The role of tumour markers in ovarian cancer

G. GARGANO (*) - M. CORREALE (**) - I. ABBATE (**) - G. FALCO (*)
N. DE FRENZA (*) - V. LORUSSO (***) - M. DE LENA (***)
A. DE LEONARDIS (*)

Summary: In the present review, the Authors have evaluated the current status of the utilization of the principle tumor markers for ovarian carcinoma in clinical gynecological practice. The major difficulty in individualizing a single marker is represented by the histological differentiation of the tumor itself. In fact, whereas for the malignant germ cell tumors, useful markers (AFP, B-HCG) are already available, for other histological forms, valid markers have been identified only because of the availability of the monoclonal antibody: CA 125. Even if this marker cannot be proposed for mass screening, it represents a useful instrument for the diagnosis and monitoring of ovarian carcinoma. The serum levels are well correlated with the clinical status of the patient and high concentrations of the marker are strongly indicative of disease progression at the second-look.

Numerous other markers such as NB 70K, IAP, PLAP, CA 15-3 and TAG 72, are actually in the clinical evaluation phase, for the most part in association with CA 125.

Key words: Markers, CA 125, Ovarian Cancer.

GENERAL FEATURES

In the last few years biological markers have taken on an ever more important role in the diagnosis, prognosis and especially in the follow-up of ovarian tumours.

Although tumour markers are now used routinely in clinical practice, it may be convenient for the purpose of this paper to mention that markers are defined as qualitatively and quantitatively detectable substances having a causal or probabilistic connection with malignant neoplasms [14].

Indirectly, biological markers give signals indicating the presence and development of a tumour, just as smoke is a signal of fire even if no fire can be seen, but simply because smoke implies fire [65]. Tumour markers are employed in clinical practice to bring out the differences between a normal subject and a patient with a neoplasm, and often to describe some differential characteristics of the same neoplasm as well.

Among the different types of markers (genetic, cytoplasmatic, metabolic and differentiating markers, surface markers), great interest has been focussed on the markers circulating in biological fluids. Such markers can be analyzed in a single sample and could reveal the existence or the behaviour of a neoplasm.

Tumour markers have a somewhat broad definition comprising both a multiplicity of substances produced by cancer cells and a great number of biohumoural variations secondary to the effects of the tumour. Moreover, no marker among all those proposed so far is absolutely speci-

(*) Division of Gynaecological Oncology
(**) Clinical Chemistry Laboratory, RIA Unit
(***) Division of Medical Oncology
Oncology Institute of Bari (Italy)

fic for a given tumour or for a neoplastic condition in general ([10]).

Marker assays yield only a quantitative determination since their presence can often be demonstrated even in normal subjects, although at lower levels. Assessing the efficacy of an assay hinges on the likelihood of categorizing a patient as neoplastic according to a discriminating threshold. Since the levels of markers commonly observed in the early phases of the disease are low (more extensive forms actually produce greater amounts of markers) and there is no perfect correlation between higher marker levels and the course of the disease, at present they cannot be proposed for the early diagnosis of tumours.

This drawback exists even in the case of gynecological neoplasms where the clinical application of markers has become remarkably important, particularly for ovarian tumours. The interest in such tumours can easily be explained considering that they now have the highest death-rate among all female genital tumours ([3]). Furthermore, the treatment of ovarian carcinoma is a great problem nowadays. Most of the cases (70%) are diagnosed at an advanced stage and in spite of the objective response obtained in 60-90% of the cases with the latest chemotherapeutic protocols, only a very low percentage of patients reaches a 5-year survival ([23, 75]).

THE FIRST MARKERS

In order to understand the problem of tumour markers and ovarian tumours, one should bear in mind that these neoplasms are widely heterogeneous and that they differ from one another according to their histogenesis, epidemiology and natural history ([32]).

According to the different histopathological entities, ovarian tumours may be divided into ([76]):

1) epithelial tumours (92% in all), which in turn may be serous (42%), undifferentiated (17%), endometrioid (15%), mucinous (12%) or clear-cell forms (6%);

2) germinal tumours (5%);

3) stromal tumours (3%).

Germinal tumours were the first ovarian tumours to have effective markers. In fact, the use of FP (alpha-fetoprotein) and of HCG (Human Chorionic Gonodotropin subunit) is undoubtedly an extremely effective tool, as with the analogous testicular forms ([29, 43]). Serum assays of these markers have significantly improved the staging of tumour development, therapy monitoring and relapse detection ([70]).

