Chromogranin A and biochemical progression-free survival in prostate adenocarcinomas submitted to radical prostatectomy

Sciarra Alessandro, Gentile Vincenzo, Autran Gomez Ana Maria, Salciccia Stefano, Gentilucci Alessandro, Monti Salvatore¹, Toscano Vincenzo¹ and Di Silverio Franco

Department of Urology, University La Sapienza, Viale Policlinico, 00161 Rome, Italy

¹Department of Endocrinology, University La Sapienza, Rome, Italy

(Correspondence should be addressed to S Alessandro; Email: sciarrajr@hotmail.com)

Abstract

The primary aim of the present study was to determine the prognostic role of elevated levels of chromogranin A (CgA) in terms of biochemical prostate-specific antigen (PSA) progression after radical prostatectomy (RRP) for prostate adenocarcinoma. Two hundred and sixty-four consecutive men with non-metastatic prostate adenocarcinoma submitted to RRP represented our population. In all cases, a blood sample for the determination of serum total PSA and CgA levels was obtained (RIA). Two different upper reference values for serum CgA levels were used: >60 and >90 ng/ml. The main end point of this study was biochemical (PSA) progression-free survival. In our population, 35.0% (91/264 cases) of cases presented a serum CgA level >60 ng/ml and only 6.4% (17/264) presented CgA >90 ng/ml. After RRP, during a mean follow-up of 64.59 ± 26.34 months (median 60 months; range 12–120 months), 59 patients (22.3%) showed a biochemical (PSA) progression. Using 60 ng/ml as upper reference value for CgA, 10.4 and 45.0% of cases showed PSA progression after RRP in the group with preoperative CgA levels ≤ 60 and > 60 ng/ml respectively. The proportion of PSA progression-free survival was significantly lower in cases with preoperative CgA > 60 ng/ml than in cases with CgA ≤ 60 ng/ml ($P < 0.0001$). In addition, at the multivariate analysis, preoperative serum CgA levels were confirmed as an independent prognostic factor for PSA progression after RRP. In non-metastatic prostate carcinomas, we described a significant prognostic role of CgA in terms of biochemical progression-free survival.

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Introduction

Approximately, 40% of prostate cancer patients who choose definitive therapy will undergo radical prostatectomy (RRP; Jemal *et al.* 2005, Simmons *et al.* 2007). While overall cancer control rates are high for localized disease, 20–30% of patients after RRP will experience recurrence, manifested initially as a rising serum prostate-specific antigen (PSA; biochemical progression), without clinical evidence (radiographic evidence of metastases) of progression (Han *et al.* 2003, Simmons *et al.* 2007). In addition, if biochemical progression after RRP at 10-year follow-up is loosely

associated with the development of clinical progression or with prostate cancer-specific mortality, this parameter is generally considered clinically significant and a treatment for patients with PSA progression after RRP is programed by most of urologists (Jhaveri *et al.* 1999, Han *et al.* 2003, Simmons *et al.* 2007). Pound *et al.* (1999) reported that the 5-year risk of clinical progression ranges from 27 to 60%, if men with PSA progression after RRP are untreated.

Neuroendocrine (NE) differentiation in prostate cancer has received increasing attention in recent years because of the prognostic and therapeutic implications.

NE differentiation is present at least focally in virtually all cases of prostate adenocarcinoma. Chromogranin A (CgA) is a valuable marker for NE tumors and it is considered to be the preferred marker for NE activity in prostate cancer cases (Shariff & Ather 2006). In patients with prostate cancer, circulating CgA has been found to reflect the immunohistochemical (Abrahamsson *et al.* 1989) and RT-PCR findings (Sciarra *et al.* 2003) at prostate cancer tissue level. It has been suggested that prostate adenocarcinoma presenting high levels of markers of NE differentiation tend to be more aggressive (Abrahamsson *et al.* 1989, Sciarra *et al.* 2003, Berruti *et al.* 2005, Taplin *et al.* 2005). More convincing data have been reported in the advanced and hormone-refractory stage of the tumor, where NE activity is highly increased (Cohen *et al.* 1991, Berruti *et al.* 2005, Taplin *et al.* 2005). Some authors, using a multivariate analysis, sustained that also in non-metastatic prostate cancer, NE differentiation and CgA can be associated with the aggressiveness of the tumor (Weinstein *et al.* 1996, Ahlgren *et al.* 2000, Sciarra *et al.* 2004). The College of American Patologists Consensus Statement 1999 (Bostwick *et al.* 2000) considered NE differentiation in prostate cancer as a category III factor for the prognostic value in patients. Although the evidence for the factors in category III is not yet good enough for their use in routine clinical practice, the role of NE differentiation is very promising, and in different countries, CgA determination started to be used and to be repeated in the clinical practice for the evaluation of prostate adenocarcinoma cases. In a population of non-metastatic prostate adenocarcinomas considered for RRP, the primary aim of the present study is to determine the prognostic role of elevated levels of CgA in terms of biochemical (PSA) progression after surgery. No data in terms of clinical progression or prostate cancer-specific mortality are available in this study. A secondary aim is to evaluate the association of CgA expression with other well-defined prognostic parameters such as pathologic stage (pT), Gleason score, and serum PSA.

