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Limitations of Using Pregnancy-associated Alpha-2 Glycoprotein as a Tumour Marker

THOMAS MÜLLER*†, MARGARET BOWEN*, JOSE DE MELLO‡, NICOLA B. PIDCOCK* and EDWARD H. COOPER*

**Unit for Cancer Research, Medical School, University of Leeds, Leeds, W. Yorkshire LS2 9NL and ‡Department of Surgery, St. James's University Hospital, Leeds, W. Yorkshire LS9 7TF, U.K.*

Abstract—*The development of a new enzyme-linked immunoassay for pregnancy-associated alpha-2 glycoprotein (α_2-PAG) has provided an opportunity to reassess the value of this protein as a tumour marker. Serum samples from 800 healthy individuals and patients with various benign and malignant diseases were assayed. There was a very wide range of α_2-PAG levels in normal females (1–>100 mg/l), and although the levels found in normal males were lower and better defined, this intrinsic variation between individuals makes a single determination in a tumour-bearing patient meaningless. Also, the levels of α_1-PAG in patients with advanced cancer were not significantly different from levels in localized cancer, benign disease or even healthy controls, and furthermore, levels before and after successful cancer treatment did not show a significant change. Our results therefore indicate that α_2-PAG is unsuitable for use as a tumour marker as there was no apparent relationship between the levels of α_2-PAG and either tumour burden or response to treatment.*

INTRODUCTION

PREGNANCY-ASSOCIATED α_2-glycoprotein (α_2-PAG), also known as pregnancy-associated macroglobulin, pregnancy zone protein and SP3, is a plasma protein with a molecular weight of 360,000, first described by MacLaren in 1959 in the serum of pregnant women [1]. Its serum level rises up to twenty times the normal level during pregnancy and returns to normal within 6 weeks after delivery, having a half-life of 5–6 days [2]. High-dose oestrogen treatment also increases its serum concentration [3, 4]. There is no universal agreement about the normal range of levels, no doubt reflecting the standards and method of assay. For instance, the upper limit of normal for non-pregnant women has been reported as being from 60 mg/l [5] to values well over 100 mg/l [6, 7]. The levels are sex-dependent and tend to rise with advancing age [8, 9]. α_2-PAG is thought to have immunosuppressive properties, summarized in a recent review of its clinical and biological significance [10].

Elevated levels of α-PAG have been reported in various non-malignant diseases such as rheumatoid arthritis [11, 12], cirrhosis of the liver, renal diseases [13], recurrent oral ulceration and Behçet's syndrome [14]. α_2-PAG has also been found to be raised in various cancers and its level has been reported by some investigators to correlate well with stage and evolution of the disease [15–18]. Opinion varies about the usefulness of α_2-PAG as a tumour marker. Several workers have reported that its concentration can increase with tumour progression even before metastases become clinically evident, and may decrease on successful treatment [15, 19]. In 1975, cautious optimism was expressed for the use of α_2-PAG as a monitor of cancer progression [20]. Later this was questioned, as no significant difference was observed between serum α_2-PAG levels in patients with disseminated disease compared to those with localized tumours or apparently healthy controls [4, 8, 21]. In view of these doubts about the potential value of α_2-PAG as a tumour marker, an enzyme immunoassay was developed to facilitate a wider scale investigation. This paper reports the observations of α_2-PAG levels in various cancers using this new assay.

MATERIALS AND METHODS

Serum samples from 800 patients, 322

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†Present address: Division of Oncology, Kantonsspital, Rämistrasse 100, 8091 Zürich, Switzerland.

females and 478 males with an age range from 18 years to 82 years, were analysed. They comprised 151 healthy blood donors, 18 patients with benign prostatic hyperplasia (BPH), 41 patients with benign lung diseases (emphysema, tuberculosis, chronic bronchitis, chronic pneumonia, lung abscess) and the following cancers: 41 head and neck (Stages III and IV), 43 prostatic, 24 breast (Stages I–III), 211 lung (Stages I–IV), 78 gastrointestinal, 16 chronic lymphocytic leukaemias, 10 advanced myelomas, 5 Hodgkin's disease, 5 non-Hodgkin lymphomas and 137 post-operative clinically tumour-free breast cancer patients, 3 months to 5 years after treatment for Stage I or II disease.

