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Variations in the Level of the Pregnancy-associated α_2-Glycoprotein in Patients with Gynaecologic Cancer

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Abstract—*α_2-PAG is a high-mol. wt glycoprotein. It occurs in trace amounts in normal human serum. The variations in serum levels of this protein were studied during treatment of 32 gynaecological cancer patients in the course of 2 yr. The periodic α_2-PAG determinations were done with an electroimmunoassay. A correspondence between α_2-PAG serum levels and the clinical course could be found in 60%. In 5 of 13 patients who did not respond to therapy α_2-PAG levels rose, and 4 had unchanged high levels, whereas 13 of 17 patients had significantly decreased levels on successful treatment. The relevance of periodic α_2-PAG determinations is discussed.*

INTRODUCTION

WHEN speaking of pregnancy-associated α_2-glycoprotein we think of carbohydrate-containing α_2-glycoprotein (α_2-PAG) having a mol. wt of about 360,000. The molecule is composed of two subunits each of a mol. wt of 180,000 being linked by sulfur bridges. The isoelectric point of α_2-globulin is at about 4.7. This protein was first described in the sera of female patients with pregnancy toxicosis [1].

Although it was at first thought to be specific for this condition [2, 3], it was subsequently detected in the sera of all pregnant women [4]. At present, α_2-PAG is considered as trace protein in human sera. Until agreement on a name for this protein [5] it appeared in the literature under a variety of terms: serum factor Xh [6], pregnancy associated (SP3) [7], pregnancy zone protein [8], pregnancy associated globulin [9] and α_2-pregnoglobulin [10].

This protein gained growing interest after detection of increased concentration levels in malignant solid tumours [11–13] and in 1975, Stimson observed a correlation between α_2-PAG values and metastasis of breast carcinoma. A recent study on breast cancer with 30 patients lead to the result that continous determination of α_2-PAG is superior to other parameters like CEA for detecting mammary micrometastases [14]. We confirmed this [15] and we found a relationship between α_2-PAG concentration and the actual stage of the disease in bronchial carcinoma [16], in melanoma [17] and in laryngeal cancer (Bauer, in preparation). In the present study we have the relationship between α_2-PAG concentration and gynaecological tumours.

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Dedicated to the 60th birthday of Prof. Dr. E. Schmiedt, Direktor of the Urologische Universitäts-Klinik in Klinikum Grosshadern, 8 München 70.

MATERIALS AND METHODS

Our examinations were performed with a group of 32 female patients having different gynaecological carcinomae. Table 1 shows the various diagnoses as well as the methods of

Table 1. Summary of the individual diagnoses and the methods of treatment of 32 gynaecological malignant growths

Diagnosis	No. of cases	Treatment: Surgery	Treatment: radiation	Treatment: Cytostatica
Cervix carcinoma	8	3	8	
Corpus carcinoma	12	12	8	
Cancer of the ovary	7	6		6
Vaginal carcinoma	1		1	
Cancer of the vulva	1	1		
Choriocarcinoma	3	1	1	3
Total	32			

The number of cases as often several methods of treatment were applied.

treatment. In all cases the diagnoses were histologically secured. Serum samples for the determination of the α_2-PAG contents were drawn before treatment, in the course of treatment and also later during after-care. The after-care included regular examinations at 3-month intervals and always included an anamnesis, a detailed gynaecological local finding and a cytologic microscopic preparation, a routine control of weight, ESR and total number of leucocytes. For cases with clinically suspicious signs the diagnosis was extended to radiological examinations, sonography, cystocopy, rectoscopy and punctures for drawing cytologic material.

After withdrawal the serum samples were coded and stored at $-20°$ C till the end of the study. For determination of the α_2-PAG content we used a modified electroimmunoassay and performed it without knowledge of the clinical dates [18].

We used an agarose solution of 1% (w/v), pH 8.6, with an antiserum content of 1% (v/v). (OP 4501—the anti-α_2-PAG from a rabbit was a preparation by Behringwerke AG, Marburg.) The applied quantity of antigen (serum of a patient) was 5 μl. For a period of 16 hr electrophoresis was executed at a voltage of 2V/cm. The detection of α_2-PAg was limited to 0.4 mg% by this method (see Fig. 1).