Materials and methods

This is a prospective, single center study. Between January 1996 and January 2002, 308 consecutive men with clinically non-metastatic prostate adenocarcinoma were considered for RRP at our clinic.

Inclusion into this study was based on the following criteria:

- no previous hormonal or radiation therapy
- no previous surgery on the prostate gland

- histologically proven adenocarcinoma of the prostate at biopsy and confirmed at RRP
- no positive surgical margins
- no adjuvant therapies after RRP

The present analysis included 264 cases from this population who fulfilled the inclusion criteria.

None of these cases presented a history of other disorders or therapies or conditions known to interfere with CgA levels as defined in previous studies (Sciarra *et al.* 2004).

Consent has been obtained from each patient after full explanation of the purpose and nature of all procedures used. The investigation was approved by the local ethical committee.

All 264 cases had a biopsy proven clinically T2-T3N₀M₀ prostate adenocarcinoma, as determined by digital rectal examination, transrectal ultrasonography, bone scan, and computer tomography (CT). All patients were submitted to RRP. Pathologic tumor stage was assigned according to the 1997 modification of the TNM classification. Tumor grade was described at RRP according to the Gleason grading system. In all 264 cases, at least 3 weeks after any prostatic manipulation and the same day of RRP, before the surgical procedure, a blood sample for the determination of serum total PSA and CgA levels was obtained. Each sample was homogeneously collected in the early morning after an overnight fast. As in previous studies (Sciarra *et al.* 2004), in each case, serum CgA was measured by RIA using a commercial kit (CIS Bio International, Cedex, France). The detection limit of this kit was 1.5 ng/ml. The inter-assay coefficients of variation of CgA assay were 5.8 and 3.8% respectively. In each case, the same serum sample was also used to determine total PSA levels (Hybritech Inc., San Diego, CA, USA). The normal range reported by the kit for CgA was 0–90 ng/ml. All samples were evaluated centrally in the laboratory of our University.

In the first 60 consecutive prostate cases, we had the opportunity to analyze CgA mRNA expression on tissue samples obtained from RRP. Prostate tissue samples were immediately frozen in liquid nitrogen and stored at –80 °C until analysis. In each specimen, the diagnosis of prostate adenocarcinoma was histologically confirmed. Each sample weighted about 1 g. Gene expression of CgA was evaluated by a semiquantitative RT-PCR, using β -actin as control. The method has been previously described (Sciarra *et al.* 2003, 2004). In this study, the tissue determination of CgA mRNA expression was used to evaluate its correlation with CgA serum levels and therefore to

support the hypothesis that serum CgA levels reflected CgA expression and NE activity at prostate adenocarcinoma tissue level.

After RRP, patients were followed at regular intervals with total PSA determinations (1-month intervals during the first year and thereafter at 3-month intervals), bone scan (1-year interval or at PSA progression), CT or MNR (1-year interval or at PSA progression). None of these cases was submitted to adjuvant therapies after RRP, and only at PSA progression a therapy was planned.

According to the literature (Ferrero-Pous *et al.* 2001), during the postoperative follow-up, a biochemical (PSA) progression was defined as the first occurrence of a PSA increase over the level of 0.2 ng/ml, with a value confirmed at two consecutive (2-week interval) determinations.

