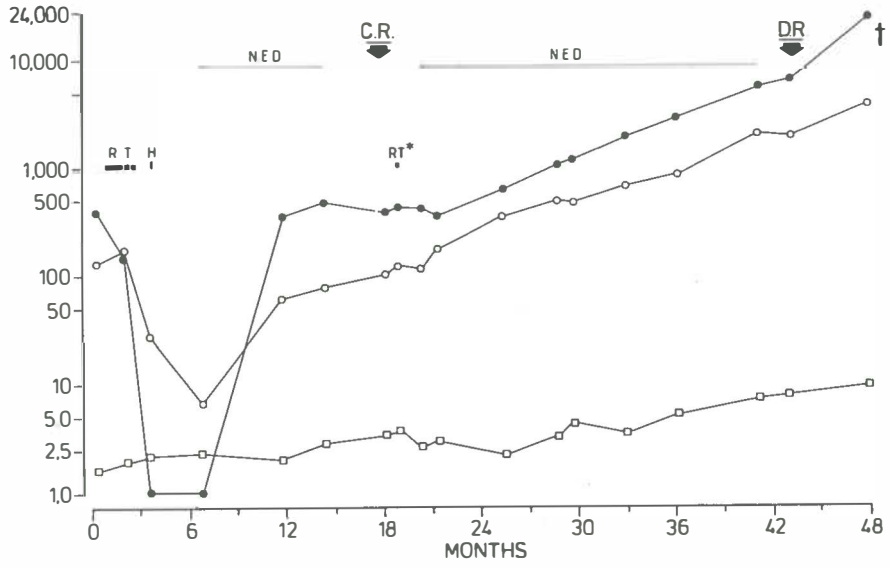
•FIG. 3. Serum profiles of CA 125 (left), Squamous Cell Carcinoma antigen (SCC;middle), and Carcinoembryonic antigen (CEA;right) in patients with progressive adenocarcinoma without squamous cell lesions (top, *N = 13*) and adenosquamous carcinoma (bottom, *N = 9*) of the cervix from whom sera were collected (22 of the 29 patients):

1. before the clinical diagnosis progressive or recurrent disease (first value) and
2. during tumor progression (second and highest value)

(86%) with central recurrence. It is notable that in two patients with adenocarcinoma, recurrence was preceded by an elevation of the SCC antigen (one) or CEA (one) serum level in the presence of normal serum CA 125 levels. The patient who had central recurrence of a well-differentiated adenocarcinoma without elevation of any of the markers is the only patient with recurrent disease (5%) who is without evidence of disease after additional treatment.

In all 15 cases of abdominal tumor recurrence, the relapse coincided with or followed increasing serum CA 125 levels. In three of these patients, a rise in the serum CA 125 level had already been noted 26 months before recurrence became clinically apparent. Figure 4 illustrates one of the most striking examples of the behavior of the three serum markers in a patient who had adenosquamous carcinoma.

In addition, we investigated the serum marker profiles of patients in complete remission. Forty-three patients who still showed no sign of tumor activity at the closing date of the study, had been followed over a period of 6 -



•FIG. 4. Serum marker trends of CA 125 (*closed circles*), Carcinoembryonic antigen (*open circles*) and Squamous Cell Carcinoma antigen (*squares*) before treatment and during follow-up of a patient with Stage IIB (FIGO) adenosquamous carcinoma of the cervix.

C.R. = central (vaginal vault) recurrence; D.R. = distant (pelvic wall) recurrence; N.E.D. = no evidence of disease; R.T. = radiotherapy; H = adjunctive hysterectomy and bilateral salpingo-oophorectomy; RT* = interstitial cesium needle implantation. †Date of death.

•TABLE V. Recurrent disease: Elevation of serum CA 125, SCC antigen, and CEA levels during follow-up in relation to the clinical site of tumor recurrence

		N	(%)	*Median lead time (mo)*	*Range*
Vaginal vault (*N* = 7)					
CA 125	>10 U/ml	4	(57)	6	3 - 8
SCC	>2.5 ng/ml	3	(43)	8.5	6 - 11
CEA	>5.0 ng/ml	3	(43)	8	3 - 8
One or more elevated		6	(86)	6	3 - 11
Abdominal (*N* = *15*)					
CA 125	>10 U/ml	15	(100)	5.5	0 - 26
SCC	>2.5 ng/ml	9	(60)	6	0 - 26
CEA	>5.0 ng/ml	10	(67)	6	0 - 25

SCC = Squamous Cell Carcinoma antigen; CEA = Carcinoembryonic antigen

72 months, with a median of 36 months. During follow-up, elevated serum marker levels were found on six occasions. Incidental elevation of SCC antigen was found in two patients (3.0 and 3.9 ng/ml) and persistent elevation of the CEA concentration was found in one patient, at a level of 6.0 ng/ml. Incidental elevation of the serum CA 125 level was not encountered. Serum levels of CA 125 were consecutively elevated in one patient, at a level of 100 U/ml. This patient underwent surgery later because of multiple follicular cysts of the left ovary, which was conserved at the primary radical operation. Ovariectomy resulted in a normalization of the serum CA 125 level. At the closing date of the study two of the 43 patients were under intensive monitoring because of gradually rising levels of both CA 125 and SCC antigen without clinical evidence of recurrent disease.

Comment

The advent of polyclonal and monoclonal antibody techniques directed against a variety of gynecologic neoplasms represents a promising new development. The detection of circulating antigen is particularly useful in the pretreatment evaluation of the patient with invasive cancer, the evaluation of

the therapeutic outcome, and the follow-up of outpatients. The results of the present study demonstrate that CA 125 is an excellent diagnostic tool in the follow-up of patients with adenocarcinoma of the uterine cervix. Additional monitoring by the determination of serum SCC antigen and CEA levels is of special interest for the detection of residual and recurrent disease, particularly in patients with adenosquamous tumor.

Studies on serial determinations of circulating antigen levels in patients with adenocarcinoma of the cervix are limited, and refer only to CEA.[1,4] We were not able to confirm the correlation between elevated serum CEA levels and tumor stage that has been observed by Kjørstad and Örjasæter.[4] The overall incidence of elevated serum CEA levels in our study group was 19%, using 5.0 ng/ml as a cutoff level. This is in contrast with the findings of Kjørstad and Örjasæter,[4] but in agreement with the observations of Van Nagell et al,[1] who found an incidence of 20%. Unfortunately, histologic subpatterns were not classified in these studies.

CA 125 has proved to be a useful tumor marker in patients with cancer of the ovaries, the endometrium, and the fallopian tube.[15,17,19-21] Niloff et al[25] reported elevated serum CA 125 levels in five of six patients with cervical adenocarcinoma. The results of the present study demonstrate that elevated serum CA 125 levels prior to treatment correlate well with the clinical stage of invasive adenocarcinoma of the cervix. Moreover, in Stages IB and II pretreatment serum CA 125 levels were elevated more frequently in patients with adenosquamous tumors than in patients with other histologic subpatterns. We were not able to relate pretreatment serum levels of SCC antigen to the clinical stage or the histologic type of the tumor. It is notable that only slight elevations of serum SCC antigen were observed, regardless of the stage.

Yet all three markers deserve a place in the follow-up of patients with cervical adenocarcinoma. After primary treatment, elevation of at least one of the three markers correlated with the finding of residual disease or the appearance of new tumor lesions during follow-up (Table IV). In addition, a combination of CA 125, SCC antigen, and CEA determination seems to increase the sensitivity of the early detection of central recurrence, as shown in Table V.

In contrast to the observations in central recurrence, serum CA 125 levels were elevated in all 15 patients with abdominal recurrence. Furthermore, CA 125 was elevated in all 27 patients with progressive disease. These findings corroborate our observations in patients with recurrent and progressive adenocarcinoma of the endometrium.[17] It is therefore crucial to note that increasing serum CA 125 levels during the follow-up of patients who under-

went treatment for these gynecologic malignancies, are highly indicative of disseminated disease.

The value of a classification into different histologic subtypes has been questioned.[26] However, with respect to the serum profiles of the three markers tested, we found some interesting differences between patients with adenosquamous tumors and those whose tumors did not contain malignant squamous cell components. First of all, the association between elevated pretreatment serum CA 125 levels and the adenosquamous tumor type was expressed again during tumor progression. Patients with adenocarcinoma and patients with adenosquamous tumors all had raised serum CA 125 levels, but these were considerably higher in the latter group. In addition, serum SCC antigen and CEA levels were unchanged or only slightly elevated in patients with progressive adenocarcinoma, whereas very high levels were determined in the patients with adenosquamous tumor. In these patients the serum levels were comparable to the observations in patients with squamous cell carcinoma of the cervix.[13,14] We have also noticed squamous dedifferentiation of a well-differentiated adenocarcinoma during tumor progression. Biochemically, this was reflected in increasing levels of SCC antigen, which were initially below 2.5 ng/ml.[14] As for SCC antigen, these observations may be explained in that adenosquamous tumor express the antigen in the squamous cell component.[10] The findings for CEA and CA 125, however, seem to indicate a difference in the biologic behavior of adenosquamous tumors. The question of whether the observations reflect a greater capacity for adenosquamous tumors to produce and release CA 125, SCC antigen, and CEA or to display a more aggressive growth pattern is presently under investigation.

It is clear from our data that recurrent adenocarcinoma of the cervix has an extremely poor outcome. Only one of the 19 patients with tumor relapse (5%) has survived. Unfortunately, with the presently available treatment options and the lack of unequivocal evidence that early detection of recurrent disease gives a better prognosis, the serial determination of serum marker levels seems to be of limited value. The majority of patients who experience recurrent or progressive disease already have a poor prognosis at presentation because of higher tumor stage, lymphatic spread, or other unfavorable prognostic characteristics. In general, these patients will receive maximal treatment, i.e. radiotherapy, with or without surgery. It is possible, however, that in a clearly defined group of patients with early-stage disease, who can still profit from additional therapy, the early detection of viable tumor burden by measurement of serum marker levels after treatment and during follow-up can influence the prognosis for the better.

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CHAPTER V

Adenocarcinoma Of The Uterine Cervix

Prognostic significance of pretreatment serum CA 125, Squamous Cell Carcinoma antigen, and Carcinoembryonic antigen levels in relation to clinical and histopathologic tumor characteristics

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SUMMARY

The prognostic value of the pretreatment serum CA 125, Squamous Cell Carcinoma (SCC) antigen, and Carcinoembryonic antigen (CEA) levels in relation to tumor type, vascular invasion by tumor cells, and lymph node metastases was investigated in 77 patients with cervical adenocarcinoma. In Stage IB (International Federation of Gynecology and Obstetrics [FIGO]), the five-year actuarial survival of patients with pretreatment serum CA 125 levels >16 U/ml was 52.4% *versus* 95.6% when normal serum CA 125 levels were determined ($P < 0.01$). Pretreatment serum SCC antigen or CEA levels had no substantial prognostic value.

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In Stage IB (FIGO), 42% of the patients with elevated serum CA 125 levels had lymph node metastases *versus* 4% when normal levels were found ($P = 0.012$). The presence of vascular invasion ($P = 0.01$) or lymph node metastases ($P = 0.001$) was associated with an increased risk for recurrent disease. Adenosquamous tumors showed a higher incidence of vascular invasion ($P = 0.05$) and a higher incidence of elevated serum CA 125 levels ($P = 0.03$). Particularly in Stage II, adenosquamous tumors were found to have a poorer prognosis than adenocarcinomas ($P = 0.0566$).

We conclude that in cervical adenocarcinoma serum CA 125 is an important prognostic factor and an implicit indicator of tumor virulence.

Introduction

Clinical methods are used in the staging of patients with cervical cancer. The International Federation of Gynecology and Obstetrics (FIGO) recommends criteria for the staging procedure.[1] Usually, there is a good correlation between the clinical stage of the tumor as assessed by an experienced clinician and patient outcome. Various other tumor-associated features, such as lesion size,[2-7] depth of stromal invasion,[6,8] and vascular invasion by tumor cells,[8-15] have been linked to survival, although discordant results sometimes emerge from these studies.

With respect to the adenocarcinomatous type of cervical cancer, there is substantial evidence that gynecologic oncologists are being confronted with a rising incidence.[5,16] Detailed studies involving epidemiologic factors and biologic behavior of cervical adenocarcinoma are needed because the prognosis of this tumor is controversial.[2-5,7,12,13,17-27]

The detection of circulating antigens in patients afflicted by malignant disease offers new opportunities in the pretherapeutic detection of metastatic disease and tumor relapse during follow-up. Recently, we showed that the serum determination of the tumor markers CA 125, Squamous Cell Carcinoma (SCC) antigen and Carcinoembryonic antigen (CEA) is valuable in the early detection of recurrent cervical adenocarcinoma.[28] We decided (1) to evaluate whether or not pretreatment serum marker levels carry prognostic information with special emphasis on Stages IB and IIA, (2) to investigate the prognostic significance of the histologic type, vascular invasion, and lymph node metastases and their mutual relationships, and (3) to analyse the relationships between the pretreatment serum marker levels and these three tumor parameters.

Patients and Methods

Between July 1978 and October 1987, 77 patients with adenocarcinoma of the uterine cervix entered the Gynecologic Oncology Service of the University Hospital of Groningen, The Netherlands. Previously, we discussed this population in a study on the clinical use of tumor markers CA 125, SCC antigen, and CEA.[28] We reviewed patients' files before serum analysis. The clinical staging was established in accordance with FIGO[1], and the histopathologic diagnosis, previously described in greater detail,[28] was made in accordance with Ferenczy and Winkler[29] (Table I). This diagnosis was assessed after careful examination of the pretreatment biopsy or cone material and on any subsequent surgical specimen. The following features were recorded: histologic type, the presence of tumor cells in endothelial-lined spaces (vascular invasion), and lymph node metastases.

• TABLE I. Classification of the study group

Stage	*N*	*Mean Age (Yr)*	*Adenocarcinoma**	*Adenosquamous*
IA	4	39	3	1
IB	41	46.5	33	8
IIA	9	46	6	3
IIB	10	54.2	6	4
III	9	56.5	8	1
IV	4	53.5	3	1
Total	77	48.6	59	18

* Adenocarcinoma variants are: endocervical, clear cell, papillary and endometrioid carcinoma

Radical hysterectomy with bilateral salpingo-oophorectomy and pelvic lymphadenectomy was the usual therapy in 22 patients with Stage IB and in one patient with Stage IIA. Nineteen patients with Stage IB and 8 patients with Stage IIA were treated by intracavitary cesium application before radical hysterectomy. After surgery, 13 patients with Stage IB and five patients with Stage IIA received whole-pelvis radiotherapy because of lymph node metastases *(N = 10)*, close margins *(N = 6)* or extension into the endometrium *(N = 2)*. Patients with Stage IIB tumors were treated using a combina-

tion of brachytherapy and external irradiation. In 6 patients adjunctive hysterectomy with pelvic node sampling was done after radiotherapy. The primary treatment of patients with Stages III and IV was radiotherapy alone. In this group radiotherapy preceded exenterative surgery in one case, was done in a second patient in combination with chemotherapy, and in addition to lobectomy of the left lung in a third patient because of a solitary metastasis.

Criteria for the judgment of the clinical course of disease were described previously.[28] Briefly, complete remission was defined as the absence of all known tumor lesions three months after treatment or during follow-up. "Residual disease" existed when tumor lesions were still known to be present after treatment. "Recurrent disease" was defined as the reappearance of disease in patients with complete remission. At the closing date of the study (January 1989) the follow-up for patients in complete remission ranged from 18 to 84 months (median 47.5). In patients with recurrent disease, the period of complete remission before clinical tumor relapse varied from 4 to 56 months (median 13).

Mean age was 48.6 years (range 18 - 81 yrs). Patients with clear-cell carcinoma were younger, with a mean age of 34.7 years. Nulliparity was noted in 20% of the cases. No patients had been exposed to *diethylstilbestrol*. Seventy-two patients were treated and followed-up at the department, including 4 patients who had undergone conization *(N = 2)* or brachytherapy *(N = 2)* before admission to our department for additional treatment. Four patients with Stage IA tumor were eliminated from further analyses. Statistical analyses were done on the data from the 68 remaining patients. Twenty-seven of the 68 patients had recurrent or residual disease. In addition, 5 patients were treated and followed-up in affiliated hospitals before admission to our department for adjunctive treatment because of recurrent disease. One of these patients is free of disease after treatment for a vaginal vault recurrence. Of the 31 remaining patients, 28 died and three patients are still alive with tumor lesions for which they are receiving palliative therapy.

Survival curves and analyses were established using a standard actuarial survival method and the log rank method.[30] "Prognosis" is used to indicate the risk for recurrent or residual disease. Since the outcome for patients with persistent or new tumor lesions was poor, no differentiation was made between patients who were "dead of disease" or "alive with tumor". In a first analysis, we studied the univariate prognostic effect of the pretreatment serum marker levels for patients with Stages IB or IIA disease because these patients received similar treatment modalities. We also investigated the prognostic impact of three well-established histopathologic parameters which might influence the pretreatment serum marker levels. These parameters

were: the histologic type, vascular invasion, and lymph node status. Subsequently, the mutual relationships between the three histopathologic characteristics and between these histologic tumor features and the initial serum CA 125, SCC antigen, and CEA levels were investigated in a correlation analysis. Multivariate analysis was inappropriate because, after stratifying into subcategories, the numbers were too small for reliable statistical evaluation.[31] The analyses were done using the X^2test and Fisher's exact test. They revealed that there were two major subpatterns: adenocarcinomas without malignant squamous cell components and adenosquamous carcinomas. This study therefore only deals with these histopathologic subtypes. In Stages IB and II, information on the lymph nodes was acquired from 50 patients. The presence or absence of vascular invasion was recorded only from viable tumor. This left 46 patients for evaluation. Serum samples collected before therapy were available in 52 patients. With respect to vascular invasion and lymph node metastasis, patients with residual disease after therapy were excluded from the survival analysis. No patients were lost to follow-up.

Serial serum samples from the patients were stored at -70°C. Pretreatment sera were available from the 68 patients who received primary treatment at our department. Serum CA 125 levels were measured using a solid-phase enzyme immunoassay (Abbott Laboratories, Chicago, IL, USA).[28] All pretreatment levels of 16 U/ml or less were considered to be normal. This cutoff value represents the 95th percentile in a population of healthy premenopausal women and is comparable to the treshold value of 30 U/ml when using an immunoradiometric assay.[32] For the measurement of serum SCC antigen levels, formerly referred to as TA-4,[33,34] we used a double antibody radioimmunoassay (Dainabot Co. Ltd., Tokyo, Japan).[28,33] An upper limit of normal of 2.5 ng/ml (the 95th percentile in a population of healthy premenopausal women) was used.[34] The CEA was measured using a one-step enzyme immunoassay kit (CEA-Enzelsa from Compagnie Oris Industrie, Bagnols sûr Cèze, France).[28] An upper limit of 5.0 ng/ml was regarded as normal, being the 99.1 percentile in a healthy population (smokers and nonsmokers).

Briefly, the incidence of elevated serum levels in Stage IB *(N = 35)* was 34% for CA 125, 40% for SCC antigen, and 14% for CEA. The corresponding figures for Stage II *(N = 17)* were 76%, 29%, and 12%, respectively.[28]

Results

The extent of the disease was a major prognostic risk factor (Table II). Particularly noteworthy is the decrease in survival once the tumor has spread

• TABLE II. Prognosis in relation to stage

Stage	*N*	*Recurrent or residual disease*	
IB	38	6	(16%)
IIA	9	3	(33%)
IIB	9	7	(78%)
III	8	8	(100%)
IV	4	3	(75%)
Total	68*	27	(39.7%)

* Excluding 4 patients with Stage IA and 5 patients admitted with recurrent disease

to the parametria. Only one patient with Stage III or IV survived: this patient had a solitary metastasis in the left lung which was succesfully resected. Residual or recurrent disease also had a poor prognosis. At the closing date of the study, only one of the 32 patients was free from disease (3%), 5 years after additional treatment for recurrence in the vaginal apex.