The reproduction of the employed method was checked by repeated analyses of coded reference sera. The rate of deviation in intra-assay reproduction was 3% and in interassay the rate of deviation was between 5 and 10%. The latter is permissable in electroimmunoassay. It is of no importance for our examinations as we are concerned with the interpretation of long period examinations of each individual patient.

RESULTS

Before treatment of 32 female patients the α_2-PAG concentrations ranged from non-detectable, i.e., below 0.4 mg%, to 14 mg% with a mean value of 6.25 mg% and a median value of 4.5 mg%. A relationship of the pretherapeutical values to age, pregnancies or histological type of the neoplastic changes could not be proved. But there was a tendency towards lower α_2-PAG values in early stages and higher α_2-PAG in more progressed stages. The female patients with cervix carcinoma [8] and corpus carcinoma [12] tended to lower α_2-PAG values than the patients with cancer of the ovary [7]. But then the observation of the course of development of α_2-PAG concentration in these patients was of greater diagnostical or prognostical value. A summary of the results is shown in Table 2. In 13 out of 17 female patients,

Table 2. Summary of the correlation of the α_2-PAG serum levels with the clinical course

Correlation positive*	18 (13+5)
False-negative results	1 (+4 unchanged high values +3 in the final stage with decreasing values)
False-positive	4
α_2-PAG not detectable	1

*As a positive correlation we chose the decrease of the level in successful treatment as well as the increase or invariability of the level in unsuccessful treatment.

with until now successful treatment (surgical intervention or surgical intervation and cytostasis) showing no signs of further progression of the disease, the α_2-PAG concentration fell in the first months of treatment and then remained at a significantly low level compared with the initial values (example see Fig. 2). Thirteen patients, however, who showed a progression in the course of disease during the observation period showed five times rising values (see as example Fig. 3) and four times constant high values compared with the pretherapeutic concentration values. The α_2-PAG level of the other four female patients fell continuously in spite of progression in the disease and exitus. At the beginning of the supervision period three female patients were already in an advanced stage of the disease. Although repeated α_2-PAG tests were performed no provable concentration of this substance could be detected in the serum of a female patient who died of tumour/cachexy. An influence by tele-cobalt radiation and radium deposits could not be proved absolutely without being able to blame other factors for this. Five female patients showed increased α_2-PAG levels at the beginning of tele-cobalt radiation (example: see Fig. 4). In contrast to this the radiation showed no changes in the α_2-PAG levels of eight female patients. Cystostatic administration, i.e., as applied in the combination of 5-fluorouracil and endoxan in carcinoma of the ovary, did not have any direct effect as the α_2-PAG values remained unchanged before and after the cytostatic application. In all the 32 female carcinoma patients who were reviewed, the results represent a correlation of 60% between the course of the disease and the α_2-PAG concentration detected in the serum. The