Statistical analysis

Descriptive statistics were used to characterize the population. For the statistical analysis, patients were classified on the basis of the pathological T stage in pT2 and pT3 patients (no pT4 and only six N+ cases were found), on the basis of the RRP Gleason score in Gleason score ≤7 (3+4) and ≥7 (4+3) and on the basis of the serum PSA levels in ≤10.0 and >10 ng/ml.

On the basis of the normal range reported by the kit for CgA and previous experiences in the literature (Sciarra *et al.* 2003, 2004), we decided to use two different reference upper values for CgA serum levels: >90 and >60 ng/ml.

Spearman correlation coefficients were calculated to measure the association among CgA and the other parameters. Differences in the parameters between groups were tested using ANOVA non-parametric test and χ^2 test. The odds ratio (OR) and 95% CI for a CgA serum levels >60 or >90 ng/ml on the basis of the stage, Gleason score and PSA classifications were analyzed. Univariate and multivariate (Cox proportional hazard method) analysis were also performed.

The main end point of this study was PSA progression-free survival, which was defined as the time between RRP and PSA progression. Patients were censored if they were known to be still progression-free or were lost to follow-up. Survival curves were estimated using the Kaplan–Meier method.

A 5% level of significance was used for all statistical testing. A Sigma-Stat and Sigma-Plot 2-2 (Jandel

Scientific software, San Rafael, CA, USA) programs have been used for all statistical analyses.

Results

General considerations

Clinical and pathological characteristics of our population are described in Table 1. Serum CgA level was significantly associated with pT stage ($r=0.1983$; $P=0.0012$) and Gleason score ($r=0.1990$; $P=0.001$) but not with PSA ($r=0.0620$; $P=0.0315$) and age ($r=0.0327$; $P=0.569$) (r =Spearman coefficient).

In all 60 prostate adenocarcinoma tissue samples examined, the expression of CgA mRNA has been found, and a significant association between serum CgA and tissue CgA mRNA levels ($r=0.4690$; $P=0.039$; r =Spearman coefficient) was demonstrated.

Serum CgA levels distribution: influence of pT stage and Gleason score

Mean serum level of CgA was 54.60 ± 22.15 ng/ml (median 52.05; range 20.0–195.0; Table 1).

In our population, 35.0% (91/264 cases) of cases presented a serum CgA level >60 ng/ml and only 6.4% (17/264) presented CgA >90 ng/ml.

Classifying cases on the basis of the pT stage (pT2 versus pT3), a statistically significant difference (Mann–Whitney test $P=0.0016$) in terms of mean serum CgA level was present. (Table 2).

According to the pT stage, the percentage of cases with CgA >60 ng/ml was 27.4% (39/142 cases) in pT2 and 44.2% (54/122 cases) in pT3 cases, whereas that of

Table 1 Clinical and pathological characteristics of our population

Number of cases	264
Age (years)	65.82 ± 5.43 (67) range 50–71
pT2	142 (53.8%)
pT3	122 (46.2%)
Gleason score ≤7 (3+4)	152 (57.6%)
Gleason score ≥7 (4+3)	112 (42.4%)
PSA (ng/ml)	7.48 ± 3.05 (7.05) range 2.5–14.0
CgA (ng/ml)	54.60 ± 22.15 (55.05) range 20.0–195.0
CgA >60 ng/ml	91/264 (35%)
CgA >90 ng/ml	17/264 (6.4%)

Number of cases; mean ± s.d. (median); range.

Table 2 Serum chromogranin A (CgA) level according to pT stage and Gleason score

	pT2	pT3	P value	Gleason ≤ 7 (3+4)	Gleason ≥ 7 (4+3)	P value
CgA (ng/ml)	52.02 $\pm$ 18.95 49.35 range 20.0–104.10	58.51 $\pm$ 25.59 (54.75) range 20.0–195.0	0.0016	51.22 $\pm$ 18.91 (49) range 20.0–102.1	60.18 $\pm$ 25.73 (58) range 20.0–195.0	0.0001
CgA > 60 ng/ml	39/142 (27.4%)	54/122 (44.2%)	0.0001	45/152 (29.6%)	52/112 (46.4%)	0.0001
CgA > 90 ng/ml	5/142 (3.5%)	12/122 (9.8%)	0.0167	6/152 (3.9%)	11/112 (9.8%)	0.0028
OR CgA > 60 ng/ml	1.0	2.470 (0.945–6.450)	0.0047	1.0	2.061 (1.238–3.429)	0.0038
OR CgA > 90 ng/ml	1.0	2.330 (0.865–6.430)	0.0451	1.0	2.650 (0.949–7.40)	0.0484

Number of cases (%); mean $\pm$ s.d. (median); range. Odds ratio (OR; 95% confidential interval). Mann–Whitney and Fisher’s exact test.

cases with CgA > 90 ng/ml was 3.5% (5/142 cases) in pT2 and 9.8% (12/122 cases) in pT3 cases (Table 2).