In a smaller group of patients, pre- and post-treatment samples were obtained: 19 with gastrointestinal tract cancers (the second sample taken 3 months after potentially curative surgery, when there was no evidence of recurrence), 5 lung cancer patients (post-operative sample taken 7–11 days after radical resection), 12 patients with advanced cancers of head and neck, each sampled before and 3–6 months after radiation therapy producing tumour remission, and 19 patients with prostatic cancer from whom samples were taken before and 2–12 months after bilateral orchiectomy.

In normal women a comparison of the levels of α_2-PAG and sex hormone-binding globulin (SP2) was made. The SP2 was measured by radial immunodiffusion [22] using specific antisera and standards provided by Dr. H. Bohn, Behringwerke AG, Marburg/Lahn.

The sera were separated and frozen at −20°C until assayed.

Immunoassay for α_2-PAG

An enzyme-linked immunoassay devised by Behringwerke AG for research purposes was used in this study. The immunoassay is similar to that described for the assay of prostatic acid phosphatase (Enzygnost PAP) [23, 24] and uses the following principle: the sample is incubated together with antibodies to α_2-PAG raised in sheep and covalently attached to the wall of polystyrene tubes. α_2-PAG in the sample is bound to the solid-phase anti-α_2-PAG. Unbound material is removed by washing. Anti-α_2-PAG (from rabbit) conjugated with horse radish peroxidase is incubated with the previously formed solid-phase antibody–antigen complex forming a 'sandwich'. Unbound material is removed by washing. The chromogen (*o*-phenylenediamine), together with the substrate (hydrogen peroxide) in buffer solution, is added. A yellow colour develops if α_2-PAG is present in the sample. The enzyme reaction is stopped by the addition of 0.5 N sulphuric acid.

In addition, many sera were assayed for α_2-PAG by radial immunodiffusion (RID) [22] using a specific antisera and standards supplied by Behringwerke AG, Marburg/Lahn, W. Germany. There was a very good correlation between values obtained using the RID method and those obtained using the new enzyme immunoassay for values of α_2-PAG > 20 mg/l ($r = 0.94$), but below this level the correlation broke down due to the insensitivity of RID when measuring low serum levels.

RESULTS

The results are summarized in Table 1. Since the distributions are not normal, the median values, interquartile ranges and whole ranges are given to help in comparison of the results. Figures 1 and 2 show the distribution of α_2-PAG values in each of the patient groups.

Blood donor controls

The median concentration in 63 healthy males was 2 mg/l, with a range of 1–55 mg/l. The levels reveal a very weak relationship between age (range 18–65 years) and α_2-PAG concentration ($r = 0.14$). The median concentration in females was 26 mg/l, with a range from 1 to > 100 mg/l for the same age group, and there was no relationship between the α_2-PAG levels and age.

Benign diseases

There was a slight difference between healthy males (median 2 mg/l) and male patients with benign lung disease (median 10 mg/l) and benign prostatic hyperplasia (median 4.6 mg/l). This may be partly due to the higher average age of the patients compared with the blood donors. The distribution of levels in females with benign lung diseases did not differ significantly from healthy controls (median 26 mg/l vs 30 mg/l).

Cancer patients

Male patients did not show significantly higher values compared with males with benign diseases. In females the levels were again similar to the control group. The weak correlation between age and α_2-PAG concentration in healthy males ($r = 0.14$) and in male patients with benign diseases ($r = 0.33$) was not present in cancer-bearing males who were about the same age group ($r = 0.017$).

In breast cancer, no difference was found between preoperative samples taken from patients with Stage I–III disease and patients

Table 1. The distribution of α_2-PAG levels in normal controls and various benign and malignant diseases

Disease	No. of patients	Median	Inter-quartile range	Range
Males				
Normal	63	2	<1–11	<1– 55
Ca. head & neck	41	7	2–28	<1–>100
BPH	18	5	3–12	<1– 71
Ca. prostate Mo	28	14	7–21	<1– 47
M+	37	10	5–23	<1– 55
Benign lung disease	31	10	2–26	<1–>100
Ca. lung	168	11	9–20	<1– 77
Ca. G.I. tract	27	8	2–21	<1– 58
Lymphoid malignancies	36	10	4–20	<1–>100
Females				
Normal	88	26	7–65	<1–>100
Ca. breast	24	17	10–30	<1– 76
Benign lung disease	10	30	24–46	10– 80
Ca. lung	43	42	20–54	2–>100
Ca. G.I. tract	23	44	15–61	5–>100

Ca.: cancer.