For all the other histopathological types, before the advent of monoclonal antibodies, the markers with the best correlation rates were CEA (Carcinoembryonic Antigen) and TPA (Tissue Polypeptide Angen). These markers had different features according to the different series but displayed a remarkable and constant lack of specificity.

CEA is a typical marker for colorectal carcinoma and was described for the first time in 1965 by Gold and Freedman ([26]). High levels of CEA are present in 30-50% of ovarian carcinoma cases, especially in the advanced and mucinous forms ([72]). This marker and TPA – a tumour antigen common to different types of neoplasms ([13]) – are currently being proposed for use in association with other markers, but with no general agreement on the matter ([8, 45, 60]).

CA 125. After Kohler and Milstein ([40]) defined the hybridoma technique in 1975 for the production of monoclonal antibodies, it became easier to detect new markers in a reproducible way and with no need to use pure antigens, at least in the initial phase. Thus, in 1981, Bast ([6]) identified CA 125 by using the monoclonal antibody OC 125, obtained by immunizing BALB/C mice with a human serous cystoadenocarcinoma cell line (OVCA 433).

CA 125 is surrently the best marker available for epithelial ovarian carcinoma.

The antigenic determinants for CA 125 are associated with mucinlike glycoproteines having a molecular weight of > 200 KD expressed by coelomic epithelium derivatives in the embryo and adult ([50, 58]). Immunofluorescence studies ([33]), using the OC 125 antibody localized the antigen in the epithelium of the Fallopian tubes, endometrium, cervix, mesothelial cells such as pleura, pericardium and peritoneum, Mullerian epithelium, foetal serosa and in amniotic fluid.

CA 125 is present on the cell surface of more than 80% of nonmucinous epithelial ovarian tumours but has not been found in sections of fetal or adult normal ovaries ([34, 57]).

Similarly, Bast ([7]) found serum high levels of CA 125 in about 80% of the patients with non-mucinous epithelial ovarian carcinoma and in only 1% of 888 seemingly healthy subjects by using an immunoradiometric method (IRMA) and setting the cut-off at 35 U/ml. CA 125 positivity was also observed in about 6% of subjects with various benign forms and on average in 30% of patients with other non-ovarian neoplasms.

Several other Authors ([1, 27, 46, 74]) have confirmed these findings and have shown that the CA 125 levels increase in pregnancy ([54]), in endometriosis [48]), acute pancreatitis, peritonitis ([67]) and in liver cirrhosis. In the last group, the increase of CA 125 is associated not with the liver disease but with the presence of ascitic fluid, above all in the cases with infection of the ascitic fluid ([5]).

CA 125 levels are correlated to the stage of ovarian neoplasms with a 50-60% positivity rate in stages I and II already, and to the tumour mass.

Canney *et al.* ([17]) reported elevation of this marker in 63% of patients with a tumor mass <2 cm, 76% in those between 2-10 cm and 100% when the mass was > 10 cm.

Table 1. – *CA 125 serum levels in ovarian cancer and percentage of correlation with clinical course.*

	No.	CA 125 >35 U/ml (%)	Percentage of correlation
Bast (1983)	101	83 (82)	93
Canney (1984)	58	48 (83)	91
Atack (1986)	20	–	88
Kivinen (1986)	166	149 (90) **	
Krebs (1986)	45	43 (96) *	95
Landoni (1986)	145	120 (83)	91
Martoni (1986)	49	43 (88)	93
Alvarez (1987)	109	96 (88)	83
Lavin (1987)	31	29 (94)	81
Vergote (1987)	114	98 (86)	92
Halila (1988)	365	322 (88)	87

(*) = cut-off >25 U/ml;
(**) = cut-off >30 U/ml.

Large discrepances exist between the different Authors ([16, 17, 21, 74]) about the relationship of CA 125 serum levels and histology. Generally mucinous carcinoma ([52]) exhibits the lowest levels of the tumour marker, and in this histological type it would also be possible to use other tumour markers such as CA 19-9 or CEA.

