

DISAPPEARANCE RATE OF BIOLOGICAL MARKERS FOLLOWING  
RADICAL PROSTATECTOMY-PROGNOSTIC IMPLICATIONS.

P. JABALAMEI,

J.E. PONTES.

Biological markers are presently widely used to diagnose and monitor neoplasias. in prostate cancer the pioneer work of Gutman and Gutman led to the wide use of prostatic acid phosphatase as a marker in this disease.(1) over the years improvements in the enzymatic assays and the development of immunochemical methods have improved the performance of this marker. (2) However, there is a certain percentage of patients with prostate cancer that will not demonstrate elevation of SPAP despite metastatic disease and although the new immunochemical methods are highly sensitive their use as a screening tool have been disappointing.(2) because of these factors, attempts to develop new markers have taken place over the years, however, have not been successful.(3-4) A new prostatic specific marker described and characterized by Wang et al in 1979(5) is a prostatic specific protein immunologically distinct from prostatic acid phosphatase but probably just a form of semino protein described by Hara in Japan in 1966.(6.7)



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Immunonochemical assays have been developed for this marker and on initial studies have shown significant advantage over SPAP. (8.9)

Another biological marker commonly used in monitoring malignancies is the Lipid-Associated Sialic Acid Assay (LASA). Although not specific for prostate cancer it may be used in conjunction with other markers. Despite the widespread use of both of these prostatic specific markers and LASA. There is very little information in the literature about the curve of disappearance of these substances from the circulation (half life) once the source of production has been removed. Such information is important biologically, since it may identify individuals at high risk of developing metastatic disease.

#### MATERIAL AND METHODS

In order to evaluate the rate of disappearance from the circulation of 3 biological markers (PAP, PSA, and LASA) we studied 6 consecutive patients with clinically localized prostatic carcinoma staged as 2 B1, 3 B2, and 1 C. All patients had serum drawn in the operating room prior to the initiation of prostatectomy and at two hour intervals for the first 12 hours, at four hour intervals for the next 12 hours, at six hour intervals for the next 12 hours, and daily for seven days. All sera were collected in heparinized solution. Kept in the refrigerator, and centrifuged daily, and kept at -20 C until analysis.

All samples were analyzed concomitantly by one investigator (P.D.H.) at Dianon Systems, and the results reported without knowledge of timing of sampling or stage of the disease.



Prostate Specific Antigen (PSA) was measured by a double antibody radioimmunoassay using Yang Laboratory Pros-Check. PSA-RIA reagents. (Yang Laboratories, 1401-140th Place, NE, Bellevue, WA 98007.)

Prostatic Acid Phosphatase (PAP) was measured in plasma using Abbott PAP-EIA reagents (Abbott Laboratories, Diagnostic Division, North Chicago, IL 60064). The method is a solid phase enzyme immunoassay based on the "sandwich" principle.

Lipid-associated sialic acid was performed by the method described by Katopodis et al (10). 50 ul of plasma is diluted with 150 ul of distilled water and extracted with 3.0 ml of 2:1 (v/v) chloroform:methanol cooled to 4-5 deg C. Following centrifugation of the mixture 1.0 ml of the upper phase is removed and treated with 50 ul of phosphotungstic acid solution (1g/ml). This mixture is then centrifuged, the supernatant removed and the precipitate suspended in 1 ml distilled water. Sialic acid is quantitatively determined by the resorcinol/HCl method of Svennerholm (11) with the colorimetric extraction described by Miettinen (12).

## RESULTS

Preoperative sampling demonstrated that all patients had normal SPAP and LASA levels. but that four of six patients had above normal levels of SPSA (3 of them above 20 mg/ml). During surgery there was a great increase of all 3 markers probably owing to manipulation of the prostate. For better analysis of the results we will describe each marker individually.



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### Serum Prostatic Acid Phosphatase (PAP)

An illustration of values of SPAP in all six patients is demonstrated in Fig. 1. There was a rapid elevation of SPAP during the operation which quickly and progressively returned to normal within 12 hours following surgery and remained normal there after.

### Lipid Associated Sialic Acid (LASA)

All patients developed highly elevated values during and following surgery, probably due to the inflammatory reaction associated with the procedure. No data is available about normalization of this marker because lack of long term follow-up.

An attempt to correlate the fluctuation of marker elevations with pathological stage of the disease was made by carefully serial sections of the prostatectomy specimens. One patient had prostatic capsule and seminal vesicles intact three patients had microscopic invasion of the capsule, and two patients had complete perforation of the capsule with seminal vesicle involvement. The two

### Serum Prostate Specific Antigen (PSA)

There was a great elevation of serum levels of PSA during the surgery including one of the two patients whose values were normal preoperatively. Fig. 2. There was a slower decrease in values towards normalization and at 36 hours four out of six patients had values at 10 ng./ml. or less, and at 72 hours all four patients had values below 10 ng./ml. Two patients with elevated values prior to surgery continued to exhibit elevated SPSA after seven days of the study.



patients with persistent elevated PSA after 72 hours had extensive carcinoma involvement of the prostate, one with microscopic invasion of the bladder neck had an incomplete resection and the other who underwent salvage prostatectomy has subsequently developed metastases to the ribs, not apparent prior to surgery.