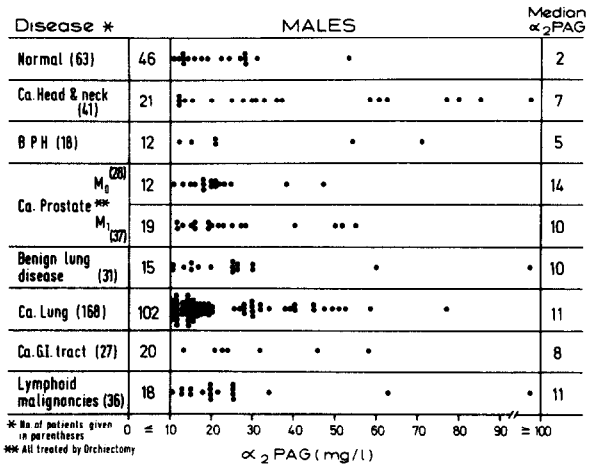

Fig. 1. The distribution of α_2-PAG levels in normal and diseased males.

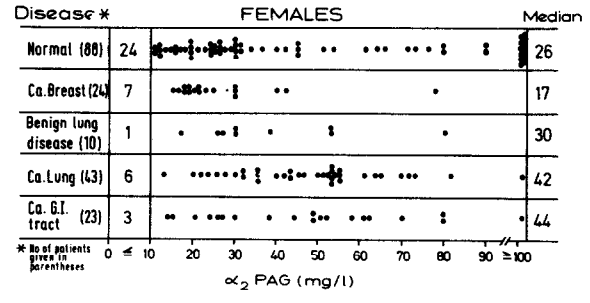

Fig. 2. The distribution of α_2-PAG levels in normal and diseased females.

who had been disease-free for 3 months to 5 years after treatment (median 17 mg/l vs 20 mg/l). Nor was there any difference between localized and advanced lung cancer median values: Stages I and II, 13 mg/l; Stage III, 12.5 mg/l; Stage IV, 18 mg/l. The prostatic cancer patients shown in Fig. 1 had all been treated by bilateral orchiectomy, and again there was no difference between those with metastases, without metastases and benign prostatic hyperplasia. Furthermore, there was no correlation between α_2-PAG levels and disease activity as indicated by prostatic acid phosphatase levels determined by enzyme immunoassay. When carcinoma of the prostate was treated with oestrogens, the patients had, as expected, very high levels of α_2-PAG (177–1532 mg/l) and were not included when the different groups were compared.

Figure 3 summarizes the change in α_2-PAG levels in patients who had had at least two samples tested, one before and one after treatment. The lung and gastrointestinal cancer patients were treated by radical excision and were considered clinically disease-free at the time of the second sample. The patients with head and neck tumours received radiation therapy and their responses were reported to be good to excellent. There was no significant difference between the pre- and post-treatment values. The post-operative samples from lung cancer patients do not support the view that α_2-PAG is an acute-phase reactant protein [25],

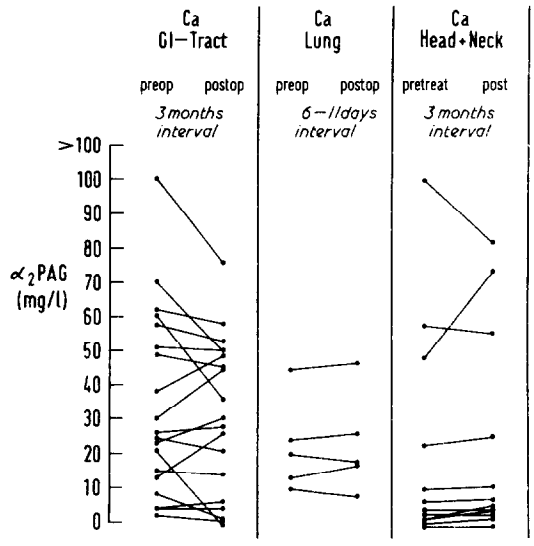

Fig. 3. The change in α_2-PAG levels in three types of cancer following potentially successful treatment.

since the second sample was taken 6–12 days after surgery when other acute phase reactants such as α_1-acid glycoprotein were still elevated. The before- and after-orchiectomy paired samples also showed no significant change in α_2-PAG concentrations.