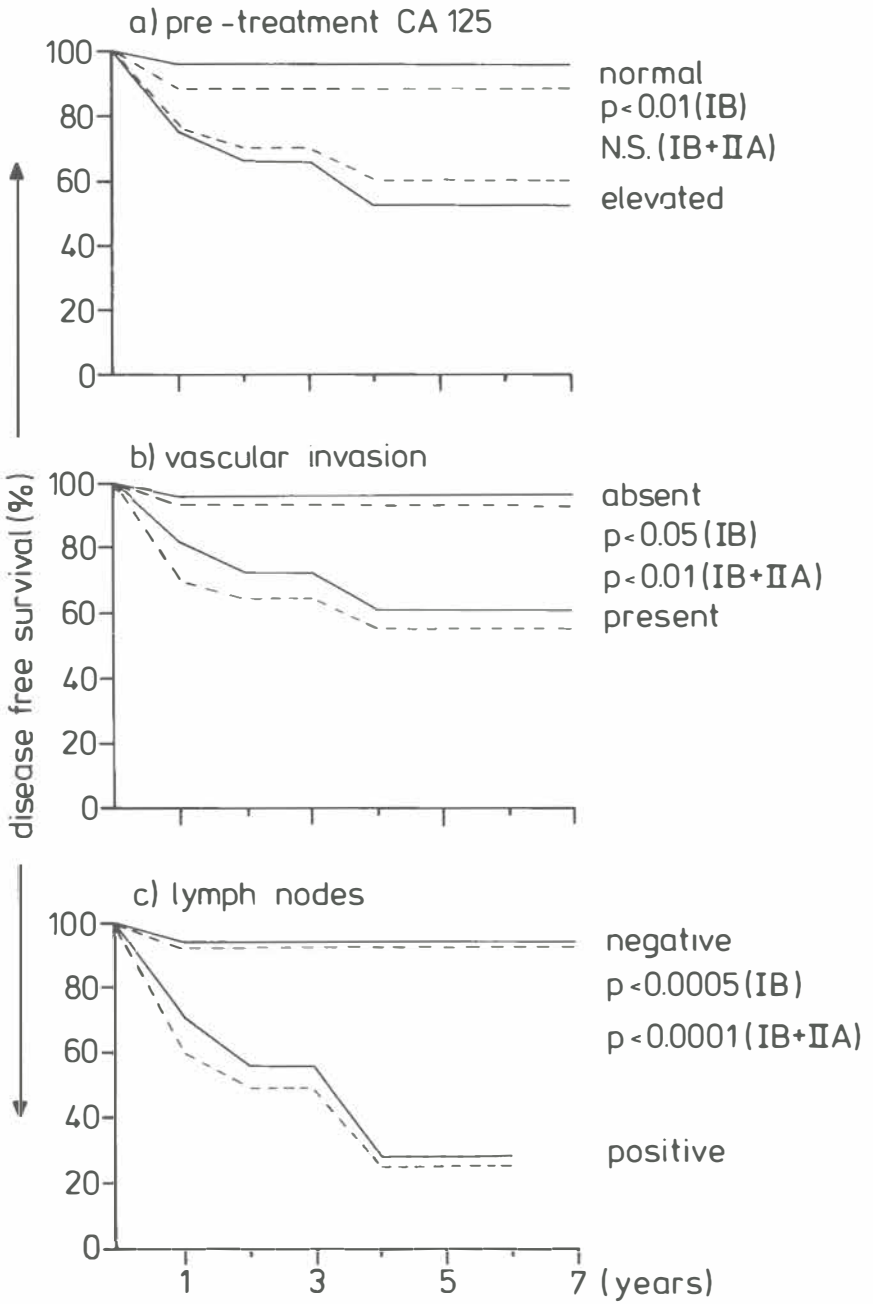
Prognostic Value of the Pretreatment Serum Marker Levels

Table III shows that in Stage IB, the finding of an elevated serum CA 125 level before treatment was associated with an increased risk for recurrent disease. The five-year actuarial survival for patients with elevated serum CA 125 levels before treatment in Stage IB was 52.4%, *versus* 95.6% for patients with normal serum CA 125 levels (Fig. 1A). When patients with Stage IIA were included, the prognostic value of serum CA 125 became less obvious: six of the 17 patients (35%) with elevated serum CA 125 levels died *versus* three of 26 patients (12%) in whom normal levels had been determined (P = 0.0694). The strong interaction between stage, serum CA 125, and prognosis is also shown in Figure 2A; patients with Stage II had a poorer prognosis

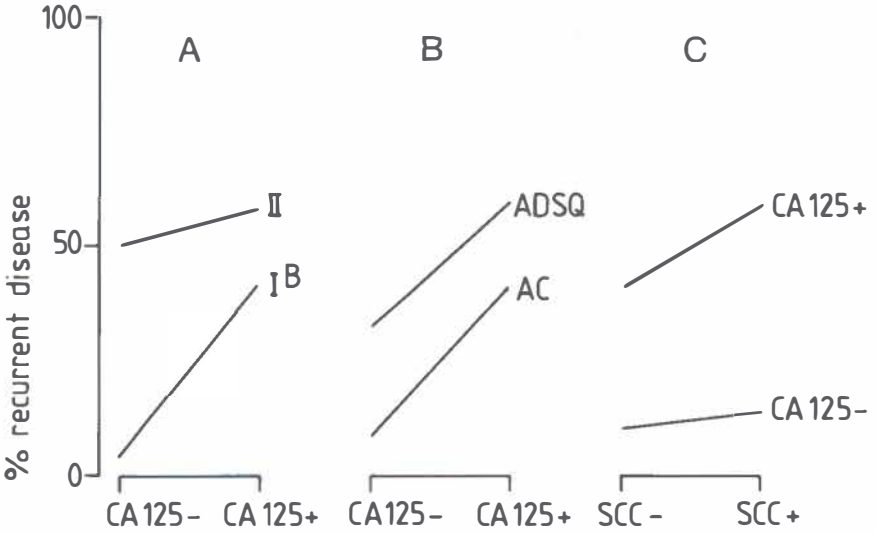
• TABLE III. Stage IB. Prognostic significance of pretreatment serum CA 125

CA 125 (U/ml)	*N*	*Recurrent disease*	*P*
>16	12	5 (42%)	
≤16	23	1 (4%)	0.012

• FIGS. 1A-1C. Cervical adenocarcinoma. Actuarial disease-free survival for patients with Stage IB (continuous line) and Stages IB + IIA (dotted line) according to (A) the serum CA 125 level before treatment, (B) the presence or absence of tumor cells in vascular spaces, (C) the presence or absence of tumor cells in lymph nodes. NS = Not significant ($X_2=3.175$, $P < 0.1$).



• FIG. 2A-2C. Stages IB and II. Interrelations between the pretreatment serum CA 125 level, the prognosis, and (A) the stage of the tumor, (B) the histologic tumor type, and (C) the pretreatment serum SCC antigen level. (+ = elevated serum marker level; - = normal serum marker level).



than patients with Stage IB. Figure 2A also shows that in Stage IB, there is a clear relationship between the pretreatment serum CA 125 level and survival. The incidence of elevated serum CA 125 levels in Stage II was 76% (13 of 17 patients). In this stage, the prognosis was virtually independent of the pretreatment serum CA 125 level. Linear regression analysis showed that the actual serum CA 125 levels had no additional prognostic value in Stage IB or II. Figure 2B shows that patients with adenosquamous carcinoma had a poorer prognosis than patients with adenocarcinoma and that, for both groups, elevation of the pretreatment serum CA 125 level was a key determinant. The serum SCC antigen level did not provide additional prognostic information (Fig. 2C).

Linear regression analysis showed that in the total study population (Stages IB - IV) the actual value of the logarithmic serum CEA level was related to the prognosis ($r = 0.36$). Six of the seven (86%) patients with serum CEA levels ≥ 15.0 ng/ml and three of the five (60%) patients who had serum CEA levels between 5.0 and 15.0 ng/ml died compared with 18 of the 52 (35%) patients with normal serum CEA levels. We were unable to find a substantial relationship between pretreatment serum CEA levels and prognosis in early stage disease.

In Stages IB and IIA, the overall prognosis for patients with adenosquamous tumors seemed to be slightly worse than that for patients with pure adenocarcinoma: four of the 11 (36%) patients with adenosquamous carcinoma died, *versus* five of the 36 (14%) patients with adenocarcinoma ($P = 0.1138$). A more detailed analysis revealed that the prognostic effect of the histologic type depended on the extent of the tumor. In Stage IB, the histologic type did not influence the prognosis ($P = 0.37$). However, in Stage II the cell type seemed to affect survival: six of the seven (86%) patients with adenosquamous tumors died *versus* four of the 11 (36%) patients with adenocarcinoma ($P = 0.0566$). The overall survival in the Stages IB and II was 47% (seven of 15) for patients with adenosquamous tumors and 80% (33 of 41) for patients with adenocarcinoma ($P = 0.016$).

In Stages IB and IIA, vascular invasion by tumor cells was related to a poor prognosis (Table IV, Fig. 1B). In Stage IB, information on this parame-

• TABLE IV. Stages IB And IIA. Prognostic significance of vascular invasion and lymph node metastasis*

		N	*Recurrent disease*	*P*
Vascular invasion	Present	14	6 (43%)	
	Absent	28	2 (7%)	0.0105
Lymph nodes	Positive	10	6 (60%)	
	Negative	37	3 (8%)	0.0013

* Excluding patients with residual disease after treatment

• TABLE V. Stages IB and IIA. Relation between histologic type and vascular invasion

	Adenocarcinoma		*Adenosquamous*		
Stage	*N*	*Vascular invasion*	*N*	*Vascular invasion*	*P*
IB	26	7 (26%)	7	4 (57%)	0.146
IIA	6	1 (12%)	3	2 (67%)	0.226
Total	32	8 (25%)	10	6 (60%)	0.050

ter was obtained from 33 patients. Four of 11 the patients with vascular invasion eventually died of their disease (36%) compared with one of 22 patients without vascular invasion (5%) ($P = 0.033$). The prognostic value of vascular invasion did not appear to be significantly influenced by the tumor stage; even when patients with Stage IIB were included in the analysis, the actuarial survival for patients without vascular invasion was 93% compared with 39% for patients with vascular invasion ($P < 0.0025$). The incidence of lymph node metastases was not higher when vascular invasion was present ($P = 0.69$). Consequently, the effect of vascular invasion on the prognosis was not influenced by the node status. Although patients with adenosquamous tumors had a higher incidence of vascular invasion (Table V), patients whose tumors did not contain squamous elements but who had vascular invasion by tumor cells were also at higher risk: three of the seven patients (43%) with pure adenocarcinoma and vascular invasion ultimately died of their disease, *versus* only two of the 24 patients (8%) whose tumors did not have vascular invasion ($P = 0.0619$). Alternatively, patients with adenosquamous carcinoma without vascular invasion seemed to have a good prognosis; all four patients are still in complete remission, but three of the six patients with vascular invasion had tumor relapse. The relationships between vascular invasion, metastatic tumor spread, and subsequently a poor survival rate were illustrated by the findings in patients with negative nodes and complete remission after primary therapy. Recurrence (pelvic wall) was found in three of 11 patients with vascular invasion *versus* none of 22 patients without vascular invasion ($P = 0.0302$). Two of the three patients with vascular invasion had adenosquamous carcinoma.

In Stage IB, seven of the 38 patients who underwent pelvic lymphadenectomy had metastasis in the pelvic nodes (18.4%) and three of the nine patients with Stage IIA tumors had lymphatic tumor spread (33.3%). The prognostic value of positive lymph nodes (Table IV, Fig. 1C) was not influenced by the clinical stage of the tumor. In Stage IB, four of the seven patients with positive nodes died *versus* two of the 31 patients who had negative nodes ($P = 0.0061$). There was no relationship between the cell type and lymph node metastasis.

Relationships Between the Pretreatment Serum Marker Levels and Histopathologic Risk Factors in the Stages IB and IIA

Table VI shows that the pretreatment serum CA 125 level of patients with Stage IB adenosquamous carcinoma was more frequently elevated. Elevated

• TABLE VI. Stages IB and IIA. Relation between serum CA 125 and histologic type

	Adenocarcinoma		*Adenosquamous*		
Stage	*N*	*CA 125 >16 U/ml*	*N*	*CA 125 >16 U/ml*	*P*
IB	28	7 (25%)	7	5 (71%)	0.033
IIA	5	3 -	3	2 -	0.714
Total	33	10 (30%)	10	7 (70%)	0.030

• TABLE VII. Stages IB and IIA. Pretreatment serum CA 125 and SCC antigen levels in relation to vascular invasion

Marker level	*N*	*Vascular invasion*	*P*
Both elevated	8	7 (88%)	
Normal or only one elevated	30	5 (17%)	0.0004

pretreatment serum SCC antigen levels were found more frequently in patients with adenosquamous carcinoma (six of 10, 60%) than in patients with adenocarcinoma (ten of 33, 30%), but this difference was not significant at the 5% level ($P < 0.1$).

The relationship between pretreatment serum marker levels and vascular invasion is shown in Table VII. A strong relationship was noted between vascular invasion and the combined elevation of both the serum CA 125 and SCC antigen levels. This relationship was not influenced by the tumor stage or node status, but it was biased, however, by the adenosquamous tumor type due to a higher incidence of both vascular invasion and elevated serum CA 125 or SCC antigen levels in these patients. The relationship was mainly based on the correlation between serum SCC antigen and vascular invasion; eight of the 13 patients (60%) with serum SCC antigen levels in excess of 2.5 ng/ml had vascular invasion compared with six of the 27 patients (22%) with normal serum SCC antigen levels ($P = 0.0191$). There was no correlation with elevated serum CA 125 levels ($P = 0.153$).

In Stage IB, the pretreatment serum CA 125 levels were related to the postsurgical finding of metastatic spread to the lymph nodes (Table VIII). This relationship was not influenced by the histologic type of the tumor; four of the seven patients with Stage IB adenocarcinoma and elevated serum

• TABLE VIII. Stages IB and IIA. Pretreatment serum CA 125 level in relation to lymph node metastasis

	CA 125 >16 U/ml		*CA 125 ≤16 U/ml*		
Stage	*N*	*Nodes positive*	*N*	*Nodes positive*	*P*
IB	12	5 (42%)	23	1 (4%)	0.012
IIA	5	1 -	3	2 -	0.28
Total	17	6 (35%)	26	3 (12%)	0.07

CA 125 levels had positive nodes (57%) *versus* none of the 21 patients with normal pretreatment serum CA 125 levels (0%) (P = 0.0017). Serum SCC antigen levels were not related to the presence of lymphatic metastases. We were unable to show any significant relationship between pretreatment serum CEA levels and histologic type, vascular invasion, or nodal spread.

The interaction between the serum CA 125 level, lymphovascular involvement, and the extent of the disease led to the identification of a low-risk and a high-risk group. In 39 patients with Stage IB or IIA data were available on pretreatment serum CA 125 and lymphovascular involvement. Seventeen patients had Stage IB without vascular invasion or nodal metastases. None of these patients had tumor relapse. The high-risk group consisted of the 22 remaining patients with one or more of the following features: Stage IIA, vascular invasion, and/or nodal spread. All nine recurrences (41%) were in this group. In the high-risk group 14 patients (64%) showed elevation of the pretreatment serum CA 125 level *versus* three patients (18%) in the low-risk group (P = 0.0009).

Comment

Decisions with regard to the optimal treatment for patients with malignant disease are based on probabilities. In cervical cancer, clinical staging provides the most vital prognostic information. Nevertheless, in clinically early stage disease, occult metastases are a challenging diagnostic problem. Tumor markers may help to identify the patient at risk. Normal and neoplastic glandular cells of the endocervix stain positively with the anti-CA 125 antibody OC 125.[35,36] Serum CA 125 determinations are useful in the monitoring of patients with cervical adenocarcinoma, and the extent of the tumor has a great impact on the pretreatment serum CA 125 level.[28] Our study shows

that in clinical Stage IB, elevated levels before treatment were indicative of the postsurgical finding of lymph nodal spread. The prognostic significance of both tumor stage[3,4,17,18,20,21,24] and lymph node involvement[3-7,20,22,24] was confirmed. Based on these observations, the poor prognosis for patients with elevated serum CA 125 levels before treatment is understandable. In Stage IB, the five-year actuarial survival for patients with elevated serum CA 125 levels was 52%, *versus* 96% when normal pretreatment levels were found.

The serum CA 125 level was also influenced by the histologic type. Patients with adenosquamous tumor had a higher incidence of elevated serum CA 125 levels. Many authors consider that the cell type has a diverging effect on patient outcome in cervical cancer.[7,12,18-25]However, Kilgore et al[3] found no differences in survival among matched patients with cervical adenocarcinoma or adenosquamous or squamous cell carcinoma. These apparently conflicting results may be explained by the fact that the stage in itself affects the prognosis to such an extent that it overwhelms other prognostic indices and renders them nonprognostic. Particularly in Stage II, however, the relative weight of prognostic factors other than the extent of the tumor may become more critical. In this respect it is noteworthy that, in accordance with Kilgore et al,[3] we found no prognostic impact for the cell type in Stage IB. In Stage II, however, our data suggest that the cell type does have an effect on the prognosis. The survival for patients with Stage II adenosquamous tumors was only 14% *versus* 64% for patients with Stage II adenocarcinoma. This observation, in addition to the higher incidence of other risk factors (Tables V and VI) and the different expression of CA 125, SCC antigen, and CEA during tumor progression[28] led us to the conclusion that adenosquamous tumors have different biologic behavior. It is feasible that these tumors expand more rapidly or have a greater tendency towards invasive extension and tumor spread than other cervical adenocarcinomas.

There is no consensus on the significance of vascular invasion as an independent prognostic factor. In squamous cell cancer of the cervix, the frequency of vascular invasion has been reported to vary widely, ranging from 9% to 73%.[15,37] Several authors reported a correlation between vascular invasion and other unfavorable prognostic tumor characteristics.[6-8,11,13-15,23-25,38] Consequently, the recurrence rate was higher and survival poorer[6-8,11,13,14,23-25] White et al[39] concluded that vascular invasion had no prognostic importance. They were supported by Gauthier et al,[6] although they noted a higher frequency of vascular invasion in deeply infiltrating tumors which were associated with a poorer prognosis. In a matched pairs analysis Boyce et al[9] clearly identified vascular invasion as a predictor of poor

outcome, which was recently confirmed by Kenter et al[10] in a multivariate analysis of risk factors in Stages IB and IIA. For adenocarcinoma of the cervix fewer data are available,[23-25] but they show an association with poor survival. In Stages IB and II, we observed an incidence of 37%. In our series, vascular invasion was not related to lymph node metastases. This is interesting because vascular invasion was shown to carry important prognostic information, even after we corrected for node status. Although vascular invasion was more frequently found in adenosquamous tumors, the prognostic value of vascular invasion did not depend on the cell type either. Adenocarcinoma patients with vascular invasion were also at risk, and conversely, patients with adenosquamous tumors without vascular invasion seemed to have a fairly good prognosis. In addition, pelvic wall recurrence was more prevalent in patients with negative nodes and vascular invasion than in comparable patients without vascular invasion. These findings support the observations made by others.[13,23-25,38] The results with regard to vascular invasion were not or only marginally influenced by the tumor stage. We believe that these observations imply a causal connection and indicate that, in cervical adenocarcinoma, vascular invasion should be regarded as an expression of the metastatic potential of the tumor involved.