Furthermore, as indicated in Table 1 ([2, 4, 37, 41, 49, 62]), the values are also very well correlated (80-100% of the cases) with the clinical course of the disease, and in various cases, CA 125 was able to predict relapses with a average of 3-5 months before the clinical finding ([11, 16, 55, 79]). Thus, it is a valid tool for the follow-up of ovarian carcinoma and monitoring therapy too ([36, 53, 59, 63]), while it cannot be used for mass screening ([9]).

CA 125 assays have two other important clinical applications: in the preoperative diagnosis of pelvic masses and before a surgical second look.

Einhorn ([21]) reported a 93% positive predictive value for CA 125 in preoperative diagnoses for malignant forms (cut-off: 65 U/ml and a 95% negative pre-

Table 2. – *Predictive value (%) of elevated (>35 U/ml) and normal (<35 U/ml) CA 125 for a positive and negative second look respectively.*

	No. pts.	CA 125 (>35 U/ml)	CA 125 (<35 U/ml)
Atack (1986)	17	100	57
Berek (1986)	55	100	56
Krebs (1986)	13	–	54 *
Niloff (1986)	50	94	88
Alvarez (1987)	26	100	50
Lavin (1987)	29	100	59
Zanaboni (1987)	36	94	62
Mogensen (1988)	81	100	64
Podczaski (1989)	45	100	50
Potter (1989)	45	100	54

(*) cut-off = 25 U/ml.

dictive value (cut-off: 35 U/ml). Other Authors ([24, 30, 47, 61]) also obtained similar results by limiting assays to postmenopausal women to avoid false positive results due to endometriosis, and by performing both clinical and ultrasonographic examinations as well. It is however, important to bear always in mind that there exists a " grey zone " ranging between 35 and 65 U/ml, while making a diagnosis of ovarian carcinoma.

Therefore, CA 125 serum levels may be useful as an aid in this case, but it is necessary to be cautions in the application of this marker for diagnostic purposes.

With regard to the predictive value of CA 125 for surgical second looks (Table 2), several Authors ([11, 51, 56, 63, 64, 78]) have observed that high CA 125 values – especially above a certain level – are a major indication for a positive second look; so much so that they have suggested postponing second looks and having patients undergo chemotherapy directly.

Recently, the Food and Drug Administration (FDA) has approved the use of CA 125 as a marker capable of identifying neoplastic residua before a second operation.

However, normal CA 125 values, on the other hand, should not exclude a positive second look, especially in cases with small-size residual masses. In fact, Rubin *et al.* ([66]) reported that there was a correlation between the maximum diameter of the largest residual tumour mass and the accuracy of the CA 125 level.

OTHER MARKERS

To complete this review on ovarian tumour markers, brief mention must be made of some other biological markers which may be of some clinical use among the numerous suggested markers (Table 3).

Nowadays they are assayed in combination with CA 125 to enhance its sensitivity and specificity ([12, 44]).

NB/70K is a 70 KD glycoprotein fraction associated with ovarian tumours and was obtained from the OCA (Ovarian Cancer Antigen) by Knauf and Urbach ([38]). The first findings from a radioimmunoassay method with one monoclonal antibody (NB 12123) seem to show that NB/70k is an antigen which is not correlated to CA 125 but quite useful when used in association with it, both to enhance its

Table 3. – *Main tumor markers in ovarian cancer.*

Epithelial ovarian cancer		
CA 125	PLAP	IAP
TAG 72	PDH	TATI
CA 15-3	OCAA	LASA
CA 19-9	OCA	Ferritin
CA 50	NB/70K	Fibronectin
CEA	MOV 2 - MOV 8	CIC
TPA	DUPAN 2	Polyamines

Germ cell ovarian cancer	Stromal ovarian cancer
Beta HCG	Estrogens
AFP	17-Ketosteroids
SP 1	

specificity and to monitor some cases with normal CA 125 levels [39].

Several substances with immunosuppressive activity have been found in the sera of tumour patients [35], like the *IAP* (Immunosuppressive Acidic Protein). IAP inhibits in vitro lymphocyte blastation induced by phytohemoagglutinin (PHA).

This glycoprotein can be assayed by means of radial immunodiffusion and is an aspecific marker for tumour activity in general [68]. It present high levels in colorectal, ovarian and pancreatic carcinomas and is produced by the macrophages of patients with ovarian and colorectal carcinomas. When associated with CA 125 it seems to enhance the diagnostic accuracy of this marker, thanks to its high sensitivity even in the first stages of the disease [18].