The OR for both CgA levels > 60 and > 90 ng/ml significantly increased from pT2 to pT3 cases ($P=0.0047$ and 0.0451 respectively; Table 2).

Classifying cases on the basis of the Gleason score (≤ 7 (3+4) vs ≥ 7 (4+3)), a statistically significant difference (Mann–Whitney test: $P=0.0001$) in terms of mean serum CgA level was present (Table 2). According to the Gleason score, the percentage of cases with CgA > 60 ng/ml was 29.6% (45/152 cases) in Gleason score ≤ 7 (3+4) cases and 46.4% (52/112 cases) in Gleason score ≥ 7 (4+3) cases, whereas that of cases with CgA > 90 ng/ml was 3.9% (6/152 cases) in Gleason score ≤ 7 (3+4) and 9.8% (11/112 cases) in Gleason score ≥ 7 (4+3) cases (Table 2).

The OR for both CgA levels > 60 and > 90 ng/ml significantly increased from Gleason score ≤ 7 (3+4) to Gleason score ≥ 7 (4+3) cases ($P=0.0038$ and 0.0484 respectively; Table 2).

At the multivariate analysis, the pT stage ($P=0.0004$) and the Gleason score ($P=0.0018$) of

the tumor but not the age and the PSA level ($P=0.845$ and 0.0937 respectively) resulted significant and independent predictors of CgA serum levels.

Serum CgA level and biochemical (PSA) progression-free survival

After RRP, the mean follow-up for our population was 64.59 ± 26.34 months (median 60 months; range 12–120 months). During this follow-up, 59 patients (22.3%) showed a biochemical (PSA) progression at a mean time of 50.73 ± 16.79 months (median 48 months; range 12–96 months) (Table 3). Table 3 shows results in terms of PSA progression, stratifying the population on the basis of pT stage, Gleason score at RRP, preoperative serum PSA, and CgA level. In particular, using > 60 ng/ml as upper reference value for CgA, 10.4 and 45.0% of cases showed PSA progression after RRP respectively in the group with preoperative CgA level ≤ 60 and > 60 ng/ml (Table 3). As reported in Fig. 1a, the proportion of cases with PSA progression-free survival was significantly lower in the group with preoperative CgA > 60 ng/ml than in the group with

Table 3 Prostate-specific antigen (PSA) progression after radical prostatectomy (RRP) in the population

	PSA progression number of cases (%)	P value	Time to PSA progression (months)
Total (264 cases)	59/264 (22.3)		50.73 $\pm$ 16.79 (48.0); 12–96
pT2	4/142 (2.8)	0.0001	54.0 $\pm$ 31.75 (48.0); 24–96
pT3	55/122 (45.0)		50.49 $\pm$ 15.68 (48.0); 12–84
Gleason ≤ 7 (3+4)	8/152 (5.3)	0.0001	63.0 $\pm$ 16.66 (60.0); 48–96
Gleason ≥ 7 (4+3)	51/112 (45.5)		48.80 $\pm$ 16.13 (48.0); 12–84
PSA ≤ 10 ng/ml	45/191 (23.5)	0.745	50.24 $\pm$ 15.98 (48.0); 12–84
PSA > 10 ng/ml	14/73 (19.1)		52.28 $\pm$ 19.75 (48.0); 24–96
CgA ≤ 60 ng/ml	18/173 (10.4)	0.001	40.0 $\pm$ 14.84 (42.0); 12–60
CgA > 60 ng/ml	41/91 (45.0)		55.44 $\pm$ 15.51 (48.0); 24–96
CgA ≤ 90 ng/ml	48/247 (19.4)	0.001	62.98 $\pm$ 20.48 (70.0); 12–96
CgA > 90 ng/ml	11/17 (64.7)		40.80 $\pm$ 11.59 (42.0); 24–60

Number of cases (%). Time to PSA progression: mean $\pm$ s.d. (median); range (χ^2 test).