Hoshina et al[40] reported that cervical adenocarcinoma cells showed no appreciable staining for SCC antigen. Nevertheless, they observed positivity in adjacent squamous metaplastic cells. These findings may explain why we observed only slight elevations before treatment and why elevations occured particularly in patients with adenosquamous tumor.[28] However, elevated serum SCC antigen levels before treatment had no prognostic significance and were not related to the extent of the tumor[28] or lymphatic tumor spread. Although 80% of cervical adenocarcinomas show positive staining for CEA,[36] pretreatment serum CEA levels had no prognostic value in early stage disease. Nevertheless, the overall survival correlated with the actual serum CEA level. This finding is consistent with the observations of Kjørstad and Örjasæter.[41]

The issue of the specificity of serum CA 125 becomes increasingly important. Studies on the interaction of serum CA 125 levels and histologic tumor features can help to explain the pathophysiology of this antigen. The results of this and of previous studies in endometrial and ovarian carcinoma[42,43] suggest that this high molecular weight antigen may gain access to the circulation because of a loss of effective natural barriers, such as the basement membranes between the epithelial site of antigen production and the peripheral bloodstream.[44,45] Invasion of the circulatory system ultimately resulting in (occult or overt) metastatic spread by antigen-shedding tumor

cells may contribute to the rise in the serum CA 125 level. Inadequate treatment will eventually result in recurrent disease. These events depend on the metastatic potential of the tumor involved[42,44,46-48] which might be illustrated by our findings in adenosquamous tumors. Moreover, proliferative mesothelial cells on the peritoneum can be an important source of CA 125 as well.[49,50] With this in mind, it is feasible that as soon as the tumor has affected the abdominal cavity, the reactive peritoneum can contribute to the elevation in the serum CA 125 level. Within this concept, the serum CA 125 level reflects the biologic behavior of the tumor and represents an implicit indicator of tumor virulence. It would therefore seem to be worthwile to consider adjusting the treatment protocols for patients with clinically early stage disease who display elevated serum CA 125 levels on admission.

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CHAPTER VI

Serum CA 125 Levels In Patients With A Provisional Diagnosis Of Pelvic Inflammatory Disease

Clinical and theoretical implications

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SUMMARY

In 50 patients with a provisional diagnosis of pelvic inflammatory disease (PID), CA 125 concentrations in serum were measured before laparoscopy and during hospitalization, using an enzyme immunoassay. The findings at laparoscopy were graded on the basis of the extent of inflammatory peritoneal involvement (grades 0 - 3; normal observations having a score of 0). On admission, 66% of the patients had serum CA 125 concentrations in excess of the cutoff value of 16 U/ml (range 20 - 1300 U/ml). The serum CA 125 concentration before laparoscopy correlated with the extent of inflammatory peritoneal involvement (eta = 0.74). The predictive value of an elevated serum CA 125 level to indicate the presence of salpingitis (grades 1 - 3) was

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97%. However, the predictive value of a normal CA 125 level indicating normal observations at laparoscopy (grade 0) was only 47%. During treatment and follow-up, the serum CA 125 concentration returned gradually to normal levels. It was concluded that the finding of an elevated serum CA 125 level confirms the diagnosis of peritoneal involvement in patients with a clinical diagnosis of PID.

Introduction

The term pelvic inflammatory disease (PID) refers to an all-embracing concept which encompasses inflammatory lesions of the upper genital tract and/or contiguous structures, caused, in most cases, by the ascending spread of microorganisms from the vagina and cervix. The estimation of intraabdominal extension of the disease is a major diagnostic problem in these patients.[1-4]

The measurement of serum CA 125 has been demonstrated to be valuable in the clinical monitoring of patients with gynecologic cancer.[5-8] However, production and release of CA 125 does not appear to be specific for malignancies alone. Other coelomic-derived cells such as reactive mesothelial cells on the peritoneal surface stain positively when incubated with the monoclonal antibody OC 125.[9,10] Benign gynecologic diseases, such as endometriosis and PID, have been reported in association with elevated serum CA 125 levels.[11-13] This study was designed to investigate whether the determination of serum CA 125 concentrations enhances the accuracy of diagnosis of peritoneal involvement in patients with a clinical diagnosis of PID.

Patients and methods

The study comprised 50 patients who were admitted to the Department of Obstetrics and Gynecology at the University Hospital of Groningen, The Netherlands, between July 1987 and April 1989 with a provisional diagnosis of PID. As minimal inclusion criteria, the patient had to present (sub)acute lower abdominal pain in association with three or more of the following symptoms and signs: increased vaginal discharge and/or irregular bleeding from the onset of the disease, noticeable tenderness of the pelvic organs, or adnexal mass at bimanual examination, a temperature in excess of 38°C, an erythrocyte sedimentation rate (ESR) exceeding 15 mm/h, and an increased white blood cell count (WBC) to over 10.0 x 10^9/l. Swabs were taken from

the urethra and cervix and cultured for *Neisseria gonorrhoea* and *Chlamydia trachomatis.*

All patients underwent a diagnostic laparoscopy within 24 hours after admission. To establish the visual diagnosis of (sub)acute salpingitis, the criteria as proposed by Jacobson & Weström were used.[1] The findings at laparoscopy were classified on the basis of the extent of inflammatory peritoneal involvement observed (Table I). All the patients were hospitalized and treated routinely with strict bedrest and an antibiotic regimen (doxycycline and metronidazole) for at least 10 days, except for those with grade 0 at laparoscopy. The patients were discharged from hospital when all the symptoms and signs had disappeared and the ESR was below 20 mm/h. Serum samples for CA 125 assay were obtained on admission (50 patients), at weekly intervals during hospitalization (41 patients), and at the routine follow-up examination 6 - 12 weeks after discharge (34 patients). The sera were coded to ensure that the results could not influence the diagnostic and treatment procedures. In patients who showed increased serum CA 125 concentrations on admission, these were considered to have stabilized when a positive or negative change in the concentration of less than 25% of the preceding value was measured in the next sample. If the preceding value differed by more than 25%, this rise or fall in the serum CA 125 concentration was defined as an increase or decrease in the serum CA 125 concentration.

Clinical data and statistics.

The age range of the 50 patients was 16 - 40 years and the period of hospitalization ranged from 2 - 37 days. Predisposing factors, such as the use of an intrauterine contraceptive device (5x) or curettage (3x) prior to the onset of the disease were found in eight patients. On admission, an ESR > 15 mm/h was observed in 41 of the 50 patients (range 20 - 90 mm/h) and a WBC

•TABLE I. Classification of acute salpingitis at laparoscopy

Grade 0: No signs of inflammation
Grade 1: Hyperemia of the surface of the fallopian tube(s) and edema of the tubal wall; hyperemia of the uterus and/or ovaries allowed to be present; seropurulent discharge at the fimbriated end or on the surface of the tube(s) and/or in the cavum Douglasi.
Grade 2: as grade 1, fresh (i.e. easily breakable nonfibrotic) intrapelvic adhesions present.
Grade 3: as grade 1 or 2, with clear evidence of local or diffuse peritonitis outside the pelvis and/or perihepatitis.

count > 10.0 x 10^9/l was found in 22 of the 50 cases (range 10.1 - 20.1 x 10^9/l). Some 3 - 7 days after commencing the treatment, the ESR showed a decrease in 46 of the 50 patients and was below 20 mm/h in 39 patients. In 49 cases, the WBC count was below 10.0 x 10^9/l within one week after admission. For the statistical data processing, Fisher's exact test, analysis of variance, and a standard discriminant analysis[14] were used. Bayes' formula has been used to determine the positive and negative predictive value of the serum CA 125 level in relation to the findings at laparoscopy.

Assay for CA 125.
Serum CA 125 levels were measured using a solid-phase enzyme immunoassay (Abbott Laboratories, Chicago, Il, USA) and the values were expressed in U/ml.[15] Variation coefficients of 10.2% and 8.9% were found at concentrations of 20 and 166 U/ml, respectively ($N = 34$). All serum levels ≤16 U/ml were regarded as normal. This cutoff value represents the 95th percentile in a population of healthypremenopausal females and is comparable to a threshold value of 30 U/ml when the measurements are performed using an immunoradiometric assay.[15]

Results

At laparoscopy, nine patients (18%) had no signs of intrapelvic inflammation or otherwise abnormal intraabdominal findings (grade 0). In 41 patients (82%) the provisional diagnosis of acute salpingitis (grades 1 - 3) was confirmed visually (Table II). Four of the 11 patients with grade 3 acute salpingitis at laparoscopy were found to have perihepatitis (Fitz-Hugh and Curtis syndrome). Three patients had periappendicitis. Currently used anamnestic and clinical parameters were related to the grade of the disease. Seven of the nine patients with grade 0 had negative cultures. In patients with acute salpingitis, there was no relationship between the culture findings and the grade at laparoscopy (Table III).

The length of the delay before admission to hospital correlated with a higher grade. Sixteen of the 19 patients with grade 0 or 1 were admitted within 11 days after the onset of symptoms and signs, as compared with nine of the 31 patients who had grade 2 or 3 at laparoscopy (Fisher's exact test, two-tailed: $P = 0.0008$).

On admission, 33 of the 50 patients (66%) had a serum CA 125 concentration in excess of 16 U/ml (range 20 - 1300 U/ml) (Table II). The actual value of the initial serum CA 125 concentration correlated with the extent of

•Table II. Initial serum CA 125 concentration in relation to the findings at laparoscopy

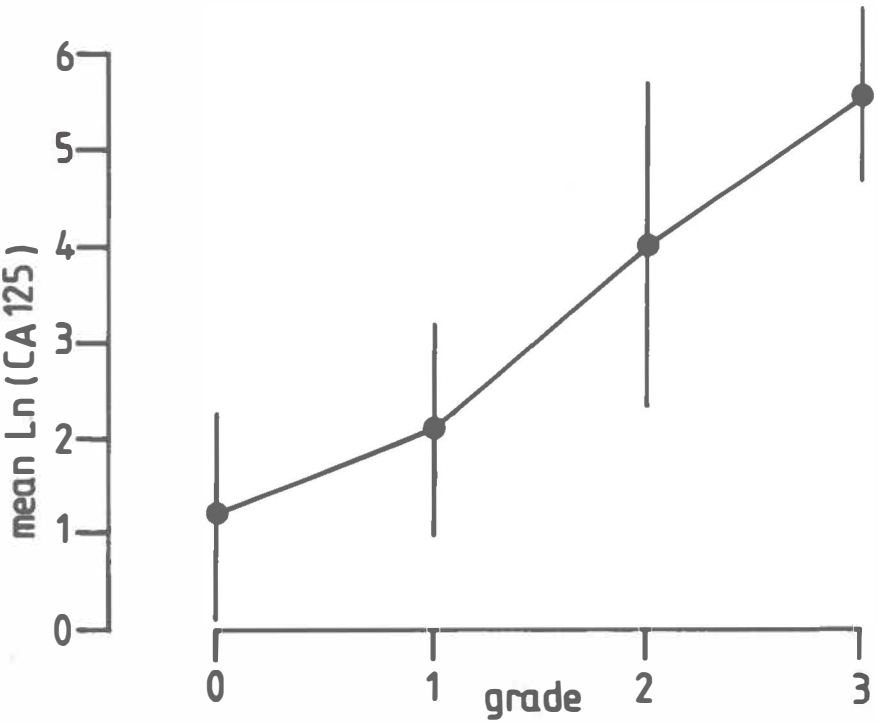
		No. of patients with CA 125:					
		Normal	*Elevated*			*Total Elevated*	*Range*
Grade	*N*	*≤16*	*17-32*	*33-160*	*>160*		*(U/ml)*
0	9	8	1	-	-	1	1 - 29
1	10	6	2	2	-	4	1 - 39
2	20	3	3	10	4	17	1 - 660
3	11	-	-	3	8	11	33 - 1300
Total	50	17	6	15	12	33	1 - 1300

•TABLE III. Culture findings in relation to the findings at laparoscopy

	No. of patients with grade:					
Micro organism	*0*	*1*	*2*	*3*	*Total*	*(%)*
CT	1	7	11	10	29	(58)
NG	1	1	2	-	4	(8)
CT + NG	-	-	2	-	2	(4)
Negative	7	2	5	1	15	(30)
Total	9	10	20	11	50	(100)

CT = *Chlamydia trachomatis*; NG = *Neisseria gonorrhoea*

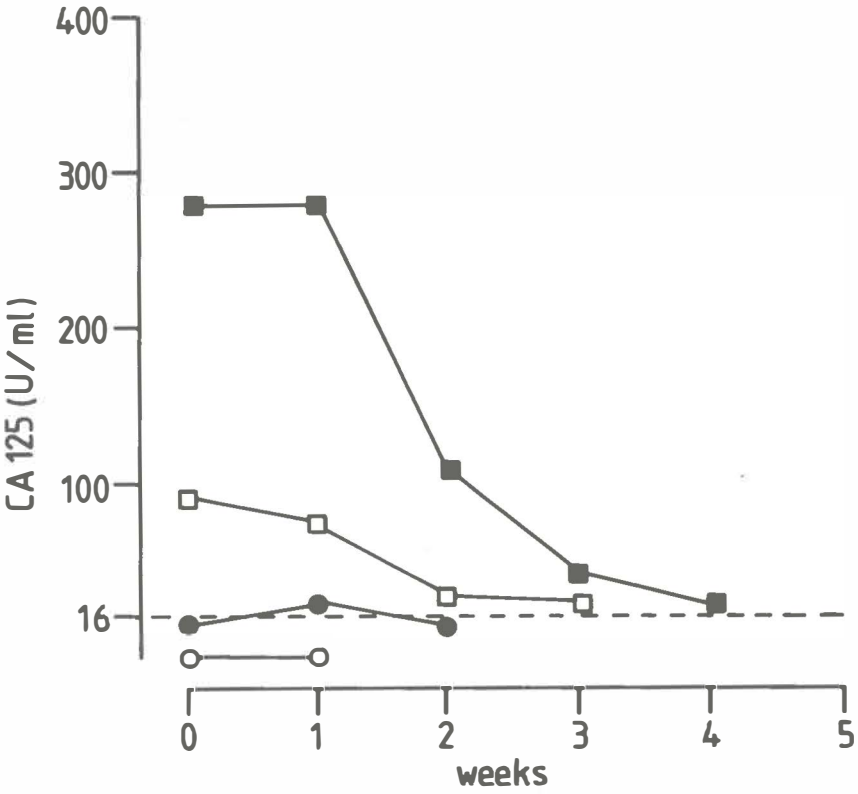
inflammatory peritoneal involvement determined at laparoscopy (after logarithmic transformation of the CA 125 values: eta = 0.743; F-test: $P < 0.00005$). It was not possible to distinguish between patients with no salpingitis (grade 0) and patients with a mild form of salpingitis (grade 1) or between patients with grade 2 or 3 salpingitis at laparoscopy (Scheffe test; $\alpha=0.05$; Fig. 1). The overall predictive value of a serum CA 125 concentration in excess of 16 U/ml for the presence of acute salpingitis at laparoscopy (grades 1 - 3) was 96.6%. However, normal serum CA 125 levels concurred



•Fig. 1. Mean natural logarithmic value of the initial serum CA 125 concentrations (± SD) according to the grade of acute salpinigitis at laparoscopy.

with normal findings at laparoscopy in only 47.3% of the cases. Discriminant analysis of the data of all the patients demonstrated that the combined function of the delay before admission to hospital and the logarithmic value of the serum CA 125 concentration on admission did not improve the accuracy of diagnosis.

On admission or during follow-up we observed serum CA 125 levels of above 16 U/ml in only one of the 19 determinations in the nine patients with grade 0 (29 U/ml). In the remaining patients (grades 1 - 3) the serum CA 125 levels mainly stabilized or increased during the first week of hospitalization (Fig. 2). In seven of the ten patients (70%) with grade 1, serum CA 125 levels exceeded 16 U/ml during the course of the disease (range of elevated serum CA 125 levels 21 - 109 U/ml), three of them showing serum levels in excess of 32 U/ml. Thirty of the 31 patients (97%) who had obvious inflammatory peritoneal involvement at laparoscopy (grades 2 and 3) presented with serum CA 125 levels higher than 16 U/ml on admission or during hospitalization (range of elevated serum CA 125 levels 18 -2600 U/ml). In



•FIG. 2. Median serum CA 125 concentrations on admission (*first sample*) and during hospitalization (*all other samples*) according to the grade of the disease. Grade 0 (*open circles*), Grade 1 (*closed circles*), Grade 2 (*open squares*), Grade 3 (*closed squares*).

27 of these patients (87%) serum CA 125 levels of over 32 U/ml were measured.

In the second week of hospitalization, the CA 125 serum concentrations started to decrease, but on the day of discharge from hospital, 26 of the 38 patients (68%) who had displayed elevated serum CA 125 levels during the course of disease, still had serum levels higher than 16 U/ml. One patient was readmitted to hospital one week after she had been discharged. A second laparoscopy once again revealed acute salpingitis. Accordingly, the serum CA 125 level had increasedfrom 27 to 48 U/ml.

Within 6 - 12 weeks after discharge, 34 patients returned to the department for a routine follow-up examination. Thirty-one patients were free from symptoms or signs. Four of these 31 patients (13%) had a serum CA 125 concentration exceeding 16 U/ml (45, 37, 22, and 17 U/ml, respectively).

One of three patients who still complained of lower abdominal pain had a level of 40 U/ml.

Comment

The diagnosis pelvic inflammatory disease is based on clinical criteria. The accuracy of this clinical diagnosis, however, is not likely to exceed 70% of the cases.[1-4] Laparoscopy is the only available diagnostic technique with which to distinguish patients with and without acute salpingitis. For this reason the validity of any test that can improve the precision of the diagnosis should be acknowledged.

The results of the present study clearly demonstrate that female genital tract infection can be associated with elevated serum CA 125 levels. This observation has been reported earlier by other investigators.[11,12] However, in these previous studies, laparoscopic evaluation had either not been described or only had been performed in a limited number of cases. In our study population, 50 patients who presented with lower abdominal pain in association with three or more symptoms or signs, which fit the provisional diagnosis of PID, underwent laparoscopy. The incidence of elevated serum CA 125 levels in this study group was 66%. Elevation of the serum CA 125 level was associated with acute salpingitis observed at laparoscopy in 32 of the 33 patients (97%) and the degree of elevation correlated with the extent of peritoneal inflammation. The finding of an elevated serum CA 125 level in a patient with PID therefore provides additional diagnostic information in that we know that there is a high probability of inflammatory peritoneal involvement. It must be noted that especially patients with infection by *Chlamydia trachomatis* occasionally present with few symptoms despite the presence of a severe intraperitoneal inflammatory reaction.[4,16] The clinician may be inclined to refrain from laparoscopy in such cases, but the finding of increased CA 125 values can give the clinician an index of suspicion high enough to justify this invasive procedure. However, it was not possible to isolate patients with normal laparoscopic findings (grade 0) on the basis of the initial serum CA 125 level. Normal levels concurred with normal laparoscopic observations in only 47% of the cases.