The Placental Alkaline Phosphatase (*PLAP*) is expressed by the syncytiotrophoblast of the placenta from the 12th week of pregnancy. PLAP was one of the first proteins found to be ectopically produced by cancer cells and is now established as a useful marker for some genital tumours (i.e. seminomas) [25].

Almost 50% of the patients with serous adenocarcinomas had elevated circulating levels of PLAP, whereas mucinous adenocarcinomas had less than 10% elevated serum levels.

Finally, many new markers have been identified by means of monoclonal antibodies, like CA 15.3, CA 19.9, CA 50, TAG 72, and CA 125 itself. They are merely epitopes of a carbohydrate nature carried by big glycoproteic molecules of the mucin family [15].

At first, they were thought to be specific for a certain type of tumour but were subsequently found in several other neoplasms as well.

Indeed, many finding suggest that the same mucin may transport many epitopes, or that several mucins carrying various epitopes may become aggregated and form complexes of a greater molecular weight.

On-going investigations are assessing whether the simultaneous rise in two markers of this type may provide a more specific test for ovarian cancer than one marker alone. The most studied markers in association with CA 125 are TAG 72 and CA 15.3, for the time being.

TAG 72 (Tumour Associated Glycoprotein 72) is an antigenic determinant expressed by a glycoprotein of high molecular weight. It was first described by Schlom *et al.* [19] and can be measured with a radioimmunoassay using two monoclonal antibodies (cc 49 and B 72.3 [31]. It is elevated in ovarian tumours as well as in tumours of the gastroenteric apparatus and of the lung [73].

CA 15.3 is also assayed by means of a RIA method with two monoclonal antibodies (115 D 8 and DF 3) [28, 42]. It is currently considered to be the most specific marker for carcinomas of the breast but it is also present in patients with carcinomas of the ovaries and lungs [69, 77].

The use of TAG 72 and CA 15.3 in combination with CA 125 seems to be useful especially in improving CA 125 specificity, which, according to some preliminary results, ranges from 84% to 90% and to 98% [22, 71].

CONCLUSION

At present there is not an ideal humoral marker for epithelial ovarian carcinoma, although CA 125 represents a useful tumor marker in the management of these patients. It could be used as an aid to the diagnosis, as a prognostic factor, in the follow up and monitoring of therapy, in the early diagnosis of relapse and in the evaluation of the need for second-look surgery. Serial determination of serum CA 125 is a non-aggressive, easy and inexpensive method for the monitoring of this disease.

Furthermore CA 125 could be suitable for other applications: measurement on ascites, immunohistochemistry on cell

smears, administration of radiolabelled antibody for immunoscintigraphy ([20]) and immunotherapy.