Figure 1 Survival curve (Kaplan–Meier method): PSA progression-free survival according to preoperative serum CgA level. Upper reference values of (a) CgA = 60 ng/ml and (b) CgA = 90 ng/ml.

CgA ≤ 60 ng/ml ($P < 0.0001$). Using 90 ng/ml as upper reference value for CgA, again the proportion of cases with PSA progression-free survival was significantly ($P < 0.0001$) lower in the group with CgA > 90 ng/ml than in the group with CgA ≤ 90 ng/ml (Fig. 1b) (19.4 and 64.7% of cases showed PSA progression after RRP respectively in the group with CgA ≤ 90 and 90 ng/ml; Table 3).

In addition, at the multivariate analysis, preoperative serum CgA level confirmed its prognostic role in terms of OR for a PSA progression after RRP. Independent significant predictors for a PSA progression after RRP resulted the preoperative CgA level (OR (95% CI): 1.85(1.24–2.56), $P = 0.001$), the pT stage (OR (95% CI): 2.83(1.23–3.05), $P = 0.0001$), the Gleason score (OR (95% CI): 2.45(1.16–2.96), $P = 0.0001$), but not the preoperative PSA level (OR (95% CI): 1.04(0.80–1.14), $P = 0.840$).

Figures 2 and 3 show that stratifying the population on the basis of pT stage and Gleason score, in all subgroups, the proportion of cases with PSA progression-free survival was significantly lower in the groups with elevated CgA level.

Discussion

To our knowledge, this is the first study in the literature that analyses the prognostic role of CgA in terms of PSA progression-free survival in a population of non-metastatic prostate adenocarcinomas submitted to RRP. Several authors (Hoosein *et al.* 1993, Cussenot *et al.* 1996, Abrahamsson 1999, Ferrero-Pous *et al.* 2001) showed that NE activity and peptide release can increase the invasive potential of prostate adenocarcinoma cells and therefore contribute to a progression and aggressive course of tumors. Fewer data evaluated the impact of NE activity in non-metastatic tumors. Weinstein *et al.* (1996), in 104 organ-confined prostate cancers treated by RRP, found that Gleason score and NE differentiation predicted progression on multivariate analysis. In a previous study (Sciarra *et al.* 2004), we showed that CgA serum levels can significantly predict, at multivariate analysis, the risk of clinical understanding in non-metastatic prostate adenocarcinomas considered for RRP.

The rationale for our analysis is based on the fact that if it is hypothesized that NE activity influences prostate cancer growth, one might expect NE markers, such as CgA, to correlate with more adverse pathological features of prostate cancer and to help in predicting the risk of progression also in non-metastatic tumors submitted to RRP.

The clinical and pathological characteristics of our population reflect those reported in similar studies (Simmons *et al.* 2007). None of our cases was previously submitted to hormone therapies, so to exclude a possible role of androgen deprivation therapy on CgA expression. Moreover, to better analyze results in terms of PSA progression-free survival after surgery, none of our cases was submitted to hormone therapies after RRP; thus, the effect of androgen deprivation therapy on CgA expression can be excluded. Similar to previous studies (Simmons *et al.* 2007), after a mean postoperative follow-up of 64.59 months, 22.3% of cases showed biochemical (PSA) progression at a mean time of 50.73 months. As in previous analysis (Abrahamsson *et al.* 1989, Sciarra *et al.* 2003, 2004), the significant association between serum CgA levels and CgA mRNA tissue expression at prostate cancer level (detected in a subgroup of cases) and the significant association between serum CgA levels and other well-defined parameters of tumor aggressiveness such as pT stage and Gleason score, all strongly suggest that, in our population, serum CgA variations reflected NE activity at prostate adenocarcinoma level.

Figure 2 Survival curve (Kaplan–Meier method). Population classified for pT stage: proportion of cases with PSA progression-free survival according to preoperative serum CgA level. Upper reference value of CgA = 60 ng/ml: (a) pT2 ($P=0.0007$) and (b) pT3 ($P=0.0001$). Upper reference values of CgA = 90 ng/ml: (c) pT2 ($P=0.0013$) and (d) pT3 ($P=0.0001$).