The results also provide additional information on the pathophysiology of the CA 125 antigen. The tissue distribution of CA 125 in fetal coelomic epithelium and in coelomic-derived cells in the adult suggests that the expression of CA 125 is related to the proliferation of these cells, whether normal (e.g. the premenopausal endometrium) or pathologic (e.g. ovarian

tumors).[7,9,10,13,17-19] Very high CA 125 concentrations have been found in the fluids inside the cavities which are lined by these tissues.[17-19] It is conceivable that the normal basic CA 125 concentrations in the blood of healthy premenopausal females is produced mainly by the epithelial lining of the genital tract. The source of increased serum CA 125 concentrations, however, may reside in any of the coelomic-derived tissues which are afflicted by disease.

The present study focuses on the fairly significant role of the peritoneum as a source of CA 125. Proliferative mesothelial cells show intense staining with OC 125, whereas normal peritoneal cells show mostly negative staining.[9,10] It is feasible that the mesothelial proliferation associated with peritonitis - and therefore the production and release of CA 125 - is more pronounced in grade 2 and 3 disease than in grade 1. It can be assumed that the rapid drainage of rather small amounts of peritoneal fluid containing CA 125 by the lymphatic capillaries lining the diaphragmatic peritoneum can cause an increase in the serum CA 125 concentration.[18-20] In the case of genital tract infection without obvious peritoneal involvement, the antigen may escape into the microcirculation due to local inflammatory erosion of the basement membrane of the epithelial lining of the endocervix, endometrium, or fallopian tube. This could explain the slight increases in some of the patients with grade 0 or 1 disease.

The above also elucidates the noticeably slower decrease in serum CA 125 concentrations during follow-up, compared with the decrease in ESR and WBC count, which are parameters of a general reaction to inflammation. The decrease in the serum CA 125 concentration may reflect the healing process of the internal genital organs and the peritoneum, resulting in the restoration of effective natural barriers and the normalization of the CA 125 production and release by reactive mesothelial cells.

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CHAPTER VII

Elevated Levels Of Squamous Cell Carcinoma Antigen In Patients With A Benign Disease Of The Skin

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SUMMARY

Squamous Cell Carcinoma (SCC) antigen, formerly referred to as TA-4, is a tumor marker for squamous cell carcinoma of the uterine cervix. Based on the findings in a patient with complete remission after treatment for cervical carcinoma, the authors decided to analyze the sera from patients with a benign dermatosis. It was found that 83% (25/30) of the patients with psoriasis and 80% (12/15) of the patients with eczema had SCC antigen levels in excess of the cutoff value of 2.5 ng/mL. In psoriasis the serum SCC antigen level correlated positively with the body surface area affected by the disease ($r = 0.64$). Seven patients with miscellaneous skin disorders, all with an inflammatory component, showed high serum SCC antigen levels as well. Thus the existence of an inflammatory skin disease or a hyperkeratotic skin disease

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with an inflammatory component interferes with the usefulness of the SCC antigen as a tumor marker in squamous cell carcinoma of the uterine cervix.

Introduction

In 1977, Kato and Torigoe[1] prepared and purified a tumor-associated antigen (TA-4) from human uterine cervical squamous cell carcinoma tissue. The antigen was characterized as a glycoprotein with a molecular weight of approximately 48,000 daltons. By means of the isoelectrofocussing method, TA-4 was shown to have 14 subfractions, sharing at least one common antigenic determinant.[2] One of the subfractions has been isolated from a liver metastasis of cervical squamous cell cancer and was designated "SCC antigen".[3] A double antibody radioimmunoassay kit has been developed in order to be able to detect antigen activity in the serum of patients with squamous cell carcinoma of the cervix. Several studies have shown that the determination of circulating antigen levels provides a promising means for monitoring the course of disease in patients with cervical cancer.[4-8]

In a prospective study at our department we are evaluating the validity of SCC antigen for the early detection of recurrent cervical cancer. During the course of this study we have been confronted with elevated serum SCC antigen levels in a patient who had been treated for cervical carcinoma in 1980 and who had a long-term history of severe psoriasis. The observations in this patient prompted us to investigate the serum SCC antigen levels in other patients with a noncarcinomatous dermatosis.

Patients and methods

Between January 1984 and July 1988, 177 patients with squamous cell carcinoma of the uterine cervix were investigated. Forty-five of these patients either had residual disease after treatment *(N = 21)* or were found to have recurrent disease during follow-up *(N = 24)* (Tables I and II). These patients will be the subject of a future report, where they will be described in more detail. In addition, 30 patients with psoriasis vulgaris, 15 patients with various types of severe eczema, and 21 patients with miscellaneous noncarcinomatous dermatoses were admitted to the Department of Dermatology between November 1987 and August 1988 (Table III).

Serum samples from all patients were collected on admission and the percentage of skin involvement by psoriasis or eczema was estimated, the head

• TABLE I. Pretreatment serum SCC antigen levels in patients with squamous cell carcinoma of the cervix

Stage	*N*	*No. of patients with SCC >2.5 (ng/ml)*	*(%)*	*Range (ng/ml)*
IB	84	31	(36.9)	0.5 - 37.6
II	71	45	(63.4)	0.8 - 62.5
III	15	14	(93.3)	2.3 - 102.4
IV	7	7	(100.0)	10.0 - 130.9
Total	177	97	(54.8)	0.5 - 130.9

• TABLE II. Serum SCC antigen levels during follow-up for squamous cell carcinoma of the uterine cervix

	N	*No. of patients with SCC >2.5 (ng/ml)*	*(%)*	*Range (ng/ml)*
Controls	136	-	(0)	0.5 - 2.2
RS/RC disease	45	39	(87)	1.3 - 51.8*

SCC = Squamous Cell Carcinoma antigen.
* Range of serum SCC antigen in all patients since 1984 at the time of the diagnosis of residual (RS) or recurrent (RC) disease

• TABLE III. Study population

Diagnosis	*N*	*Female*	*Male*	*Mean age (yr)*	*Range (yr)*
Psoriasis vulgaris	30	11	19	52	11 - 74
Atopic dermatitis	6	2	4	33	3 - 71
Contact allergic eczema	3	1	2	52	46 - 59
Eczema on hands or feet	6	2	4	45	22 - 84
Miscellaneous	21	11	10	53	18 - 82
Total	66	27	39	50	3 - 84

accounting for 10%, the arms for 20%, the trunk for 30%, and the legs for 40% (body surface area [BSA]). In patients with psoriasis the correlation between the serum SCC antigen level and the percentage of skin involvement was calculated by regression analysis. Serum samples were taken longitudinally from four patients with psoriasis and related to the clinical course of the disease during topical treatment with anthralin using a severity index for disease activity (i.e. percentage of body surface involved x the grade of intensity of erythema of the psoriatic lesions; scale 0-4, normal skin having a score of 0).

The SCC antigen levels were measured using a double antibody radioimmunoassay kit (Dainabot Co. Ltd., Tokyo, Japan). The sensitivity of this assay system was 0.5 ng/ml. Variation coefficients of 12.8% at a low level (1.5 ng/ml) and 5.9% at a high level (13.3 ng/ml) were determined in 20 different assays. A level of 2.5 ng/ml was taken as the upper limit for normal, representing the 95th percentile in a population of 63 healthy females.[8] In another control group of 136 patients without known dermatologic disease, the 95th percentile was 2.0 ng/ml. This control group consisted of patients who had been in complete remission for at least 12 months after treatment for Stage IA, IB, or IIA (International Federation of Obstetrics and Gynecology [FIGO]) cervical carcinoma and who had attended the outpatients station between November 1987 and May 1988.

Tissue sections from two patients with psoriasis were cut from snap frozen tissue blocks (liquid freon). The air-dried sections were fixed for ten minutes in acetone and stained in accordance with standard procedures using an indirect immunoperoxidase technique. The monoclonal anti-SCC antigen-antibody (F_2H_7) was a gift from Dr H. Kato (Ube, Japan) and Dr I. Ikeda (Dainabot Co. Ltd., Tokyo, Japan) and was used in a concentration of approximately 4 $\mu g/cm^2$. Peroxidase conjugated rabbit antimouse antibodies (Dakopatts a/s, Glostrup, Denmark) were used in the second step. Postmortem skin biopsy specimens from two individuals without dermatologic disease were used as a control.

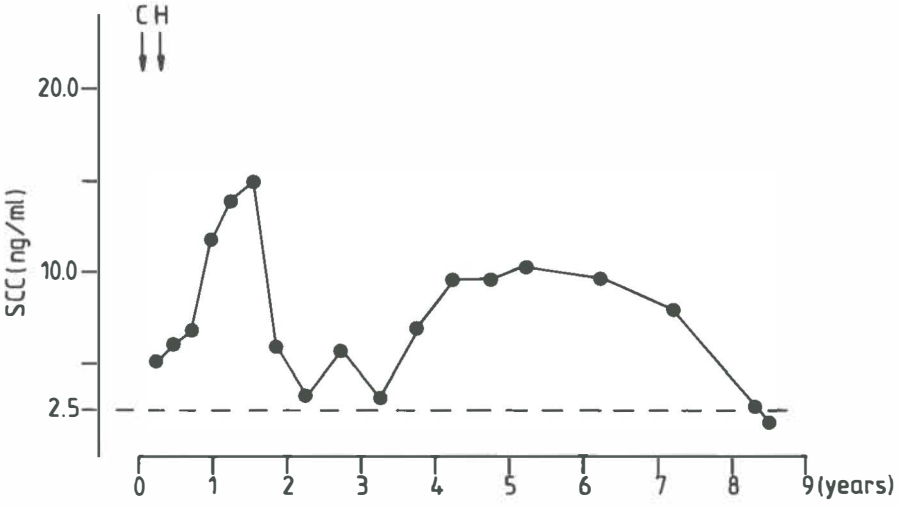
Results

The preliminary findings in patients with cervical cancer are summarized in Tables I and II. The incidence of elevated serum SCC antigen levels is related to the clinical stage of the disease, ranging from 37% in Stage IB to 100% in Stage IV (Table I). Normal serum SCC antigen levels were found in a control group of 136 patients without tumor activity or dermatologic disease, whe-

reas at the time of the diagnosis of residual or recurrent disease 87% (39/45) of the patients showed elevated serum SCC antigen levels (Table II). During tumor progression this figure rose to 98% (44/45) (range 1.3 - 305.0 ng/ml).

Dermatologic disease can be a major cause of elevated serum SCC antigen levels as well. The characteristics of our study population are shown in Table III. Figure 1 presents the high serum SCC antigen levels during 8 years of follow-up in a patient in complete remission after treatment for cervical cancer but with severe psoriasis. Table IV shows that 83% of the patients with psoriasis and 80% of the patients with eczema showed serum SCC antigen levels in excess of the cutoff value of 2.5 ng/ml. In addition, elevated serum SCC antigen levels were observed in seven of the 21 patients with miscellaneous noncarcinomatous skin disease. The highest level measured was 73.2 ng/ml in a patient with psoriasis.

Regression analysis revealed that the logarithmic value of the serum SCC antigen level of patients with psoriasis correlated positively with the percen-



• FIG. 1. Serum SCC antigen levels in a 54-year old female with severe psoriasis vulgaris who had been treated in 1980 at the Gynecologic Oncology Service for a Stage IB (FIGO) SCC of the cervix. During 7 years of routine follow-up, the patient had been free from tumor activity. In 1987, a level of 8.0 ng/ml was determined. Additional analyses of the patient's sera (stored at the Serum Bank of the Department) showed that high SCC antigen levels had been present since 1980. In 1988 the serum SCC antigen level decreased to normal after successful oral methotrexate treatment. At that time the estimated body surface area affected by psoriasis was less than 5%. C= conization; H= radical hysterectomy and pelvic node dissection.

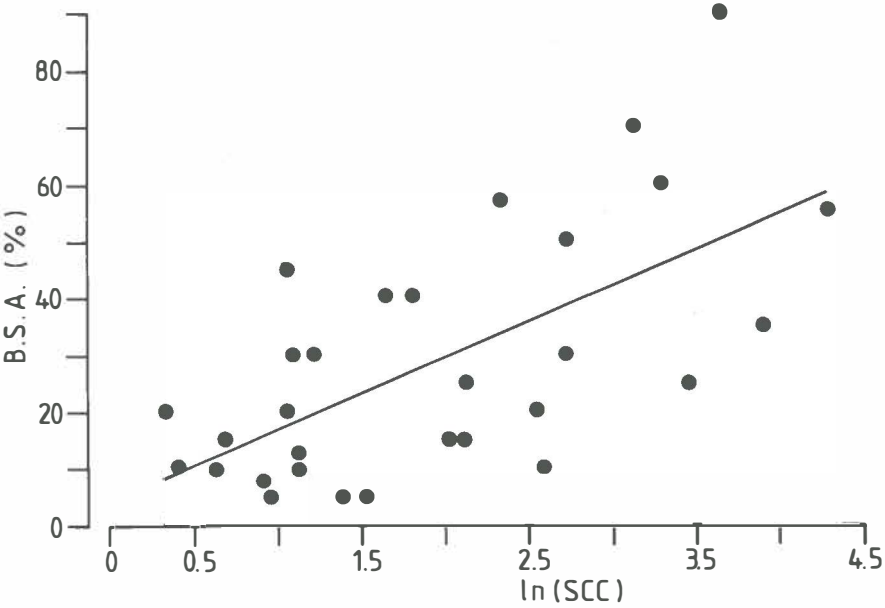
• TABLE IV. Elevation of serum SCC antigen levels in patients with noncarcinomatous skin disorders

Diagnosis	*Total Number*	*No. of patients with SCC >2.5 (ng/ml)*	*(%)*	*Range (ng/ml)*
Psoriasis	30	25	(83)	1.4 - 73.2
Eczema	15	12	(80)	1.3 - 56.6
Miscellaneous:				
Lamellar ichtyosis	1	1	-	30.0
Disseminated non-actinic porokeratosis	1	1	-	4.3
Dyskeratosis follicularis	1	1	-	40.6
Erythrodermic pityriasis rubra pilaris	1	1	-	19.5
Hypereosinophlic syndrome	1	1	-	20.1
Erysipelas	1	1	-	8.7
Cutaneous T-cell lymphoma	1	1	-	3.5

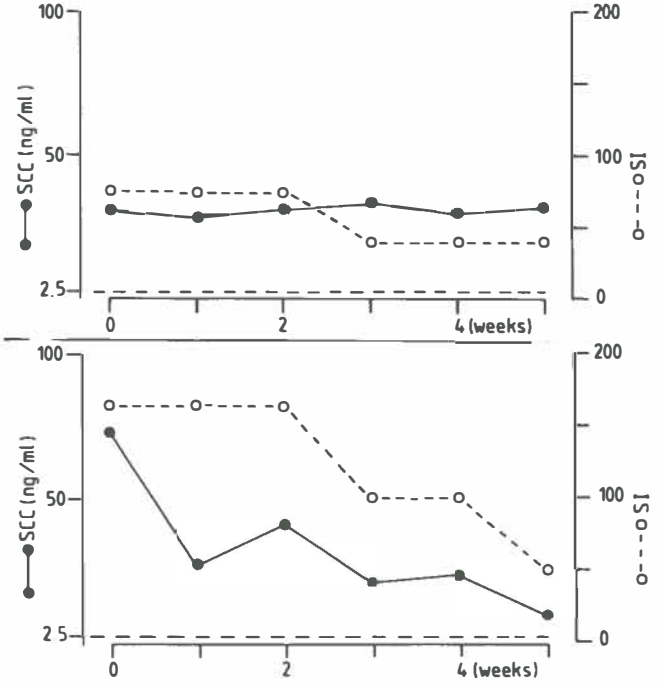
SCC = Squamous Cell Carcinoma antigen

tage body surface area affected by the disease (r = 0.64) (Fig. 2). The association between the percentage of skin involvement and the serum SCC antigen level is also demonstrated in Table V: in patients with eczema it was the extent of the disease which influenced the serum SCC antigen level rather than the type of eczema.

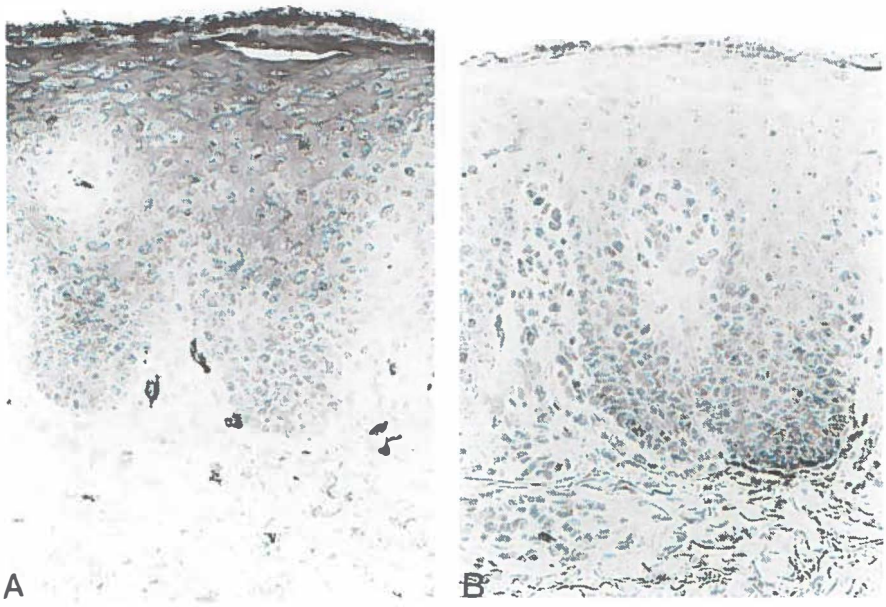
The results of four patients with psoriasis who were treated by topical anthralin, suggest a relationship between the clinical course of the disease and the serum SCC antigen level (Fig. 3). In two patients with psoriasis who were treated succesfully, the serum SCC antigen level decreased accordingly. In the two other patients, treatment had only a marginal effect. In these patients serum SCC antigen remained at the level which had been determined on admission. Immunohistochemical staining of psoriatic skin showed a positive reaction in the cytoplasm of the keratinocytes of the stratum spinosum (Figs. 4A and 4B). The cells of the basal layer did not show positive



• FIG. 2. Correlation between the initial serum SCC antigen level and the percentage body surface area (BSA) affected by psoriasis.



• FIG. 3. Serum SCC antigen levels in two patients with psoriasis during topical treatment with anthralin. SI = severity index of disease activity. (*Top*) Stabilization of the serum SCC antigen level in a patient with only marginal reponse to therapy. (*Bottom*) Decreasing serum SCC antigen levels in a patient who responded well to therapy.



Figs. 4A and 4B. Frozen tissue section. Psoriatic skin. (A) Immunoperoxidase using anti-SCC antigen-antibody F_2H_7 (H. Counterstain, x225). Note the intense staining of macrophages. (B) Negative control.

staining. In the dermis intense staining was observed in dendritic cells, most likely macrophages. In contrast to these findings, normal skin sections were completely negative for SCC antigen.

Comment

The results of this pilot study clearly demonstrate that elevated serum SCC antigen levels can be found in a variety of noncarcinomatous skin disorders, in particular dermatoses with an inflammatory component. Consequently, the determination of SCC antigen in patients with cervical carcinoma is of only limited prognostic value if the patient has been affected by an inflammatory dermatosis. This observation is of the utmost importance, because until now the finding of increased serum SCC antigen levels in a patient after treatment or during follow-up for cervical squamous cell carcinoma, implies that she will be subjected to thorough examination because of the high sus-

picion of recurrent disease.[6-8] It should be noted that these examinations have serious psychologic consequences for the patient involved. Nevertheless, our preliminary results in patients with cervical cancer indicate that the determination of the serum SCC antigen level can be of great help to the clinician (Tables I and II).