REFERENCES

1) Ai-Ru W., En-Yu W., Ling L.: *J. Exp. Clin. Cancer Res., 8,* 29. 1989.
2) Alvarez R. D., To A., Boots L. R. *et al.: Gin. Oncol., 26,* 284, 1987.
3) "American Cancer Society": *Cancer Facts,* 1987.
4) Atack D. B., Nisker J. A., Allen H.H. *et al.: Am. J. Ost. Gyn., 154,* 287, 1986.
5) Ballesta A. M., Molina R., Filella X. *et al.: Tumor Biol., 7,* 283, 1986.
6) Bast R. C., Feeney M., Lazarus M. *et al.: J. Clin. Invest., 68,* 1331, 1981.
7) Bast R. C., Klug T. L., John S. T. *et al.: N. Engl. J. Med., 309,* 883, 1983.
8) Bast R. C., Klug T. L., Schaetzl E. *et al.: Am. J. Obst. Gyn., 149,* 553, 1984.
9) Bast R. C., Hunter V., Knapp R. C.: "Pros and Cons of Gynecologic Tumor Markers", *Cancer, 60,* 1984, 1987.
10) Bates S. E., Longo V. L.: *Sem. Oncol., 14,* 102, 1987.
11) Berek J. S., Knapp R. C., Malkasian G. D. *et al.: Obst. Gyn., 67,* 685, 1986.
12) Bhargava A. K., Lele S. B., O'Donnell A. *et al.: J. Tumor Marker Oncol., 3,* 379, 1988.
13) Bjorklung B.: *Tumor Diagnosti, 1,* 9, 1980.
14) Bombardieri S.: " Biomarcatori ". In: *Manuale di Oncologia Medica,* Bonadonna G., Robustelli Della Cuna G. (eds.), Masson Italia Editore Milano, *147,* 1987.
15) Bombardieri E.: *The Ligand Quarterly, 7,* 299, 1988.
16) Brioschi P. A., Irion O., Bischof P. *et al.: Br. J. Obst. Gyn., 94,* 196, 1987.
17) Canney P. A., Moore M., Wilkinson P. M. and James R. D.: *Br. J. Cancer, 50,* 765, 1984.
18) Castelli M., Romano P., Atlante G. *et al.: Int. J. Biol. Markers, 2,* 187, 1987.
19) Colcher D., Horan H., Nuti P. *et al.: Cancer Invest., 1,* 127, 1983.
20) Crowther M. E., Britton K. E., Granowska M., Shepherd J. H.: *Br. J. Obst. Gyn., 96,* 516, 1989.
21) Einhorn N., Bast R. C., Knapp R. C. *et al.: Obst. Gyn., 67,* 414, 1986.
22) Einhorn N., Zurawski V. R., Knapp R. C. *et al.: Proc. Am. Assoc. Res., 28,* 357, 1987.
23) FIGO Committee, 1986.
24) Finkler N. J., Benacerraf B., Lavin P. T. *et al.: Obst. Gyn., 72,* 659, 1988.
25) Fisken J., Leonard R. C. F., Shaw G. *et al.: J. Clin. Pathol, 42,* 40, 1982.
26) Gold P., Freedman S. O.: *J. Exp. Med., 121,* 439, 1965.
27) Halila H., Lehtorvirta P., Stenman U. H., Seppala M.: *Acta Obst. Gyn. Scand., 67,* 53, 1988.
28) Hilkens J., Buijs F., Hilgers J. *et al.: Int. J. Cancer, 46,* 2582, 1984.
29) Ishiguno T., Yoshida Y., Tenzaki T. *et al.: Cancer, 48,* 2480, 1981.
30) Jacobs I., Oram D.: *Biomed. Pharmac., 42,* 589, 1988.
31) Johnston W. W., Szapk C. A., Lottich S. C. *et al.: Cancer Res., 45,* 1894, 1985.
32) Joung R. C., Knapp R. C., Perez C. A.: " Cancer of the ovary". In: *Cancer, Principles and practice of Oncology,* De Vita V. T., Hellman S., Rosenberg S. A. (eds.), Lippincott Philadelphia, 884, 1982.
33) Kabawat S. E., Bast R. C., Bhan A. K. *et al.: Int. J. Gyn. Bathol., 2, 275,* 1983.
34) Kabawt S. E., Bast R. C., Welch W. R. *et al.: Am. J. Clin. Pathol., 79,* 98, 1983.
35) Kamo I., Friedman H.: *Adv. Cancer Res. 25,* 271, 1977.
36) Kikuchi Y., Kizawa I., Koyama E., Kato K.: *Obst. Gyn., 63,* 561, 1984.
37) Kivinen S., Kuoppala T., Leppilampi M. *et al.: Obst. Gyn., 67,* 468, 1986.
38) Knauf S., Urbach G. I.: *Am. J. Obst. Gyn., 127,* 705, 1977.
39) Knauf S., Bast R. C.: *Int. J. Biol. Markers, 3,* 75, 1988.
40) Kohler G., Milstein C.: *Nature, 256,* 495, 1975.
41) Krebs H. B., Goplerud D. R., Kilpatrick J. *et al.: Obst. Gyn., 67,* 473, 1986.
42) Kufe D., Inghirami G., Abe M. *et al.: Hybrodoma, 3,* 223, 1984.
43) Kurman R. J., Scardino P. T., Waldmann T. A. *et al.: Ann. Cl. Lab. Sci., 9,* 462, 1979.
44) Lahousen M., Stettner H., Pickel H. *et al.: Cancer, 60,* 2228, 1987.
45) Lahousen M., Stettner H., Purstner P.: *Baillieres Clin. Obst. Gyn., 3,* 201, 1989.
46) Lavin P. T., Knapp R. S., Malkasian G. *et al: Obst. Gyn., 69,* 223, 1987.
47) Maggino T., F. Sopracordevole, Matarese M. *et al.: Eur. J. Gyn. Oncol., 8,* 590, 1987.
48) Malkasian G., Podratz K. C., Stanhope C. R. *et al.: Am. J. Obst. Gyn., 155,* 515, 1986.
49) Martoni A., Bellucco A., Marabini A. *et al.: Int. J. Biol. Markers, 3,* 121, 1986.
50) Masuho Y., Zalutsky M., Knapp R. C., Bast R. C.: *Cancer Res., 44,* 2813, 1984.
51) Mogensen O., Mogensen B., Jakobsen A., Sell A.: *Eur. J. Cancer Clin. Oncol., 24,* 1835, 1988.
52) Neunteufel W., Breitenecker G.: *Gyn. Oncol., 32,* 297, 1989.
53) Ng L. W., Homesley H. D., Barrett R. J. *et al.: Am. J. Clin. Oncol., 12,* 106, 1989.