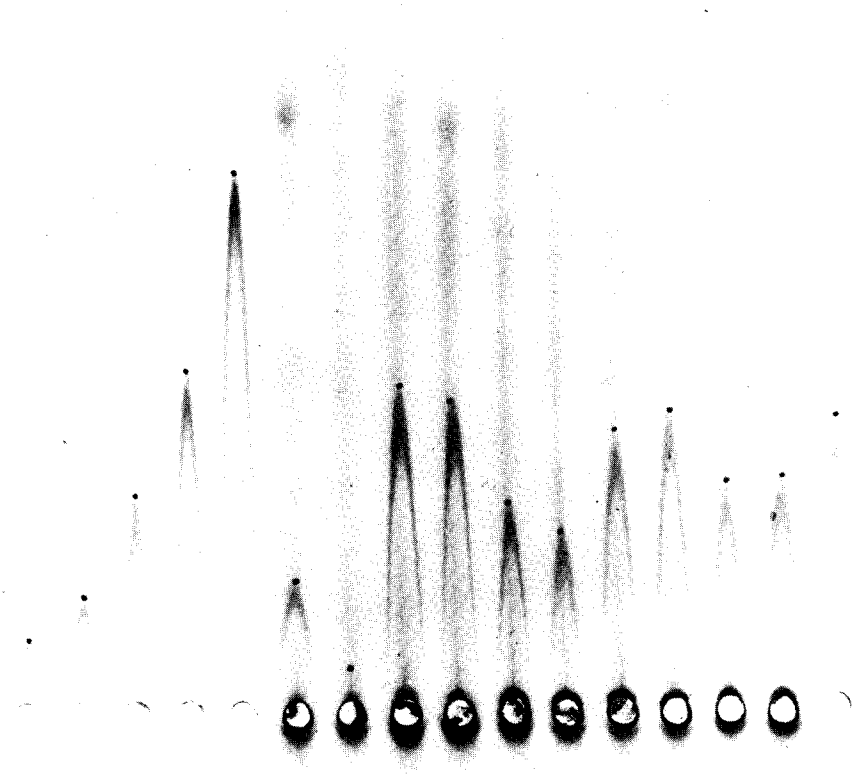

Fig. 1. Plate of an electroimmunoassay for the determination of α_2-PAG. The gel contains 1% (v/v) rabbit anti-α_2-PAG (OP 4501, Behringwerke, Marburg).

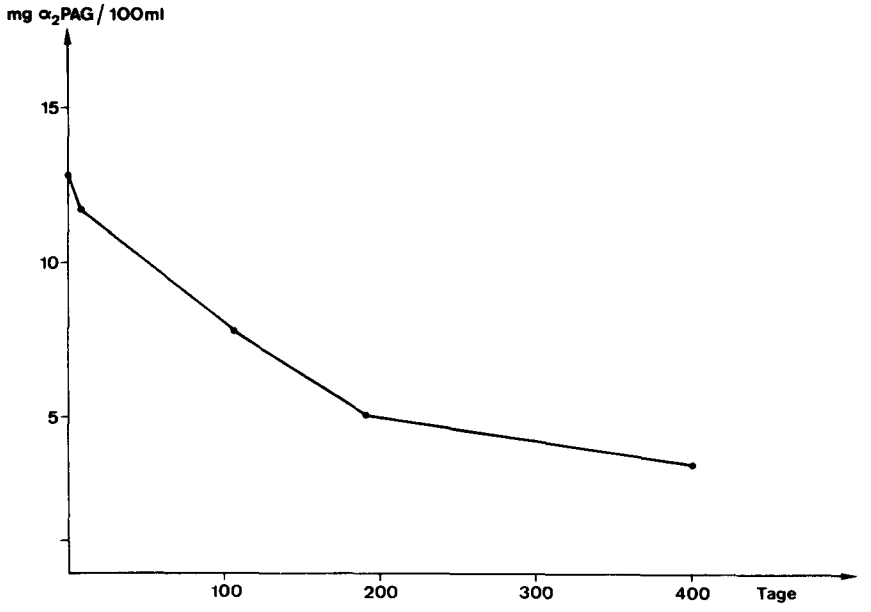

Fig. 2. Female patient E. R., age: 68 yr. *Diagnosis: corpus carcinoma. Histological finding: highly differentiated adenocarcinoma. Therapy: abdominal hysterectomy including adnexa. Without any further treatment the female patient is without relapse.*

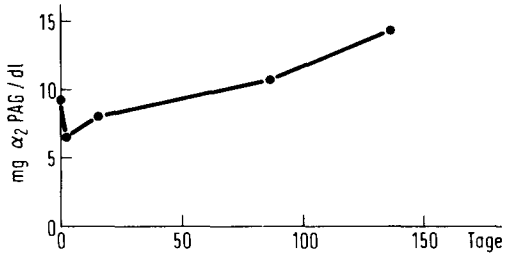

Fig. 3. Female patient A. G., age: 41 yr. *Diagnosis: collum carcinoma I, intraoperative collum carcinoma III. Histological finding: non-hornifying squamous cell carcinoma. Therapy: surgical intervention after Wertheim-Meigs. Intraoperative a more advanced tumour stage was noticed. The surgical intervention had to be incomplete. Post radiation with tele-cobalt in two series total* 3000 rad *focus dosage. Further progression of the tumour.* Five months *after beginning of therapy exitus. The female patient showed a continous increase of the α_2-PAG. The brief decrease compared with the pretherapeutical value on the* 2 *post operative days is obviously due to the diluting effect of transfusions and infusions.*