Elevated serum SCC levels have been observed in squamous cell carcinomas other than cervical carcinoma, such as squamous cell carcinoma of the lung and head or neck region.[8] Yagi et al[9] observed high serum SCC antigen levels in three patients with advanced stagesquamous cell carcinoma of the skin, whereas the SCC antigen levels remained within normal limits in patients with a benign dermatosis. In contrast, the findings in the present study confirm the observation made by Kudo et al[10] that elevation of the serum SCC antigen level is not specific for squamous cell cancer. They found high levels of this antigen in the blister fluid of patients with pemphigus in association with increased serum SCC antigen levels. Our study also confirms their observation of a relationship between the serum SCC antigen level and the disease activity.

The elevated serum SCC antigen levels in the 15 eczema patients and the seven patients with miscellaneous skin disorders indicate that it may be the inflammation which stimulates the expression of this epidermal cell antigen rather than abnormal keratinization. In this respect, the observation that epidermal growth factor (EGF) enhances the production of TA-4 (SCC antigen) by a cervical carcinoma derived cell line *in vitro*, is of particular interest, as EGF is produced by macrophages.[11]

In contrast to the observations made by Hoshina et al,[12] our results show that the expression of this antigen is not solely restricted to the squamous cervical epithelium. Although the exact nature and pathophysiology of the SCC antigen have not yet been fully clarified it may be speculated that the antibody recognizes an epitope on one or more of the "small" cytokeratins. The cytoplasmatic staining pattern and tissue localization, the absence in normal epidermis in contrast to the expression in hyperproliferative and neoplastic epithelium, the approximate molecular weight and the demonstration of an acidic and a basic subfamily are all arguments in favor of this hypothesis.[12-15] In addition, the intense staining of macrophages suggests that tissue damage and cell lysis may cause liberation of the antigen into the extracellular matrix followed by phagocytosis.[16] In our opinion this hypothesis merits further investigation.

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CHAPTER VIII

Cancer Of The Uterine Cervix: Sensitivity And Specificity Of Serum Squamous Cell Carcinoma Antigen Determinations

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SUMMARY

Between 1978 and 1989, 451 patients with cervical squamous cell carcinoma were referred to our department, of whom 143 experienced persistent or recurrent disease. Serial serum samples of the patients were analyzed for the presence of Squamous Cell Carcinoma antigen (SCC). The incidence of elevated pretreatment serum SCC antigen levels ranged from 37% in Stage IB (*N = 173*) to 90% in Stage IV (*N = 19*). Multivariate analysis showed that deep stromal infiltration and lymph node metastases were associated with significantly higher serum SCC antigen levels.

Serum SCC antigen trends correlated with the course of disease: after treatment the sensitivity (% positive results in patients with persistent disease) was 79% and the specificity (% negative results in patients with no evidence of disease) was 91%. During follow-up, the sensitivity of the assay was 85.5% in patients with recurrent disease. However, the positive predictive

This study has been accepted for publication in Gynecologic Oncology, *1990.*

value of a single serum SCC antigen value > 2.5 ng/ml for tumor recurrence was only 49%. This figure rose to 76% when two consecutive elevations were determined.

The stage and pretreatment serum SCC antigen level were the only factors which were found to influence survival, using Cox's regression analysis with five pretreatment variables.

Introduction

Once the diagnosis of cancer has been made, the patient will be subjected to thorough tests to delineate the extent of the tumor. Generally, the spread of the disease determines the treatment planning and is a denominator for the prognosis. The clinical staging of cervical cancer includes an examination under anaesthesia, blood analysis and radiographic and/or endoscopic techniques.[1] Despite efforts to detect extracervical tumor growth, a significant discrepancy still exists between clinical and surgical staging.[2] Although surgical staging may have failed to demonstrate extracervical lesions, the patient is still at risk for recurrent disease due to the growth of subclinically metastasized tumor cells. If we were able to track down and treat patients at the very earliest stage of metastatic growth, this might lead to improved survival rates.

In the past decade, the role of tumor markers in gynecologic oncology has become the subject of much research. The serum determination of tumor-associated antigens is a potential aid for the staging and monitoring of patients with gynecologic malignancies. The application of such markers may be particularly useful for the detection of occult disease immediately after completing primary treatment or during follow-up. However, many of these markers lack sensitivity or specificity and studies on factors which influence the serum levels of these markers are few. This makes it difficult for the clinician to correctly interpret the results from such investigations.

Several studies have indicated that serum values of Squamous Cell Carcinoma (SCC) antigen, a subfraction[3] of TA-4,[4] are of significance for the management of squamous cell carcinoma[5-11] and adenosquamous carcinoma[12,13] of the cervix. Immunohistochemistry has demonstrated the presence of this antigen mainly in keratinizing and large-cell non-keratinizing tumors, whereas undifferentiated or small cell carcinomas show no appreciable staining.[5,9,14]

In the present study, we used the sera and clinico-histopathologic profile of 451 cervical cancer patients, to investigate (1) the impact of tumor-related

parameters on the serum SCC antigen level (2) the sensitivity and specificity of the SCC antigen assay during the course of the disease (3) the prognostic significance of pretreatment serum SCC antigen values.

Patients and Methods

The study population comprised 451 patients with invasive carcinoma of the uterine cervix who were referred to the Gynecologic Oncology Service of the University Hospital of Groningen, The Netherlands, between January 1978 and March 1989. The study was initiated in March 1987.

Serial serum samples of the patients were collected on up to 42 occasions and stored at the Serum Bank of the department at -70°C before being analyzed for the presence of SCC antigen. Since March 1987, 99 patients entered the study prospectively. The files of all the patients referred before that date were reviewed for history and age, diagnostic and staging procedures, histopathologic diagnosis, treatment protocols and follow-up.

The mean age of the patients was 56.5 years (SD: 15.3 years). The staging was in accordance with the recommendations of the International Federation of Gynaecology and Obstetrics (FIGO)[1] (Table I). Bimanual examination was performed under general anaesthesia. During this procedure, the lesion size

•TABLE I. Study population

FIGO Stage	*N*	*(%)*	*RC/RS disease**	
			n	*(%)*
IB	208	(46.1)	30	(14.4)
IIA	85	(18.8)	23	(27.1)
IIB	95	(21.0)	43	(47.3)#
IIIA	6	(1.3)	4	(66.7)
IIIB	38	(8.4)	26	(70.3)#
IVA	9	(1.9)	7	(77.8)
IVB	10	(2.2)	10	(100.0)
Total	451	(100)	143	(32.1)#

* RC = recurrent disease; RS = residual disease.

5 patients unevaluable (4 Stage IIB, 1 Stage IIIB).

(largest diameter) was estimated routinely and expressed in centimeters for the large majority of patients.

Patients with (FIGO) Stage IB or IIA were mainly treated by preoperative intracavitary radiotherapy followed by a radical hysterectomy with bilateral salpingo-oophorectomy and pelvic lymphadenectomy 4 weeks later. The Stage IB patients who presented with small tumors (largest diameter ≤3 cm) were generally treated by primary radical surgery.

Postoperative whole-pelvis radiotherapy was applied in cases where lymph node metastases, marked vascular invasion, and/or positive resection margins were present. The total radiation dose was 45 Gy (fractions of 1.8 Gy daily; 8 MV photons) with central shielding after 30 Gy if the patient had received preoperative intracavitary irradiation. Patients with positive common iliac nodes also received paraaortic irradiation.

Patients with Stage IIB and most of the patients with Stage III were treated by combined external and intracavitary radiotherapy or external radiotherapy only. A small number of these patients were subjected to an adjunctive hysterectomy. In the remaining Stage III patients and in all the Stage IV patients, the treatment was individualized using combinations of radiotherapy, surgery, and/or chemotherapy. In seven patients with Stage III or IV, therapy was declined.

Complete remission (no evidence of disease) was defined as the absence of all tumor lesions, three months after treatment or during follow-up. Partial remission was defined as a 50% or more reduction in tumor size, without the appearance of new lesions following therapy. Stable disease was defined as no alteration in the clinical status of a patient who was known to have tumor. Progression of disease was defined as the appearance of new lesions, the growth of a pelvic mass, or other known tumor lesions or a distinct deterioration in the clinical status of a patient who was known to have tumor (such as vaginal hemorrhage, progressive uremia, or poor liver function tests) ultimately resulting in death. Recurrent disease was used to indicate the reappearance of disease in patients who experienced complete remission.

Statistics.

The calculations focussed on three issues:

1. Does the pretreatment serum SCC antigen level reflect tumor burden and can we define other factors which influence the pretreatment serum SCC antigen level?
2. Are serum SCC antigen determinations reliable for monitoring treatment results and the clinical course of the disease?

3. Do pretreatment serum SCC antigen levels carry prognostic information?

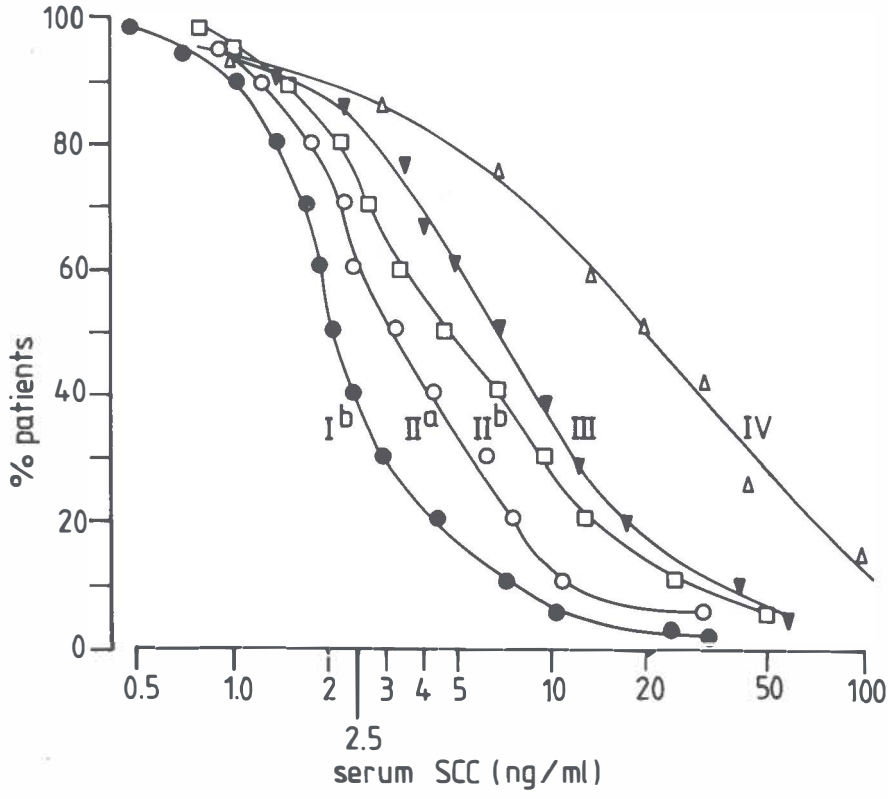
To answer these questions, one of the authors (HH) carefully reviewed all the available biopsy or cone material and the subsequent surgical specimens of the 232 patients who had been referred since 1984 plus those of the 143 patients who were referred since 1978 with residual (*N = 72*) or recurrent disease (*N = 71*). We classified the tumors into: well (grade 1), moderately (grade 2), and poorly (grade 3) differentiated squamous cell carcinoma, in accordance with the criteria laid down by Ferenczy and Winkler.[15] A small number of tumors showed severe nuclear anaplasia and/or a sarcomatous growth pattern and were categorized as undifferentiated carcinoma (grade 4).

The relationship between the clinical stage of the tumor and the pretreatment serum SCC antigen level was calculated for all the patients. In 20 patients, the pretreatment blood sample had been drawn after conization. They were excluded from the analyses. Three more patients with psoriasis were excluded, for this disease has proved to be a major cause of false-positive serum SCC antigen levels.[16] The data used for multivariate analysis[17] included the stage of disease (FIGO), lesion size, tumor grade, lymph node status, vascular invasion, depth of infiltration, and the pretreatment SCC antigen value (because of its skewed distribution the analysis required logarithmic transformation of this value). The clinicohistopathologic characteristics were eliminated from the multiple regression model by a 'step-down' procedure if they did not have a significant effect on the pretreatment serum SCC antigen level, given the other characteristics.

Differences between survival curves were computed by means of the survival procedure of the SPSS-X programme using a standard actuarial survival method and the Lee and Desu test.[17] The simultaneous effect of prognostic factors on survival was analyzed by means of Cox's proportional hazard model of regression analysis using the BMDP computer programme.[18] Five patients with a follow-up of less than six months were excluded. Survival on the basis of the pretreatment serum SCC antigen value was calculated using the method of moving average, taking intervals of 20 consecutive serum SCC antigen values for all stages and ten consecutive values for Stage IB. The median follow-up time for patients in complete remission was 62 months (range 6 - 133 months). Patients who experienced recurrent disease had a median follow-up period of 26 months (range 8 - 151 months). The disease-free interval in these patients, i.e. before the relapse became clinically apparent, was 79 weeks (range 21 - 584 weeks).

SCC antigen radioimmunoassay.

A double antibody radioimmunoassay (Dainabot Co. Ltd., Tokyo, Japan) was used, developed from a subfraction of a TA-4 preparation, isolated from a liver metastasis of a cervical squamous cell carcinoma.[4] In 30 different assays, the variation coefficients were 13.5 per cent at a low level (1.5 ng/ml) and 5.7 per cent at a high level (13.3 ng/ml). An upper limit of 2.5 ng/ml was regarded as normal, being the 95th percentile in a population of 85 healthy premenopausal women. Pretreatment sera were available from 421 patients (in 20 cases, serum had been obtained after conization).



•FIG. 1. Distribution of serum SCC antigen levels in 401 untreated patients with cervical squamous cell carcinoma in relation to (FIGO) stage. The vertical axis gives the sensitivity in terms of the percentage of patients with elevated serum levels at a particular serum concentration of SCC antigen.

•TABLE II. Data on all patients referred since 1984 with Stage IB (*N = 105*) or IIA (*N = 40*)

Parameter	*Stage IB*		*Stage IIA*		*Total No.*	
	N	*(%)**	*N*	*(%)**	*N*	*(%)**
Grade						
1	17	(17)	6	(16)	23	(17)
2	60	(61)	20	(53)	80	(58)
3	21	(21)	12	(32)	33	(24)
4	1	(1)	-	-	1	(1)
incalculable	6		2		8	
Infiltration						
≤5 mm	23	(43)	-	-	23	(33)
6-10 mm	14	(26)	4	(25)	18	(26)
≥10 mm	17	(31)	12	(75)	29	(41)
incalculable	51		24		75	
Vascular invasion						
Present	42	(57)	12	(60)	54	(57)
Absent	32	(43)	8	(40)	40	(43)
Incalculable	31		20		51	
Lymph Nodes						
Positive	19	(19)	6	(23)	25	(20)
Negative	80	(81)	20	(77)	100	(80)
Incalculable	6		14		20	
Lesion size						
<3 cm	33	(41)	1	(3)	34	(30)
3,4 cm	29	(36)	14	(44)	43	(38)
5,6 cm	18	(22)	12	(38)	30	(27)
>6 cm	1	(1)	5	(16)	6	(5)
unknown	24		8		32	

* Percentage of calculable patients

Results

The Correlation between Clinicopathologic Factors and the Serum SCC Antigen Level

The serum SCC antigen level prior to treatment was predominantly influenced by the extent of the tumor (Fig. 1). The overall incidence of elevated serum SCC antigen levels was 57% (227 of the 401 patients; range 0.6 - 131 ng/ml; median 3.1 ng/ml; SD = 15.5 ng/ml). This incidence varied from 37% in Stage IB (*N = 173*) to 90% in Stage IV (*N = 19*).

Subsequently, we investigated the effect of: lesion size, the depth of infiltration, grade, the presence or absence of vascular invasion, and the node sta-

•TABLE III. Stages IB and IIA. Patients referred since 1984. Univariate correlations between tumor-related parameters and the pretreatment serum SCC antigen level

		Serum SCC antigen > 2.5 ng/mL		
Parameter	*N*	*n*	*(%)*	*P*
Grade				
1, 2	82	40	(49%)	
3, 4	26	6	(23%)	0.0209
Infiltration				
≤5mm	14	1	(7%)	
>5mm	35	18	(51%)	0.0040
Vascular invasion				
Absent	26	8	(31%)	
Present	43	20	(47%)	0.1969
Lymphnodes				
Negative	78	26	(33%)	
Positive	21	15	(71%)	0.0017
Lesion size				
<3cm	28	7	(25%)	
≥3cm	73	34	(47%)	0.0481

tus, on the pretreatment serum SCC antigen level in Stages IB and IIA. The data on these patients are presented in *Table II*. In an initial univariate analysis, the lesion size, tumor grade, depth of stromal infiltration, and node status all correlated well with the pretreatment serum SCC antigen level (Table III). After multivariate analysis, however, only the node status ($P = 0.05$) and the depth of infiltration of the tumor ($P = 0.002$) were found to have a significant effect on the pretreatment serum SCC antigen level. Despite the bias which occurred when the data on all the patients (Stages IB - IV) were included, the multiple regression analysis nevertheless showed once again that, apart from the clinical stage ($P = 0.03$), the only factors which still influenced the pretreatment serum SCC antigen level were the depth of infiltration ($P = 0.04$) and the node status ($P = 0.0005$). It is important to note, however, that these three factors only explained 22% of the variability of the pretreatment serum SCC antigen level, indicating that the latter was also influenced considerably by other - unknown - factors.

The significant correlation with the node status was of particular interest. From Table III it can be concluded that 37% of the 41 patients with elevated pretreatment serum SCC antigen levels were found to have positive lymph nodes, compared to 10% of the 58 patients with normal pretreatment serum SCC antigen determinations. This finding proved especially relevant in patients with small tumors: none of the 18 patients with a tumor size of less than 3 cm and a serum SCC antigen level of below 2.5 ng/ml had lymph node metastases, *versus* three of the seven comparable patients (43%) with an elevated pretreatment serum SCC antigen level ($P = 0.015$).

Correlation analysis of the 5 parameters showed that a lesion size of ≥3 cm ($P = 0.057$), stromal infiltration of >5 mm ($P = 0.0009$) and vascular invasion by tumor cells ($P = 0.039$) all correlated with the finding of positive lymph nodes. Of the remaining (seven) interrelationships, only lesion size (≥3 cm) correlated with stromal infiltration (≥5 mm) ($P = 0.0001$). The lack of any association between the tumor grade and the other parameters was noteworthy.

Serum SCC Antigen Levels in Relation to the Treatment Results

Serum SCC antigen levels appeared to be a valuable diagnostic tool for evaluating the short-term effect of therapy. *Table IV* shows that the overall sensitivity of the posttreatment serum SCC antigen level was 78.7% (37 of the 47 patients with known tumor lesions showed elevated serum SCC antigen levels). The overall specificity of the posttreatment serum SCC antigen level was 91.3% (158 normal SCC antigen determinations in 175 patients with no

•TABLE IV. Serum SCC antigen levels 3 months after completing therapy in relation to the clinical diagnosis

		Serum SCC levels >2.5 ng/ml				
*Clinical diagnosis**	*N*	*n*	*(%)*	*Median*	*Maximum*	*SD*
Complete remission	145	8	(5.5)	1.5	4.0	0.6
Recurrent disease	51	14	(27.5)	1.8	43.2	10.2
Partial remission	25	18	(72.0)	3.3	132.3	26.0
Stable disease	7	5	(71.4)	6.1	96.1	34.3
Progression	15	14	(93.3)	14.0	156.0	47.9

* Complete remission: data on all patients referred since 1984;
recurrent or persistent disease: data on all patients referred since 1978

evidence of disease; patients referred since 1984) and was 94.5% in patients who were still disease-free at the closing date of the study. Patients who showed elevation of the serum SCC antigen level after therapy, in the absence of identifiable tumor lesions, were at greater risk: nine of the 17 patients (53%) developed a recurrence, compared to 21 of the 158 patients (13%) with a normal serum SCC antigen determination after treatment ($P < 0.0005$; patients referred since 1984).