54) Niloff J. M., Knapp R. C., Schaetzl E. *et al.: Obst. Gyn., 64,* 703, 1984.
55) Niloff J. M., Bast R. C., Schaetzl E., Knapp R. C.: *Am. J. Obst. Gyn., 151,* 981, 1985.
56) Niloff J. M., Knapp R. C., Lavin P. T. *et al.: Am. J. Obst. Gyn., 155,* 56, 1986.
57) Nouwen E. J., Hendrix P. G., Dauwe S. *et al.: Am. J. Pathol, 126,* 230, 1987.
58) O'Brien I. J., Hardin J. W., Bannon G. A. *et al.: Am. J. Ost. Gyn., 155,* 50, 1986.
59) Onetto M., Bruzzone M., Conte P. F. *et al.: Oncology, 46,* 117, 1989.
60) Panza N., Pacilio G., Campanella L. *et al.: Cancer, 61,* 76, 1988.
61) Patsnet B., Mann W. J.: *Am. J. Obst. Gyn., 59,* 873, 1988.
62) Pecorelli S., Favalli G., Sartori E. *et al.: Quad. Lig. Quart., 4,* 75, 1985.
63) Podczaski E., Whitney C., Manetta A. *et al.: Gyn. Oncol., 33,* 193, 1989.
64) Potter M. E., Moradi M., To A. C. W.: *Gyn. Oncol., 33,* 201, 1989.
65) Prodi G.: *G. I. Pat. Clin., 2,* 26, 1987.
66) Rubin S. C., Hoskins W. J. Hakes T.B. *et al.: Am. J. Obst. Gyn., 160,* 667, 1989.
67) Ruibal A., Encabo G., Martinez-Miralles E.: *Bull. Cancer, 71,* 145, 1984.
68) Sawada M., Okudaira Y., Matsui Y. *et al.: Cancer, 54,* 2081, 1984.
69) Scambia G., Benedetti Panici G., Baiocchi L. *ea al. Oncology, 45,* 263, 1988.
70) Seppala M.: " Ovarian and uterine cancer markers ". In: *Human Cancer Markers,* Sell S., Wahren B. (eds.), Humana Press. Clifton, 233, 1982.
71) Soper J. T., Hunter V., Tanner M. *et al.: Proc. Am. Assoc. Cancer Res., 28,* 205, 1987.
72) Stall K. E., Martin E. W.: *J. Repr. Med., 26,* 75, 1981.
73) Thor A., Gorstein F., Ohuchi N. *et al.: J. Natl. Cancer. Inst., 76,* 995, 1986.
74) Vergote I. B., Bormer O. P., Abeler W. M.: *Am. J. Obst. Gyn., 157,* 88, 1987.
75) Vogl S. E., Berenzweig M., Kaplan B. H. *et al.: Cancer Treat. Rep., 63,* 311, 1979.
76) Weiss N. S., Homonchuk T., Young J. C.: *Gyn. Oncol., 5,* 161, 1977.
77) Yedema C., Massuger L., Hilgers J. *et al.: Int. J. Cancer Suppl., 3,* 61, 1988.
78) Zanaboni F., Accinelli G., Colombo P. *et al.: Int. J. Biol. Markers, 2,* 105, 1987.
79) Zanaboni F., Presti M., Scarfone G., Bolis G.: *Tumori, 75,* 69, 1989.

Address reprint requests to:
Dr. G. GARGANO
Gynecology Division - Oncology Institute
Via Scipione l'Africano, 191
70124 Bari