SP2 is a very sensitive indicator of oestrogen levels, and even the weak oestrogenic stimulus found in tamoxifen treatment for breast cancer causes an increase in the level of this protein [26]. When the levels of α_2-PAG in the normal females were compared to those of SP2 (Fig. 4) there was no correlation, making it improbable that the higher levels of α_2-PAG were the result of an excessive oestrogenic stimulus.

DISCUSSION

Several investigators have reported α_2-PAG to be a useful tumour marker and have stressed its potential importance as a biochemical

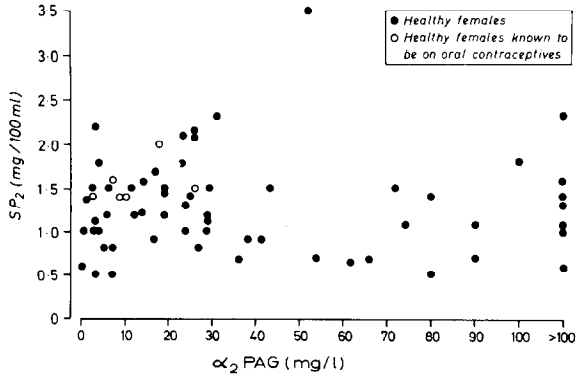

Fig. 4. The relationship between α_2-PAG and sex hormone-binding globulin (SP2) levels in normal females.

aid in monitoring response to cancer therapy. We have not been able to reproduce their promising results. It appears that α_2-PAG is unsuitable as a tumour marker for several reasons.

The normal range is different in males and females [8, 9]. Females show a wide individual variability whose basis is uncertain except that an elevation of α_2-PAG invariably occurs in pregnancy. The levels in healthy women and female patients with various benign diseases do not differ markedly from cancer-bearing women. The stage of the tumour does not seem to influence the level of serum α_2-PAG and after potentially successful treatment no decrease can be observed. The same applies to male patients, with the exception that our control group had lower levels than any of the male patient groups included. This could possibly be due to the large differences in age between the groups as we have already shown a correlation between α_2-PAG and age in the normal and benign groups of males. We were, however, unable to show this age-dependent variation of α_2-PAG levels in women as previously reported [8, 9], possibly due to an insufficient number of samples from disease-free females in the older age group (65–95 years).

The variability of α_2-PAG levels in healthy controls means that comparison of an individual patient level to a mean or median value in a control group has little meaning, and so percentage change was introduced [15, 19]. In this way each patient serves as his own control. This is a good theoretical approach, but examination of the published examples show there can be marked fluctuations in the levels, making it unlikely to be useful in clinical routine; it also seems to be prone to pitfalls, especially in the low ranges which are now easily detectable by enzyme immunoassay, as a small actual change in α_2-PAG would appear as a large percentage change.

α_2-PAG has been shown to have an immunosuppressive effect on T-lymphocytes *in vitro*, but the pathophysiological effects of this action in pregnancy and disease are unknown. It would be very interesting to know whether patients with high α_2-PAG levels carry a different prognosis compared to those with normal levels; we are studying this prospectively in the lung cancer patients. However, in prostatic carcinoma, patients treated with oestrogens have very high levels of α_2-PAG (range 177–1532 mg/l) which can be sustained for many years, and these patients do not show signs of marked immunosuppression. On the

other hand, in the haematological malignancies in the series, which are known to be associated with immunosuppression, the α_2-PAG levels were within the normal limits for elderly males. Further knowledge of the normal function of α_2-PAG is needed before the reason for its elevation in some diseases can be elucidated.

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