Sensitivity and Specificity of the Serum SCC Antigen Level during Follow-up

Progressive disease, ultimately resulting in death, was noted in 121 of the 143 patients who had persistent ($N = 72$) or recurrent ($N = 71$) disease during follow-up. Nineteen patients were alive with tumor at the closing date. Only three patients (4%) had no evidence of disease two to nine years after adjunctive treatment for recurrent disease. Two of these three patients experienced a tumor relapse in the vaginal apex, one patient showed multiple metastases in the lungs but is still in complete remission eight years after adjunctive chemotherapy.

The basic characteristics of the patients with recurrent disease are listed in Table V. The median follow-up time before the clinical detection of recurrent disease was 79 weeks (18 months). Recurrence within two years of the primary diagnosis of cervical cancer was observed in 50 patients (70.4%). In eight patients (11.3%) the tumor recurred more than five years after the initial diagnosis. The serum SCC antigen level before or at the time of the clini-

•TABLE V. Serum SCC antigen levels according to the site of tumor recurrence

		Interval after primary diagnosis (weeks)		*Serum SCC level (ng/ml)*	
Site of recurrence	*N*	*Median*	*Range*	*Median*	*Range*
Vaginal vault	10				
+ pelvic wall	2	55	(35 - 408)	3.2	(1.1 - 14.9)
+ distant	4				
Pelvic wall	12	71	(34 - 107)	7.8	(2.8 - 11.7)
Thorax	14	99	(53 - 374)	3.2	(1.4 - 11.4)
Liver	3				
+ pelvic wall	4	48	(30 - 210)	3.8	(2.8 - 5.7)
Bones	3				
+ pelvic wall	4	81	(52 - 150)	2.7	(0.9 - 5.6)
Supraclavicular	5	105	(48 - 365)	4.1	(3.3 - 5.0)
Multiple	10	92	(21 - 584)	3.5	(1.2 - 42.5)
Total	71	79	(21 - 584)	3.5	(0.9 - 42.5)

cal diagnosis did not depend on the site of the relapse (Table V) and it was not possible to distinguish between patients with local, local and distant, or only distant recurrence on the basis of the serum SCC antigen level.

Serial serum samples were available in 55 of the 71 patients with a tumor relapse, allowing a reliable follow-up study. In 47 of the 55 patients, elevated serum SCC antigen levels preceded (*N = 35*) or coincided with (*N = 12*) the clinical detection of tumor recurrence, providing a sensitivity of 85.5%. In three patients, the serum SCC antigen level began to increase during tumor progression, four to eight weeks after the clinical diagnosis of recurrent disease. In the total group of 50 patients who showed elevated serum SCC antigen levels, the median lead time of the SCC antigen increase was 14 weeks. In the group of patients who showed elevated serum SCC antigen levels prior to the detection of recurrence, the median lead time was 25.5 weeks (*N = 35*;

range 6 - 173 weeks). Five patients with recurrent disease had normal serum SCC antigen determinations during follow-up. It is important to note that these five patients included the three patients who were without evidence of disease after adjunctive treatment for the recurrence.

Serum SCC antigen levels were measured on 1399 occasions during the follow-up of 303 patients with a duration of complete remission of at least six months following therapy. Forty patients (13%) showed elevation of the serum SCC antigen level on one or more occasions. In three patients, psoriasis was recognized as the cause of persistent elevation of the serum SCC antigen level. In the 37 other patients, an incidental elevation of the serum SCC antigen level was measured on 64 occasions (4.6%). In this group, 23 patients only had a single elevation of the serum SCC antigen level which could not be confirmed during later follow-up. Thus, 14 of the 303 patients (4.6%) in complete remission showed multiple serum SCC antigen levels which exceeded the cutoff value of 2.5 ng/ml, providing a specificity of the SCC antigen assay of 95.4%. One of these 14 patients had been subjected to multiple vulvar biopsies one week before the measurement of an elevated serum SCC antigen level which decreased to normal when the biopsy lesions had healed. Two patients showed symptoms and signs of lymphedema in one or both legs in association with varicose veins. One patient was known to have an ovarian cyst in the right ovary which was left *in situ* at primary sur-

•TABLE VI. Course of serum SCC antigen and clinical outcome during follow-up of 72 patients in complete remission and an abnormal serum SCC antigen value

		Serum SCC level during follow-up (ng/ml):					
		First observation		*Next sample*			
Ultimate Diagnosis	*N*	*Median**	*Range**	*No. Elevated*		*Median*	*Range*
Recurrence	35°	3.6	2.6 - 42.5	28	(80%)	6.7	1.6 - 43.2
Complete remission	37Δ	3.1	2.6 - 6.6	9	(24%)	1.8	0.7 - 3.3

* Median and range of elevated serum SCC antigen levels

° Increase in serum SCC antigen prior to the clinical diagnosis of recurrence

Δ Three patients with psoriasis excluded

gery. The cyst had been present for four years. In the seven other patients, the possible cause of the increased serum SCC antigen levels remained obscure. Apart from the patients with psoriasis, only the patient with an ovarian cyst showed a once-off elevation of the serum SCC antigen level in excess of 4.5 ng/ml (6.6 ng/ml).

Although these elevations were only slightly above the cutoff level of 2.5 ng/ml, they represented an important interfering factor for the early detection of tumor activity. Table VI shows that the first abnormal serum SCC antigen level determined during the follow-up of patients with recurrent disease, did not differ significantly from those which were determined in the 37 patients who were in complete remission. The predictive value of a single elevation of the serum SCC antigen level for the early detection of recurrent disease was therefore only 48.6% (35 of 72 patients).

Table VI nevertheless shows that the serum marker trends in both groups were quite different. In the next serum sample, patients who showed recurrent disease exhibited a significantly higher incidence of serum SCC antigen elevations (80% *versus* 24%) in addition to considerably higher absolute serum SCC antigen values than the patients without evidence of disease. The predictive value of two consecutive elevations of the serum SCC antigen level for recurrent tumor was 75.7% (28 of 37 patients).

Serum SCC Antigen Levels during Tumor Progression

A variety of serum SCC antigen trends could be noted in patients with progressive disease. The course of the serum SCC antigen level in the group of patients who experienced tumor recurrence was particularly interesting: the median serum SCC antigen value during follow-up increased from 3.6 ng/ml (first abnormal value) to 6.7 ng/ml (next observation) (Table VI) to 8.3 ng/ml (range 0.8 - 136 ng/ml) at the time of the clinical detection of recurrence. During clinical progression of the tumor, the median serum SCC antigen level rose to 14.8 ng/ml (range 0.6 - 305 ng/ml). The overall sensitivity was 93% (98 of the 105 calculable patients).

Three of the seven patients who displayed normal serum SCC antigen levels before treatment and during tumor progression had undifferentiated carcinoma, one patient had poorly differentiated carcinoma and the remaining three patients had moderately differentiated carcinoma.

Pretreatment Serum SCC Antigen Levels and Patient Outcome

Elevation of the pretreatment serum SCC antigen level was associated with a poor prognosis. Using 2.5 ng/ml as the cutoff level and regardless of the

stage, 150 of the 172 calculable patients (87.2%) with normal pretreatment serum SCC antigen levels were in complete remission at the closing date, compared to 117 of the 224 patients (52.2%) who showed elevated pretreatment serum SCC antigen levels.

In a stage by stage analysis, no difference was found in the incidence of elevated pretreatment serum SCC antigen levels or absolute serum SCC antigen values in the patients who experienced recurrent or persistent disease: the overall incidence of elevated serum SCC antigen levels was 83% (106 of 128 patients) ranging from 79% in Stage IB to 90% in Stage IV; the overall median serum SCC antigen level prior to treatment was 7.6 ng/ml (range 0.8 - 131 ng/ml). In Stage IB alone, the five-year actuarial survival for patients with normal pretreatment serum SCC antigen levels was 95.8%, *versus* 70.0% when elevated pretreatment serum SCC antigen levels had been determined ($P < 0.0001$).

An analysis of the pretreatment data on the 153 patients referred since 1984 using Cox's regression model, controlling for age, stage, lesion size, grade and SCC antigen value, showed that the stage and the pretreatment serum SCC antigen value were the only factors which had a significant effect on survival, although older patients also fared slightly better (Table VII). Figure 2 shows that the survival rate decreases when a higher pretreatment serum SCC antigen value was determined.

•TABLE VII. Pretreatment prognostic variables of 153 patients referred since 1984 in Cox's regression model

Variable	*Coefficient*	*SE**	*Coeff/SE*	*Improvement* X^2	*P*
Stage	1.51	0.34	4.47	38.03	< 0.00005
LnSCC°	0.42	0.13	3.28	10.40	0.0013
Age	-0.02	0.009	-1.84	3.47	0.06

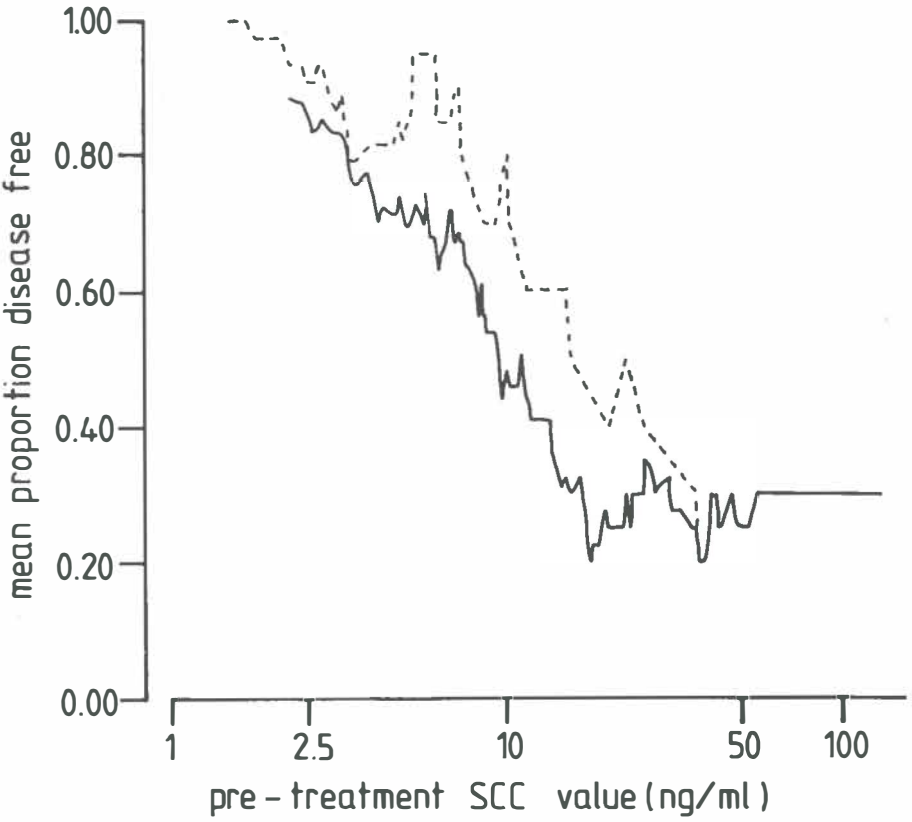
Global $X^2 = 61.04$; degrees of freedom = 3; $P < 0.00005$

* Standard error

° SCC antigen value after logarithmic transformation

Comment

Our results confirm the good correlation between serum SCC antigen levels and the extent of the disease.[5-11,19,20] The incidence of elevated serum SCC



•FIG. 2. Survival of patients with cervical squamous cell carcinoma in relation to the SCC antigen concentration in serum before treatment.

antigen levels ranged from 37% in Stage IB to 90% in Stage IV. Apart from tumor mass, also the grade of the tumor[9] and lesion size[10] have been reported to correlate with the serum SCC antigen level. Our univariate analysis showed that the depth of tumor infiltration into the cervical stroma and the node status had an important effect on the serum SCC antigen level as well. In fact, the multivariate analysis demonstrated that, in addition to higher stage, the only factors which significantly contributed to the increase in the serum SCC antigen level were deep infiltration of the tumor and positive lymph nodes. The latter was shown to be especially relevant in patients with tumors of less than 3 cm diameter. At our hospital, these patients are considered to be optimal candidates for primary surgery. The data indicate that particularly in such patients, pretreatment serum SCC antigen can help to distinguish between patients with and without a high risk for lymph node metastases.

Two other findings seem to have provided more insight into the pathophysiology of the SCC antigen. First, it was found that the larger tumors had infiltrated more deeply into the cervical stroma. Second, the presence of larger tumors, deep stromal infiltration and vascular invasion all carried an increased risk for lymph node metastases. The strong interaction between tumor extent, histopathologic findings, and the serum SCC antigen concentration provides supportive evidence that a major determinant contributing to the increase in the serum antigen concentration is formed by "leaky barriers", such as loose basement membranes between the site of antigen production and the peripheral circulation, rather than the local amount of antigen production. This hypothesis is supported by the findings of Crombach et al[9] who found significantly higher SCC antigen concentrations in cytosol preparations of normal squamous epithelia of the exocervix than in those obtained from squamous cell carcinoma, whereas serum levels in healthy females were below 2.5 ng/ml. The hypothesis is also in agreement with observations regarding the tumor-associated antigen CA 125: in noninvasive conditions, serum levels remain within the normal range despite sometimes extraordinarily high local antigen levels in tissues or body fluids.[21-24] Within this concept, it is feasible that serum concentrations of tumor-associated antigens do indeed reliably reflect the invasive (and ultimately metastatic) course of a tumor mass.[5,9,21,25,26]

The present study on a large number of patients with complete follow-up data, confirms that serum SCC antigen determinations are a valuable tool for monitoring the treatment results and clinical course of carcinoma of the uterine cervix.[5-13,19,20,27] After completing therapy, the sensitivity of the serum SCC antigen level was 79% and the specificity was 91%. The sensitivity was 85.5% in patients who experienced recurrent disease, with a median lead time of 25.5 weeks in patients where the increase in the serum SCC antigen level preceded the clinical detection of the recurrence. Nevertheless, the predictive value of a single elevation of the serum SCC antigen level during follow-up was only 49%. A proportion of the patients who showed false-positive serum SCC antigen elevations were found to have skin-related lesions, (an observation which has been documented previously[16]).

Our data support the suggestion made by Maiman et al[10] that sequential serum determinations might serve to increase the sensitivity and specificity of tumor markers. The predictive value for the early detection of tumor recurrence increased to 76% when two consecutive elevated serum SCC antigen levels were measured. In addition, the serum SCC antigen value measured in the second sample was considerably higher in patients who showed tumor relapse than in those who had no evidence of disease. After the exclu-

sion of the patients with psoriasis and the one with an ovarian cyst, all the patients with serum SCC antigen levels in excess of 4.5 ng/ml were found to have recurrent or persistent disease.

Five patients with a recurrence showed normal serum SCC antigen levels during follow-up. It is interesting that the only three patients with a relapse who have survived were among these five patients. Although it was not possible to distinguish between patients with local or distant recurrence on the basis of the serum SCC antigen value, this observation seems to be in agreement with the finding that the serum SCC antigen level reflects tumor burden. In endometrial and cervical adenocarcinoma, similar observations have been reported with regard to serum CA 125 and local or distant tumor recurrence.[12,25]

During tumor progression, the overall positivity of serum SCC antigen levels rose to 93%. The majority of the seven patients with normal serum SCC antigen levels had poorly differentiated or undifferentiated carcinoma. In fact, the noticeably low serum SCC antigen values in these patients suggest that these tumors did not express the antigen, which is concurrent with the immunohistochemical distribution of the antigen.[5,9,14]

Not surprisingly, the stage of the disease had a great impact on survival. This study shows that the absolute pretreatment serum SCC antigen value provides important additional prognostic information, which may be particularly useful in clinically early stage disease where occult tumor spread confronts the physician with a discouraging situation. It should be acknowledged that recurrent disease is in fact the ultimate clinical manifestation of the growth of metastasized tumor cells which were already present on admission and which routine diagnostic procedures have failed to detect during primary staging. The use of a set of clinical, biochemical, and histopathologic prognostic factors might serve to select patients who are at risk for recurrence. In advanced cervical cancer, there is some evidence that the combined use of radiotherapy and chemotherapy may increase the response rate.[28] Whether the application of combined local and systemic treatment in high-risk patients with early stage disease may help to improve the survival rate, merits further investigation.

Conclusion

For the majority of patients with cervical cancer, serial serum SCC antigen analysis represents a most sensitive, specific and cost-effective diagnostic tool

during treatment and follow-up and inflicts virtually no physical harm on the patient involved.[29]

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CHAPTER IX

General discussion

Introduction

There is a great need for sensitive, specific, and cost-effective methods for diagnosing cancer and evaluating the treatment of cancer patients. For the clinician, research into such methods should specify the indications for conducting the test, the sensitivity and specificity, and the way in which the test results could influence patient management. Moreover, the test should not be harmful to the patient.

Clinically, the most important characteristic of a tumor marker is that the test result should provide information on the course of the disease up to the moment of analysis and possibly thereafter. This data will generally have diagnostic characteristics. In the most optimal circumstances, a positive result will automatically mean that the patient has a certain type of cancer. Such a test would make it possible to screen certain target populations for the presence of malignant cells. Although the hormonal markers αFP and hCG come close to the concept of the ideal marker, such tests are not available. This thesis demonstrates that this is also valid for modern tumor marker assay systems in various fields, including gynecologic oncology in particular. Generally, such tests would only be carried out on the grounds of complaints or other symptoms which were directly indicative or suspicious of malignant disease. The (desired) information obtained in this way would therefore have a different character. The test result would help to confirm a provisional diagnosis; it would provide important prognostic data and assist in treatment monitoring or the early detection of recurrent disease.

Although low 'overall-specificity' is a disadvantage, it should be realized that, given a certain symptomatology, the test can nevertheless be useful provided there is high test sensitivity in the target population. Moreover,

research into substances which are produced by cells which have undergone malignant transformation, will increase our insight into the biologic behavior of tumors. The events which are associated with the process of tumor infiltration and metastasis will ultimately define the sensitivity of a tumor marker assay. The observations regarding the presence of CA 125 or SCC antigen in tissue and body fluids support the view that the production and release of these substances represent phenotypic manifestations of normal and deviated cell growth and differentiation. These observations contribute to the views regarding the specificity of such tests.