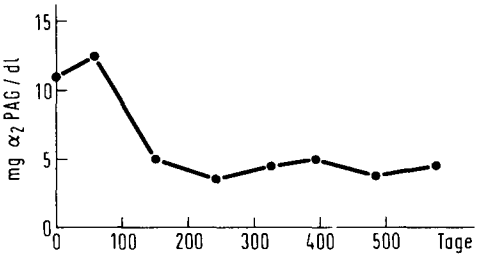

Fig. 4. Female patient M. O., age: 68 yr. *Diagnosis: corpus carcinoma. Histological finding: adenocarcinoma. Therapy: abdominal hysterectomy including adnexa. First continuation of the administration of Gestagen (Depostat)* 200 mg/week. *Tele-cobalt radiation, in two series, total* 5000 rad *focus dosage. Clinically and cytologically the female patient is free of relapses.*

results were distinctly less false-negative [1 (1 +4) of 13] than false-positive (4 of 17) and allowed a more favourable association with adenocarcinoma.

DISCUSSION

Another 2-yr study of 30 patients with bronchial carcinoma revealed in the course of the disease a confirmity between α_2-PAG values and the clinical data. Also here the results were that 2/3 of the patients showed a correlation between α_2-PAG and therapy response. Except for the prefinal days the α_2-PAG values increased continously in proportion to the growth of the tumours [16]. A second bronchial carcinoma study led to the result that simultaneous determination of CEA and α_2-PAG are superior to other parameters and represent for the time being the most efficient control of therapeutical effects in this sort of cancer [19]. In this connection it would be advisable to point out that there could not be a connection with the hormone dependence of different tumours. All bronchial carcinoma patients for example were male patients.

Additionally we determined in parallel the oestrogen fractions (oestron, oestriol, oestradiol) in three patients of this study, and did not find any correlation with α_2-PAG. A recent study on breast cancer with 30 patients led to the result that continous determination of α_2-PAG is superior to other parameters, like CEA, for detecting mammary micrometastases [14].

Very early on, considerable attention was given to the glycoprotein in case of malignant neoplasm [20, 21]. Due to the lack of detailed studies, these positive earlier results and subsequent attempts at interpretation soon disappeared again from the scene [22]. What remained was the question about the part some glycoproteins had as so-called "acute phase reactants". In case of inflammatory disease the concentration of these factors rises suddenly and intensively, but, as yet, little is known about their biological functions. That α_2-PAG is not 'acute phase' protein could be proved by comparing studies of α_2-PAG and two acute phase proteins—the acid glycoprotein and the α-antichymotrypsin in a group of 20 patients over a period of 2 yr (Bauer *et al.*, in preparation).

On the basis of its immunosuppressive properties [23, 24] the α_2-PAG is regarded as the inhibitory factor of the antigen diagnosis of sensitised lymphocytes [25]. The presence of such an element indeed promotes fetal or malignant growth. Thus the α_2-PAG cannot be a carcinoma–embryonal antigen. The site of origin of this protein is still unknown. In analogy to other proteins it would seem possible that the liver is the site of synthesis.

The biological function of α_2-PAG and its absence in tumours cannot be already associated to α_2-PAG increases and neoplastic growth, but allow the consideration of multifactoral dependencies. The results of the submitted 2-yr study of a group of 32 female patients with gynaecological carcinoma have to be interpreted on this background. Then the missing correlation of α_2-PAG and the tumour size, tumour site and histological differentiating is not at all surprising. The greater number of false-positive results compared with false-negative results is more easily understood, except for the final stages [3]. According to its function as a pioneer for fetal and malignant growth α_2-PAG increases inevitably occur before possible tumour recidives. This, however, does not need to be an automatic mechanism. During post-care α_2-PAG increases help to select the risk patients.

The finding of one female patient will remain vague, as she showed no other specific signs. In her case neither in the early stage nor in the final stage α_2-PAG could be detected. The effects of cytostasis on α_2-PAG, which had been described by other authors, could not be observed by us. This will be subject of further investigations. Regular α_2-PAG detection in gynaecological carcinomae during post-care could be, if these results conform with those of other authors, a considerable help in judging the therapeutical results.

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