An undefined problem for the use of serum CgA as marker of NE activity in prostate cancer is the determination of a CgA upper reference value. We must remember that, unlike pure NE tumors, in prostate adenocarcinomas only a focal NE expression

is present (Abrahamsson *et al.* 1989). Therefore, in prostate adenocarcinomas, we cannot expect to find the same CgA levels as in pure NE tumors. Again, in non-metastatic tumors, where NE activity could start to condition tumor progression and aggressiveness, a

Figure 3 Survival curve (Kaplan–Meier method). Population classified for Gleason score: proportion of cases with PSA progression-free survival according to preoperative serum CgA level. Upper reference value of CgA = 60 ng/ml: (a) G < 7 = Gleason score: ≤ 7 (3 + 4) ($P=0.0001$) and (b) G ≥ 7 = Gleason score ≥ 7 (4 + 3) ($P=0.0001$). Upper reference value of CgA > 90 ng/ml: (c) G < 7 = Gleason score: ≤ 7 (3 + 4) ($P=0.001$) and (d) G ≥ 7 = Gleason score ≥ 7 (4 + 3) ($P=0.0001$).

CgA upper reference value lower than that used in metastatic cases should be available. At present, an accepted serum CgA cut-off value in prostate adenocarcinoma cases has not been defined. Therefore, in our analysis, we used mean and median levels of CgA as control to verify differences in the various groups. Moreover, as upper reference value of serum CgA level we used either >60 or >90 ng/ml. The value of CgA >90 ng/ml is that reported by the RIA kit, which is also often used in the clinical practice to define a clinically significant NE activity in either metastatic prostate or pure NE tumors (Hoosein *et al.* 1993, Ferrero-Pous *et al.* 2001). Based on the literature (Sciarra *et al.* 2004), we also used a previously proposed CgA upper reference value of 60 ng/ml.

The first interesting data obtained from our analysis is that, in our population, 35% of non-metastatic prostate adenocarcinomas showed levels of CgA higher than the upper reference value of 60 ng/ml, whereas only 6.4% higher than 90 ng/ml. Moreover, the presence of a CgA value >60 or >90 ng/ml was significantly associated with pT stage and Gleason score. The significant association between elevated CgA levels and the other parameters related to the aggressiveness of the tumor should suggest that CgA measurement may preoperatively predict the risk of progression in this kind of population. Recently, Berruti *et al.* (2005), in a longitudinal analysis on metastatic prostate cancers, showed that CgA-elevated levels are significantly associated to the risk of progression and to survival. We showed that also in a population of non-metastatic prostate adenocarcinomas submitted to RRP, CgA measurement could significantly predict the risk of biochemical (PSA) progression after surgery. As described by the Kaplan–Meier analysis, the cumulative proportion of cases with PSA progression-free survival was significantly lower in the groups with preoperative CgA level over both the reference values (60 and 90 ng/ml). On the contrary, no significant difference in terms of time to PSA progression was found in the various groups. It is important to underline that independently to the pT stage and Gleason score stratification (also if more evident in pT3 and Gleason score ≥ 7 (4+3) groups), the cumulative proportion of cases with PSA progression-free survival remained significantly lower in the groups with preoperative CgA level over the reference values (Figs 2 and 3).

Some limits of the study must be underlined. Our results are related to a prospective analysis in a limited population, but they could support the need for larger

multicentric studies. In this study, we limited the analysis to the predictive value of CgA in terms of biochemical (PSA) progression after surgery and we have no data in terms of clinical progression or overall survival. The reason is the very low percentage of cases with clinical progression or prostate cancer-related death at our follow-up. This is not an unexpected finding, because several studies (Simmons *et al.* 2007) have shown that at 10-year follow-up, approximately 90% of cases with biochemical PSA progression after RRP are free of clinical progression. Otherwise, our data are clinically relevant also if limited to a biochemical progression-free survival analysis. The parameter of PSA progression after surgery is considered clinically significant and a treatment for these patients is programmed by most of urologists.

Conclusions

Our analysis in non-metastatic prostate adenocarcinomas submitted to RRP showed a relevant percentage of cases (35%) with preoperative serum CgA level over the upper reference value of 60 ng/ml. A possible prognostic role of preoperative CgA determination in terms of risk for biochemical progression after RRP has been shown.

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