The clinical application of CA 125 and SCC antigen in epithelial cancer of the corpus or cervix uteri

I. Interpretation of pretreatment serum marker levels.

In epithelial ovarian cancer patients the incidence of serum CA 125 elevations is approximately 90%, regardless of stage or histology.[1] Compared to this figure the rather low overall-incidence of elevated pretreatment serum CA 125 levels in endometrial or endocervical adenocarcinoma patients (25% and 52%, respectively) seems somewhat discouraging. This difference in incidence may be explained by the fact that, in contrast to patients with uterine cancer, most patients with ovarian carcinoma present with Stage III or IV tumor. Although information on CA 125 in Stage I epithelial ovarian cancer is still scarce, the sensitivity of serum CA 125 in this stage is not likely to exceed 50% anyway.[1]

The close correlation between the serum concentration of CA 125 prior to treatment and the clinical stage of the disease in patients with uterine adenocarcinoma led to a more detailed study on the significance of increased serum CA 125 concentrations in early stage disease. First, it was found that both in clinically early stage endometrial and cervical adenocarcinoma elevated pretreatment serum CA 125 levels correlate with prognostically unfavorable clinicopathologic parameters. Clinically, the correlation between elevated serum CA 125 and (overt) extracorporeal or extrauterine tumor spread is of special interest. In (FIGO) Stage IB cervical adenocarcinoma all patients with lymph nodal spread except for one were among the patients who showed an increased serum CA 125 level at presentation. In (FIGO) Stage I endometrial carcinoma the predictive value of an elevated serum CA 125 level for the detection of metastasized tumor cells was rather low, however (27%). In a report by Soper et al,[2] the sensitivity for the detection of extrauterine disease

in patients with (FIGO) Stage I disease was 65% with a specificity of 88%. The positive predictive value was 54% and the negative predictive value 92%. Data from a prospective study[3] carried out at Stony Brook University, New York, showed higher sensitivity and specificity rates (90% and 98%, respectively). In papillary serous carcinoma, a highly malignant form of endometrial adenocarcinoma, nine of 10 patients with elevated serum CA 125 levels had extrauterine disease.[4] In addition, in an immunohistochemical study on the expression of CA 125 in endometrial carcinoma, Berchuck et al[4] found that tumors with a high degree of CA 125 expression were associated with a higher metastatic potential than tumors with low CA 125 expression.[5]

Although pretreatment serum CA 125 cannot be used to distinguish patients with metastasized disease in (FIGO) Stage II cervical adenocarcinoma (because of the high incidence of 76% elevated levels at this stage), 80% of patients with elevated pretreatment serum CA 125 in (FIGO) Stage II endometrial adenocarcinoma were understaged versus 20% of patients with normal pretreatment determinations. In addition, all patients who were downstaged after surgery had normal serum CA 125 levels. Whether the combined measurement of CA 125 in serum and peritoneal fluid enhances the sensitivity of detecting extrauterine tumor spread merits further investigation.[6,7]

Both in endometrial and in endocervical adenocarcinoma, pretreatment serum CA 125 has been found to provide additional prognostic information. In this respect, it is not only worthwile to acknowledge the association between elevation of serum CA 125 and poor patient outcome but it is also equally important to note the good prognosis for patients who present with normal serum CA 125 levels. Most recurrences were found among patients who showed an increased serum CA 125 concentration at presentation. In (FIGO) Stage I or II endometrial cancer the recurrence rate was 29% for patients with elevated pretreatment serum CA 125 levels versus 7% when a normal serum CA 125 had been determined. The corresponding numbers for (FIGO) Stage IB cervical adenocarcinoma patients were 42% and 4%, respectively.

In patients with squamous cell carcinoma of the cervix there was a good correlation between pretreatment serum SCC antigen and the clinical stage of the disease at presentation. Apart from the stage multivariate analysis revealed that the depth of stromal infiltration and the lymph node status had a significant impact on the pretreatment serum SCC level as well. As a con-

sequence, pretreatment serum SCC antigen values correlated well with patient survival. In a Cox regression model, controlling for five pretreatment variables (age, stage, lesion size, grade, and pretreatment serum SCC antigen value), only the clinical stage and the pretreatment serum SCC value provided prognostic significance. It is worthwile to note that, in contrast to the findings on CA 125 in uterine adenocarcinoma patients, the absolute pretreatment serum SCC antigen value provided additional information: higher levels were associated with considerably poorer patient survival.

II. Monitoring the course of the disease.

The results presented in Chapters 3 and 4 show that the serum CA 125 assay forms a sensitive and specific means of following the course of the disease in patients with uterine adenocarcinoma. In endometrial adenocarcinoma, recent reports from other institutions[4,8-12] have confirmed most of the observations made in Chapter 3. An increase of the serum CA 125 concentration is indicative for the occurence of renewed tumor activity. Although in our study the overall sensitivity in the 12 patients who experienced a relapse was only 42%, it is important to note that an increase of serum CA 125 before the clinical detection of the recurrence was observed in all patients who showed intraabdominal tumor recurrence. In agreement with this observation, Scharl et al[9] and Patsner et al[12] found a sensitivity of 92% and 100% in patients with distant metastases, respectively. In both studies, mainly normal serum CA 125 levels were noted in the presence of a vaginal recurrence. During the follow-up of patients treated for endometrial cancer the specificity was 95% in patients who had elevated serum CA 125 levels before treatment (Chapter 3). In all other patients in complete remission only once a slight elevation of the serum CA 125 level was found. The studies cited above[4,9-12] confirm this observation; these authors reported a specificity rate of between 98.2% and 100%, respectively.

For the monitoring of patients with cervical adenocarcinoma, CEA is the only marker which has been found to have clinical value.[13-15] The present investigation shows that CA 125 meets most of the criteria of a good marker in the staging and follow-up of patients with cervical adenocarcinoma. Most of the findings regarding the significance of serum CA 125 determinations in patients with cervical adenocarcinoma were comparable to the observations in patients with endometrial adenocarcinoma. There was good correlation between serum CA 125 and the stage of the disease according to FIGO

criteria, ranging from 34% increased CA 125 serum concentrations in Stage IB to 100% in Stage IV, whereas pretreatment serum CEA did not correlate with the clinical extent of the tumor. In addition, all patients (100%) who experienced recurrent disease at distant tumor sites showed an increase of serum CA 125 before or at the time of the clinical detection of the recurrence compared to 67% for CEA.* Two other findings were comparable to the observations in endometrial cancer: the sensitivity of the CA 125 assay for the detection of a vaginal recurrence was relatively low (57%) but the overall specificity of the test was high (97.5%). Nevertheless, all three markers investigated (CA 125, SCC antigen, and CEA) deserve a place during the follow-up of patients with cervical adenocarcinoma. Elevation of one or more serum marker levels following treatment was found in 74% of the patients who ultimately died of the disease. Although we noted that rising serum SCC antigen or CEA levels can precede or coincide with the increase of serum CA 125 in a few patients with pure adenocarcinoma or during squamous dedifferentiation of the adenocarcinomatous tumor,[16] serum CEA or SCC antigen determinations are especially useful in patients who present with an adenosquamous tumor. So far, data from prospective studies on the use of serum CA 125 assay in patients with cervical adenocarcinoma remain anecdotal, but they nevertheless seem to indicate high sensitivity and specificity rates.[9,11,17]

Chapter 8 shows that serial serum SCC antigen determinations are of value for the evaluation of treatment results in patients with squamous cell carcinoma of the cervix. After treatment the sensitivity of the assay was 79% and the specificity 91%. The risk of recurrence in patients who were clinically free from disease but who had an abnormal serum SCC antigen determination was 53% versus 13% when a normal serum SCC antigen level was found. During follow-up, increasing serum SCC antigen levels preceded or coincided with tumor relapse in 86% of the patients, whereas 96% of the patients in complete remission showed normal antigen levels. The median lead time before the recurrence was clinically detected was 25.5 weeks. These

* In Chapter 3 we have defined a level of 10 U/ml as a treshold value for CA 125, using an enzyme immunoassay. This value is based on the 95th percentile in 199 patients who underwent hysterectomy or radiotherapy for uterine cancer and were in complete remission for at least 12 months. No difference was found, however, when 16 U/ml was used as a cutoff: the sensitivity of the assay for the detection of tumor recurrence was still 57% in patients with central recurrence and 100% in patients with tumor sites located in the abdominal cavity.

observations are consistent with the recent findings of Holloway et al.[18] The positive predictive value of a single elevation of the serum SCC concentration during follow-up was only 49%, however. This figure rose to 76% when two consecutive elevations had been determined. These calculations were carried out on a heterogeneous group of patients who only shared one common denominator, i.e. the clinical absence of tumor cell activity. It should be emphasized, that in the case of an individual patient, a test result must be interpreted within the context of the patient's history and clinical performance status. The preliminary results from a prospective study presently underway at the University Hospital of Groningen, indicate that the informative value of a serum marker determination may be greatly augmented by carefully interviewing and examining the patient to exclude any lesions which have already been recognized as a cause of false positive test results. This study, in combination with the results presented in Chapters 7 and 8, show that the diseases which are most frequently found to interfere with the specificity of the serum SCC antigen assay include those affecting the skin or mucous membranes (such as severe lymphedema with varicous venes or tissue repair reactions localized, e.g. in the vaginal vault after radical hysterectomy) and abnormal liver or renal function tests (confirming the previous observations of Fischbach et al[19]). In addition, high specificity can be achieved by using a slightly higher treshold value of 4.5 ng/ml at the cost of a relatively minor decrease in sensitivity.

The results from this thesis raise some critical questions with regard to the management of patients who, regardless of the clinical stage, have been recognized to have a substantial risk to die of their disease. The crucial issue is whether it is legitimate to alter the treatment modalities for such patients. The present study illustrates that the earliest possible recognition of tumour recurrence is in fact synonymous with the accurate detection of metastases during the primary staging procedure. If undetected and/or untreated, such metastases will grow relentlessly until they manifest themselves clinically, as 'recurrent disease', during follow-up. These ultimate 'metastases of metastases' are characterized by increased genetic lability and intratumoral heterogeneity (Chapter 2). The subpopulations within these tumours differ greatly in therapeutic response and may readily become resistant to any treatment mode. This partly explains in part the poor treatment results in patients with recurrent cervical or endometrial cancer. It is doubtful whether the early detection of these recurrences (e.g. by using tumor marker assays) will have a significant positive effect on survival or on the quality of life (preliminary data). In patients with colorectal cancer, the early detection of recurrent dis-

ease by means of serial serum CEA determinations did not result in an improvement of survival.[20] On the other hand and despite the sometimes severe toxic side-effects, approximately 70% of the patients consider life-extension by palliative chemotherapy worth-wile.[21]

Nevertheless, there is little or no evidence that the application of systemic regimens has any beneficial effect on the prognosis or quality of life of patients with high stage or recurrent endometrial or cervical carcinoma. However, studies are now underway on the concomitant administration of chemotherapy and radiation in cervical cancer patients. Preliminary data suggest encouraging high initial response rates indicating that cytotoxic drugs, at least in combination with radiotherapy, do have an effect on the cells which populate these tumors[22,23] (own observations). So far, prospective randomized studies on the application of combined systemic and local treatment in patients with early stage endometrial or cervical cancer who are at high risk for recurrent disease have not been conducted. Selection of such patients should use clinical, biochemical and histopathologic parameters. For the next decade, it is the challenge to set up large cooperative studies to investigate the effect of combination therapy in these selected patients.

A possible route of antigen transfer into the circulation

Neither CA 125 or SCC antigen is tumor specific. As is valid for all known tumor-associated substances these antigens are products of normal, benign or malignant cells. A number of nonmalignant tissues (including normal tissue) express antigen activity and elevated serum concentrations of these antigens can be observed both in healthy individuals or in patients with a benign disease. For instance, CA 125 is present in the normal epithelial lining of the female genital tract.[24] In healthy women these epithelial cells are very likely the main source of the basic serum CA 125 level. Clearly, intact barriers exist between these tissues and the peripheral bloodstream, because the median CA 125 concentration in normal cervical and uterine fluids is 65,000 U/ml in the presence of low blood antigen levels.[24,25] For SCC antigen the same observation is probably true. At the Department of Pathology of the University Hospital of Groningen, The Netherlands, antigen activity has been demonstrated in a variety of normal tissues, e.g. bladder mucosa or liver cells. Urine of both healthy males and females contain SCC antigen concentrations up to 250 ng/ml (unpublished data).

In order to correctly interpret the results from a serum marker assay, it is mandatory to answer the question of why increased serum marker concentra-

tions are only observed under certain conditions. Studying factors which (may) contribute to the increase of serum antigen concentrations, both in healthy individuals and in patients afflicted by benign or malignant disease, may help to elucidate the pathophysiology of tumor markers and provide more insight into the biologic behavior of malignant tumors.

This study has revealed an intricate interaction between serum antigen levels and tumor-related characteristics (Chapters 3, 5 and 8). With respect to endometrial carcinoma, recent studies support this observation.[2-5,9-11] The results suggest that the infiltrative and ultimately metastatic process is a key determinant for the increase in both serum CA 125 and SCC antigen levels in epithelial uterine cancer. It must be emphasized, however, that these data should be interpreted with caution, because there is no general agreement between the results from the different studies. For instance, although we found a close correlation between elevation of serum CA 125 and vascular invasion in endometrial cancer, we were not able to confirm this finding in patients with endocervical cancer. Furthermore, despite the good correlation between elevated serum SCC antigen levels and vascular invasion in patients with cervical adenocarcinoma, this correlation was absent in patients with squamous cell cancer of the cervix. In the latter case, stromal infiltration was shown to significantly influence the pretreatment serum SCC antigen level, whereas there was a lack of correlation between pretreatment serum CA 125 and the depth of myometrial infiltration in patients with endometrial cancer. Moreover, multivariate analysis was not always appropriate or showed that other - unknown - factors had not been included in the analysis. The observations on CA 125 in patients with undifferentiated endometrial carcinoma (Chapter 3) and SCC antigen in patients with dedifferentiated cervical squamous cell carcinoma (Chapter 8) give some evidence that a loss of antigen expression in low grade tumors may play a limited but significant role.

The results nevertheless support the hypothesis that tumor-associated antigens may gain access to the circulation after the tumor has eroded natural barriers between the local site of antigen expression and the peripheral circulation. Tumor infiltration into surrounding tissues after destruction of basement membranes represents an early step in the metastatic cascade.[26,27] In addition, basement membranes within the tumor have been shown to be absent or poorly formed.[27-29] Further - but implicit - arguments in favor of the hypothesis can also be derived from observations in healthy individuals or patients affected by benign disease. For instance, significantly higher serum CA 125 levels have been reported in healthy females during menstruation[30] and a sharp rise in serum CA 125 levels has been noted shortly after delivery.[31] In addition, elevated serum CA 125 levels have been determined

in serum from patients with endometritis or cervicitis.[24] Although the content of both benign and malignant ovarian cysts contain large amounts of antigen, a significant elevation of the CA 125 concentration is predominantly found in patients with infiltrative epithelial ovarian cancer.[32] With regard to SCC antigen, this study has shown that skin lesions, particularly those with an inflammatory component, can be associated with an increase of the serum antigen concentration. As is also the case with malignant disease these conditions are associated with impairment of tissue barriers or increased vascular permeability.

With respect to the serum CA 125 concentration the drainage of the abdominal or pleural cavity may play an important role as well. CA 125 is produced by proliferative coelomic-derived cells, such as reactive mesothelial cells or the epithelium of ovarian cysts.[32-35] High antigen concentrations have been measured in the fluids produced by these tissues.[6,7,32,36-38] The study on serum CA 125 levels in patients with pelvic inflammatory disease (Chapter 6) provides evidence that antigen production and release by reactive mesothelial cells can give rise to a significant increase of the serum CA 125 concentration. Levels up to 2600 U/ml were measured in the study. Paavonen et al[39] reported similar observations. The presumption has recently been supported by the study results of van der Zee et al[40] who found that 82% of patients who were subjected to abdominal surgery show an increase of the serum CA 125 level, irrespective of sex, primary diagnosis, or type of operation. In addition, elevated serum CA 125 levels can frequently be observed in patients who have a tumor which is immunohistochemically negative for CA 125, such as uterine sarcoma.[41,42] Elevations were predominantly found in patients with wide spread disease. It may also explain why elevated levels can occasionally be observed during pelvic radiotherapy or in patients with radiation injury[12] (own observations).

Conclusions

This investigation has disclosed a good correlation between the serum CA 125 concentration and the clinical or surgical stage of the disease both in endometrial and in cervical adenocarcinoma (Chapters 3, 4, and 5). In clinically early stage disease (FIGO) the results show that the measurement of serum CA 125 levels before the start of the therapy might be an additional tool for distinguishing between patients with or without metastatic disease. Pretreatment elevations were associated with poor patient survival. In addition, the course of the CA 125 serum trends were consistent with the treat-

ment results and the serum CA 125 levels were shown to reflect the disease process during follow-up. Rising serum CA 125 levels in patients in complete remission were an early indicator of tumor recurrence.

Serum SCC antigen determinations provide important clinical information with regard to the staging, monitoring of the treatment results and the follow-up of patients with cervical cancer whose tumors contain squamous cell components. Pretreatment serum SCC antigen values in patients with cervical squamous cell carcinoma correlate with the patient outcome (Chapter 8). Moreover, in clinically early stage disease they may help to recognize patients at risk for metastazised disease and tumor recurrence.

The studies on the interaction between serum marker concentrations and tumor-related characteristics in patients with epithelial uterine cancer (Chapters 3, 5, and 8) in addition to the studies on CA 125 in patients with pelvic inflammatory disease (Chapter 6) and on SCC antigen in patients with a benign skin disease (Chapter 7), have yielded a concept which may help to explain how and under what circumstances tumor markers may gain excess to the peripheral circulation.

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SAMENVATTING

Tumorcellen produceren een grote verscheidenheid aan stoffen (tumor-geassocieerde antigenen), waartegen antilichamen kunnen worden opgewekt. Met behulp van deze antilichamen kan de aanwezigheid van dergelijke stoffen in tumorcellen en lichaamsvloeistoffen met zeer gevoelige immunologische technieken worden aangetoond. In de laatste vijfentwintig jaar bestaat er een toenemende belangstelling voor de klinische toepassingsmogelijkheden van deze bepalingstechnieken. Deze belangstelling is o.a. gebaseerd op de waarneming dat tumorcelactiviteit kan leiden tot een abnormale (in casu, verhoogde) serum-concentratie van deze stoffen. Door het meten van deze serum-concentratie kan aanvullende informatie worden verkregen bij het stadiëringsonderzoek en ontstaat de mogelijkheid om op een voor de patiënt weinig belastende wijze de resultaten van de behandeling te evalueren. Klinisch staat bij het meten van de serum-concentratie van tumor-geassocieerde antigenen de vroege opsporing van tumorrecidief centraal. Deze bepalingen zijn vooral nuttig in een situatie waarin aanvullende behandelingsmodaliteiten beschikbaar zijn. Voorwaarde hiervoor is, dat de test een hoge sensitiviteit heeft. Tumor-geassocieerde antigenen komen echter niet alleen tot expressie in maligne getransformeerde cellen, maar ook in cellen zonder infiltratieve of metastatische potentie. Dit is een belangrijke limiterende factor bij de klinische toepassing van deze bepalingsmethoden. Naast een hoge sensitiviteit is immers ook de specificiteit bepalend voor de klinische bruikbaarheid van de test.

In de inleiding van dit proefschrift (Hoofdstuk 1) wordt ingegaan op een aantal algemene aspecten van het biologisch gedrag van maligne tumoren die mede bepalend zijn voor de prognose van de patiënt. De klinische mogelijkheden van de toepassing van tumormerkstoffen en de voorwaarden waaraan de bepalingsmethode dient te voldoen, worden aangestipt. De doelstellingen van het onderzoek zijn: (1) het verkrijgen van inzicht in de toepassingsmogelijkheden van de tumormerkstoffen "Cancer Antigen (CA) 125" en "Squamous Cell Carcinoma (SCC) antigen" voor de stadiëring, behandeling en follow-up van patiënten met endometrium- of cervixcarcinoom en (2) onderzoek naar factoren, die de serum-concentraties van deze tumormerkstoffen beinvloeden, ten einde een beter inzicht te krijgen in de pathofysiologie van deze stoffen en het biologisch gedrag van endometrium- en cervixcarcinoom.

Veranderingen in het genoom van de cel liggen ten grondslag aande geleidelijke transformatie van een normale cel in een cel met infiltratieve en uiteindelijk metastatische eigenschappen. Hoofdstuk 2 geeft een korte uiteenzetting van de genetische en phenotypische veranderingen, die zich tijdens dit proces kunnen voordoen. Inzicht in deze gebeurtenissen maakt het mogelijk om de waarnemingen die betrekking hebben op de expressie van tumormerkstoffen in (a) het weefsel en serum van oncologische patiënten (sensitiviteit) en in (b) cellen en lichaamsvloeistoffen van gezonde individuen of patiënten met een niet-oncologische aandoening (specificiteit), beter te begrijpen.

Daarna wordt een literatuuroverzicht gegeven met betrekking tot de tumormerkstoffen "Cancer Antigen (CA) 125" en "Squamous Cell Carcinoma (SCC) antigen".

In Hoofdstuk 3 wordt de klinische toepasbaarheid van serum CA 125 bepalingen beschreven in een retrospectieve studie van 121 patiënten met een carcinoom van het endometrium. Deze patiënten werden tussen 1978 en 1985 behandeld in het Academisch Ziekenhuis Groningen. Bij immunohistochemisch onderzoek bleek CA 125 zowel in normaal endometrium als in carcinomen van het endometrium tot expressie te komen. Terwijl het antigeen in normaal endometrium gelocaliseerd was aan de luminale zijde van de celmembraan, waren in epitheliale endometrium tumoren zowel CA 125 positieve als negatieve klierstructuren aantoonbaar. Solide structuren waren geheel negatief. Voor deze studie werd gebruik gemaakt van een immunoradiometrische bepalingsmethode.

Voor de start van de behandeling had 25% van de 110 patiënten een verhoogde concentratie van het serum CA 125 (> 35 U/ml). Er bestond een samenhang met de klinische- en chirurgische uitbreiding van het proces. In de Stadia I en II (FIGO) werd bij patiënten met een verhoogde serum CA 125 waarde significant vaker tumorcellen in vaten en tumorweefsel buiten het corpus uteri aangetroffen. In (FIGO) Stadium I had 27% van de patiënten met een abnormale serum CA 125 uitslag tumorgroei buiten het corpus uteri. Patiënten met een klinisch Stadium II en een verhoogd CA 125 hadden in 80% van de gevallen extrauteriene metastasen. Voor patiënten met normale bepalingen waren deze getallen respectievelijk 6% en 20%.

Door analyse van follow-up sera kon worden aangetoond, dat de serumconcentratie van het CA 125 parallel liep met het ziekteproces. De sensitiviteit van de bepaling in patiënten met tumorrecidief was 42%. Stijgende serum CA 125 spiegels werden gemiddeld 1,8 maand voor de klinische herkenning van het recidief waargenomen. Bij alle patiënten met een abdomi-

naal gelocaliseerd recidief en bij alle 15 patiënten met tumorprogressie werd een stijgende CA 125 concentratie waargenomen. Bij de analyse van de sera van patiënten in complete remissie werd in slechts één monster een licht afwijkende serum CA 125 concentratie gemeten. Patiënten met een Stadium I of II carcinoom en een abnormale serum CA 125 concentratie voorafgaand aan de behandeling hadden een slechtere prognose: 29% van deze patiënten overleden in vergelijking tot 7% van de patiënten met een normale CA 125 uitslag.

In Hoofdstuk 4 worden de resultaten beschreven van een retrospectief onderzoek naar de betekenis van serum CA 125, SCC antigeen en Carcino-embryonic Antigen (CEA) bepalingen in een groep van 77 patiënten met een cervicaal adenocarcinoom met of zonder squameuze componenten. Deze patiënten werden tussen 1978 en 1987 in het Academisch Ziekenhuis Groningen behandeld. Van de drie onderzochte tumormerkstoffen correleerde alleen de serum-concentratie van CA 125 significant met het tumorstadium. Voor de evaluatie van de behandelingsresultaten bleken echter alle drie de markers betekenis te hebben: indien de serum-concentratie van één of meer merkstoffen na therapie verhoogd was, had 74% van de patiënten tumorresidu of kreeg een recidief tijdens follow-up. Indien de markers na behandeling normaal waren, was dit in 15% van de patiënten het geval.

De serum-concentratie van de drie merkstoffen correspondeerde met het klinisch beloop van het ziekteproces. Bij 43 patiënten in complete remissie was de specificiteit van de bepaling voor zowel CEA als voor CA 125 97,7%. De specificiteit van de SCC antigeen assay was 95,4%. Twee patiënten die bij de afsluiting van deze studie in complete remissie waren, maar een stijgende serum CA 125 en SCC antigeen concentratie vertoonden, kregen later tumorrecidief (Hoofdstuk 5). Alle patiënten met tumorprogressie hadden een verhoogde serum CA 125 concentratie, terwijl abnormale serum SCC antigeen of abnormale serum CEA uitslagen werden aangetroffen bij 56% van deze patiënten. Vroege opsporing van tumorrecidief is mogelijk door de longitudinale bepaling van alle drie merkstoffen tijdens follow-up. Verhoging van minstens een van de drie merkstoffen werd aangetroffen bij 86% van de patiënten met een recidief in de vagina blindzak. Bij alle patiënten met een abdominaal gelocaliseerd recidief werd een abnormale serum CA 125 concentratie gemeten. Het tijdsinterval tussen de eerste waarneming van een stijgende serum marker concentratie en het moment waarop het recidief zich klinisch openbaarde, lag tussen 5,5 en 8,5 maanden, met een spreiding van 0 tot 26 maanden.

Met betrekking tot de serum-concentratie van de drie merkstoffen beston-

den er verschillen tussen patiënten met zuivere adenocarcinomen en patiënten met een adenosquameuze tumor. Vóór behandeling had een significant groter aantal patiënten met een adenosquameus carcinoom een verhoogde serum CA 125 concentratie. Voor SCC antigeen bestond er eveneens een verschil, hoewel dit verschil net niet significant was op 5% niveau. Dit patroon werd ook gedurende tumorprogressie waargenomen: alle patiënten met een progressief adenosquameus carcinoom hadden verhoogde serum-concentraties van de drie merkstoffen. Bij patiënten met een adenocarcinoom zonder squameuze partijen werden vooral abnormale serum CA 125 concentraties gemeten. Bovendien waren ook de absolute waarden van de serum-concentraties beduidend hoger bij patiënten met een adenosquameuze tumor. Dit gold met name óók voor de hoogte van de serum CA 125 concentraties.

Dezelfde studiepopulatie wordt in Hoofdstuk 5 beschreven. In dit hoofdstuk staat het onderzoek naar de prognostische betekenis van de pretherapeutische serum CA 125, SCC antigeen en CEA concentraties in relatie tot histopathologische tumorparameters centraal. De mediane follow-up periode voor patiënten in complete remissie was 47,5 maanden. De actuariële 5-jaars overleving voor patiënten met een (FIGO) Stadium IB cervicaal adenocarcinoom en een verhoogde serum CA 125 concentratie was 52,4% vergeleken met 95,6%, indien de serum CA 125 concentratie normaal was. De slechtere prognose voor patiënten met een hoger klinisch stadium (Stadia II - IV [FIGO]) kwam tot uiting in een hoge incidentie van verhoogde serum CA 125 concentraties (72%). Hoewel CEA bepalingen (verhoogd versus niet verhoogd) niet gerelateerd waren aan de prognose, werd wel vastgesteld, dat een hogere absolute waarde van de serum CEA concentratie gepaard ging met een lager overlevingspercentage. Serum SCC antigeen bepalingen hadden geen prognostische betekenis.

Daarna werden in de Stadia IB en II (FIGO) de onderlinge verbanden tussen de serum marker concentraties, het histologische tumortype en het al of niet aanwezig zijn van vaatinvasie of lymfkliermetastasen in relatie tot de prognose geanalyseerd. In een univariate analyse bleek dat patiënten met vaatinvasie of positieve klieren een significant slechtere actuariële 5-jaars overleving hadden. Deze waarneming bleek onafhankelijk te zijn van het tumortype of het klinisch stadium van de tumor. Wel werd in de groep patiënten met een Stadium IB of II tumor (FIGO) vaatinvasie door tumorcellen vaker aangetroffen in adenosquameuze tumoren. Met name in Stadium II was de prognose voor patiënten met een adenosquameuze tumor dan ook slechter dan voor patiënten met een adenocarcinoom. Patiënten met een adenocarcinoom met vaatinvasie hadden echter ook een minder goede prognose

dan patiënten met een adenocarcinoom zonder vaatinvasie. Alhoewel er geen correlatie tussen vaatinvasie en lymfkliermetastasen werd gevonden, bleken patiënten met vaatinvasie maar met negatieve klieren vaker een bekkenwandrecidief te krijgen dan patiënten zonder vaatinvasie.

In (FIGO) Stadium IB had 42% van de patiënten met een verhoogde serum CA 125 concentratie positieve klieren in vergelijking tot 4% van de patiënten met een normale serum CA 125 uitslag. De gecombineerde verhoging van de serum CA 125 en de serum SCC concentratie hing in sterke mate samen met de aanwezigheid van vaatinvasie door tumorcellen in het histologische preparaat. Zoals al gememoreerd werd in Hoofdstuk 4, hadden patiënten met een adenosquameuze tumor vaker serum CA 125 of serum SCC verhogingen dan patiënten met een adenocarcinoom. In de Stadia IB en IIA (FIGO) was het door het combineren van klinische- en histologische parameters mogelijk alle patiënten met een recidief in een 'high-risk' categorie onder te brengen. In deze groep was de incidentie van verhoogde serum CA 125 waarden voorafgaand aan de behandeling 64% vergeleken met een incidentie van 18% in de 'low-risk' categorie.

In 1987 werd in twee artikelen melding gemaakt van verhoogde serum CA 125 concentraties bij patiënten met het klinisch beeld van "pelvic inflammatory disease" (PID). Reactieve mesotheliale cellen tonen een sterke kleuringsreactie na incubatie met het monoclonale antilichaam OC 125. Deze cellen vinden, evenals de epitheliale bekleding van de vrouwelijke tractus genitalis, hun oorsprong in het embryonale coeloomepitheel. Hoofdstuk 6 beschrijft een studie die werd verricht bij 50 patiënten die op basis van 4 criteria voldeden aan de waarschijnlijkheidsdiagnose PID. Bij alle patiënten werd een diagnostische laparoscopie verricht. De intraabdominale bevindingen, zoals die bij deze ingreep werden aangetroffen, werden geclassificeerd op basis van de betrokkenheid van het peritoneum in het onstekingsproces. Voorafgaand aan de ingreep en gedurende de opname werd bloed bij de patiënten afgenomen. De CA 125 concentratie in het serum werd bepaald en gerelateerd aan de waarnemingen bij de laparoscopie. De hoogte van de serum CA 125 concentratie bleek sterk samen te hangen met de mate waarin het peritoneum in het ziekteproces was betrokken. Benadrukt wordt dat in deze studiepopulatie de betrouwbaarheid van de waarschijnlijkheidsdiagnose PID toenam van 82% naar 97%, indien de serum CA 125 concentratie bij opname als criterium werd toegevoegd.

Tijdens de opname werden serum CA 125 concentraties tot 2600 U/ml gemeten. Deze resultaten ondersteunen de veronderstelling dat geprikkelde mesotheelcellen een belangrijke bron van CA 125 produktie kunnen zijn.

Tijdens de follow-up van een patiënte in complete remissie na behandeling wegens een planocellulair cervixcarcinoom, werd een sterk verhoogde serum SCC antigeen concentratie gemeten. Deze patiënte leed in ernstige mate aan psoriasis vulgaris. Naar aanleiding van deze waarneming werd besloten tot een nadere analyse van de serum SCC antigeen concentraties van 66 patiënten met een goedaardige aandoening van de huid. Hoofdstuk 7 beschrijft de resultaten van dit onderzoek. Een significante toename van de serum SCC antigeen concentratie werd aangetroffen bij 83% van de patiënten met psoriasis en 80% van de patiënten met een eczemateuze huidaandoening. Serum SCC antigeen concentraties tot 73.2 ng/ml werden gemeten. Ook 7 patiënten uit een heterogene groep patiënten met andere huidaandoeningen vertoonden significante stijgingen. Evenals bij de patiënten met psoriasis en eczeem stond bij deze patiënten een inflammatoire reactie op de voorgrond. Immunohistochemisch onderzoek van huidbiopten die bij twee patiënten met psoriasis vulgaris waren afgenomen, toonde een sterk positieve reactie na incubatie met het tegen SCC antigeen gerichte monoclonale antilichaam F_2H_7. Geconcludeerd wordt dat SCC antigeen niet specifiek is voor maligne planocellulaire aandoeningen en dat huidaandoeningen de specificiteit van de serum SCC antigeen assay kunnen beinvloeden. Argumenten worden aangevoerd voor de veronderstelling dat het SCC antigeen een antigene determinant is van een of meerdere cytokeratines.

Hoofdstuk 8 beschrijft de resultaten van een onderzoek naar de sensitiviteit en specificiteit van serum SCC antigeen bepalingen bij 451 patiënten, die tussen 1978 en 1989 in het Academisch Ziekenhuis Groningen werden behandeld wegens een planocellulair cervixcarcinoom. De mediane follow-up periode van de patiënten in complete remissie was 62 maanden. De incidentie van verhoogde serum SCC antigeen concentraties correleerde significant met het klinisch stadium en nam toe van 37% in Stadium IB tot 90% in Stadium IV (FIGO). Hoewel in een univariate analyse dedifferentiatie van de tumor, grotere tumorlaesies, diepe infiltratie in het onderliggend stroma en de aanwezigheid van positieve klieren alle positief correleerden met verhoogde serum SCC antigeen concentraties, droegen in een multivariate analyse alleen de laatste twee parameters en het klinisch stadium van de tumor bij tot een stijging van de serum SCC concentratie. Deze bevindingen bleken vooral nuttig voor de selectie van 'high-risk' patiënten met een (FIGO) Stadium IB tumor, die kleiner is dan 3 cm: in deze subpopulatie werden alle patiënten met positieve klieren aangetroffen in de groep met serum SCC antigeen waarden hoger dan 2,5 ng/ml (3 van de 7 patiënten, 43%). Alle 18 patiënten in de groep met normale serum SCC antigeen waarden hadden negatieve klieren.

Na behandeling was de serum SCC concentratie bij 79% van de patiënten met bekend tumorresidu verhoogd (sensitiviteit). De specificiteit was 91%. Patienten die na behandeling in complete remissie waren, maar waarbij een abnormale serum SCC concentratie werd gemeten, hadden een verhoogd risico om tijdens follow-up een recidief te ontwikkelen. Van deze patienten kreeg 53% een recidief. Het risico was 13%, indien een normale serum SCC concentratie werd gemeten.

Gedurende tumorprogressie werd bij 98 van de 105 patiënten (93%) een verhoogde serum SCC concentratie gemeten. De specificiteit van de bepaling tijdens de follow-up van 303 patiënten in complete remissie was 95.4% (indien twee of meer bepalingen als 'fout-positieve uitslag' werden gedefinieerd en patiënten met psoriasis werden uitgesloten). Huidlaesies werden herkend als de belangrijkste oorzaak voor deze 'fout-positieve uitslagen'. Voor de vroege herkenning van recidief tumorgroei vormen deze aandoeningen een belangrijke beperkende factor: de voorspellende waarde van een enkele verhoging was slechts 49%. De voorspellende waarde van twee opeenvolgende abnormale bepalingen was 76%, waarbij uitslagen hoger dan 4,5 ng/ml alleen werden waargenomen bij patiënten die een recidief ontwikkelden. Het tijdsinterval, gemeten vanaf de eerste abnormale bepaling tot het moment waarop het recidief klinisch manifest werd, was 25,5 week (berekend in de groep waarin het optreden van recidief voorafgegaan werd door een stijging van de serum SCC antigeen concentratie).

De actuariële 5-jaars overleving van patiënten met een abnormale serum SCC concentratie hoger dan 2,5 ng/ml was 52%. Voor patiënten met een normale uitslag was dit 87%, ongeacht het stadium. In een Cox regressie analyse met vijf - pretherapeutisch bekende - parameters (leeftijd, serum SCC waarde, stadium, tumorgrootte en differentiatiegraad) werd de prognose alleen door het stadium en de serum SCC waarde beinvloed. Hogere absolute waarden van de serum SCC concentratie bleken, ongeacht het stadium, gepaard te gaan met een daling van de levensverwachting.

In Hoofdstuk 9 worden de resultaten van de verschillende studies samengevat en getoetst aan de resultaten van andere studies, die inmiddels op dit gebied zijn gepubliceerd. Geconcludeerd wordt dat, beschreven in termen van sensitiviteit en specificiteit, CA 125 een geschikte tumormerkstof is voor de evaluatie van de behandelingsresultaten bij patiënten met een adenocarcinoom uitgaande van het klierepitheel van het endometrium of de endocervix. Voorts wordt geconcludeerd dat de serum SCC antigeen bepaling een gevoeligeen specifieke methode is ter evaluatie van patiënten met een planocellulair cervixcarcinoom. Bepaling van de serum-concentraties van deze antige-

nen voor de aanvang van de therapie kan een additioneel hulpmiddel zijn bij de herkenning van de patiënt met een verhoogd risico op tumormetastasen en een slechte prognose. Tevens wordt uitgelegd waarom de tumormerkstoffen SCC antigeen en CEA een plaats verdienen in de follow-up van patiënten met een cervicaal adenocarcinoom.

Gesignaleerd wordt, dat de resultaten van het onderzoek naar factoren die de serum-concentraties van de onderzochte tumormerkstoffen beinvloeden, niet geheel coherent zijn. Toch lijken er argumenten aan ontleend te kunnen worden, die ervoor pleiten dat een verstoring in de weefselbarrieres kan leiden tot het optreden van verhoogde concentraties van deze tumor-geassocieerde antigenen in de perifere circulatie. Voor oncologische patiënten zou dit zowel de samenhang van de serum-concentraties van deze stoffen met prognostisch ongunstige histopathologische parameters als ook de prognostische betekenis van de onderzochte merkstoffen kunnen verklaren. Hoewel andere factoren, zoals toename van locale productie waarschijnlijk een belangrijke bijdrage leveren, zou door deze veronderstelling mede kunnen worden verklaard waarom 'vals-positieve uitslagen' (abnormale uitslagen bij niet-oncologische patiënten) juist in geval van ontsteking en weefseldestructie werden waargenomen.

Serum marker bepalingen maken de vroege opsporing van tumorrecidief bij patiënten die zijn behandeld wegens een carcinoom van het corpus- of de cervix uteri mogelijk. Twijfels worden geuit, of deze vroege opsporing de prognose bij de huidige stand van zaken - het ontbreken van adekwate therapie - gunstig kan beinvloeden. Dit zou misschien het geval kunnen zijn voor bepaalde subgroepen, waarbij aanvullende therapie mogelijk is. Benadrukt wordt, dat de vroegst mogelijke opsporing van recidief tumorgroei in het algemeen samenvalt met de detectie van metastatisch tumorweefsel tijdens de primaire stagering. Eerder ingrijpen in het proces van tumorprogressie zou op theoretische gronden een verbetering van de overlevingscijfers tot gevolg kunnen hebben. Daarom wordt in Hoofdstuk 9 gepleit voor het selecteren en behandelen van 'high-risk' patiënten die zich klinisch in een vroeg stadium van het ziekteproces bevinden. Bij een dergelijke selectie zou gebruik gemaakt kunnen worden van klinische, biochemische en histologische parameters die geassocieerd zijn met een verhoogd overlijdensrisico. In prospectieve, gerandomiseerde studies zou het effect van locale therapie in combinatie met systeembehandeling op de overleving van deze 'high-risk' patiënten onderzocht moeten worden.

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