#### DISCUSSION

The use of biological markers in malignancies can assume several aspects. Markers can be used for screening, diagnosis, prognosis, or monitoring response to therapy. Presently available markers in prostate cancer have little use in screening general population. (2) These markers however have been used extensively to monitor response to therapy. Although some work has been done in the fluctuation of SPAP in patients with metastatic disease, very little has been done in monitoring the disappearance of these markers from the circulation following radical prostatectomy. (13.14) These studies are important in order to define the "normal" levels in patients without prostate and to establish the prognostic significance of a persistent positive marker in the development of metastatic disease. There is evidence that an elevation of prostatic specific acid phosphatase may precede radiological diagnosis of metastatic disease. (13.14.16) However, the fact other pathological conditions may interfere with the measurements of SPAP have made this marker not completely reliable. (2) Furthermore, there is a percentage of patients with metastatic prostatic carcinoma and negative SPAP. (2) The advent of SPSA as a new specific marker for prostate cancer has improved significantly our capa-

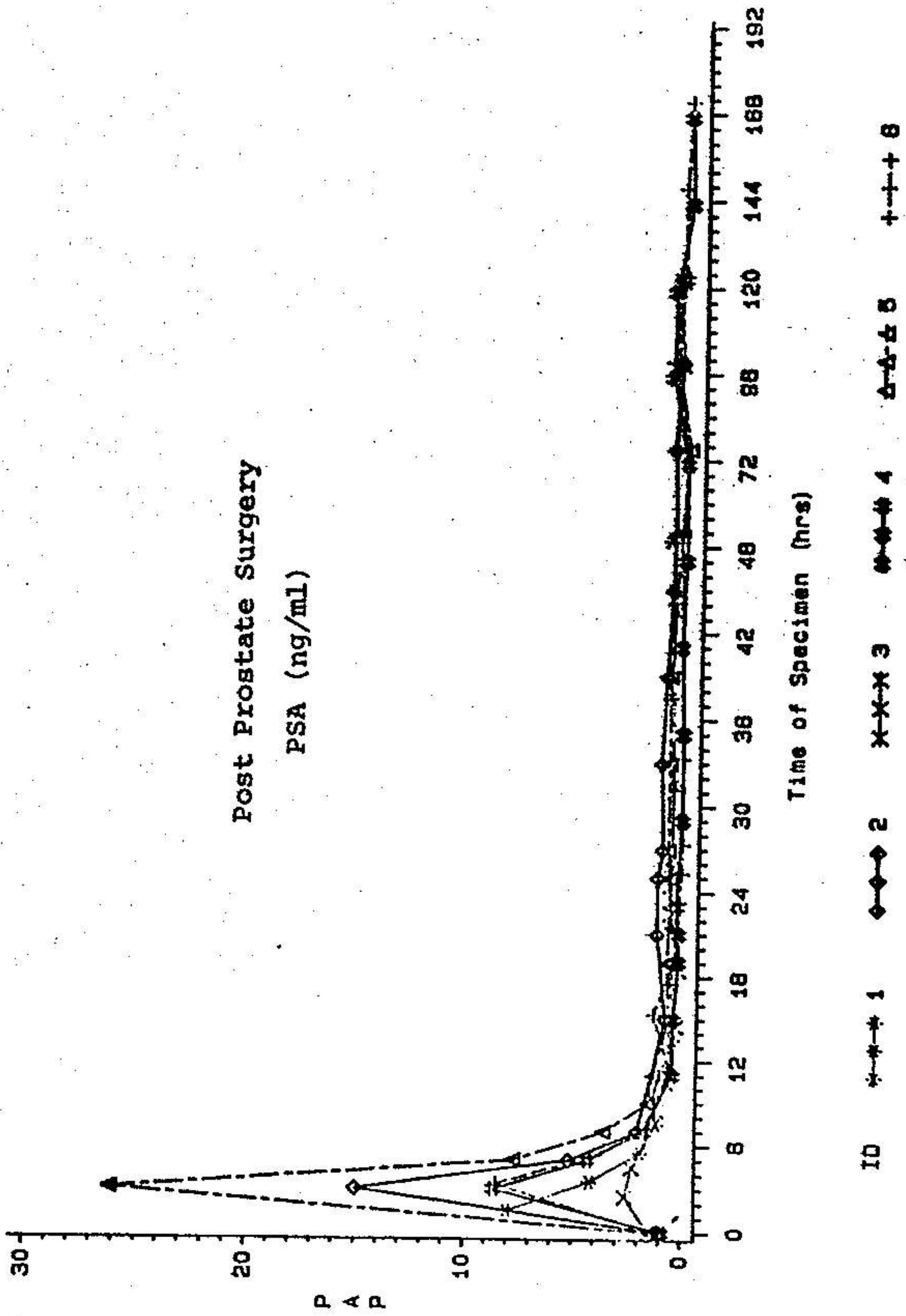


Fig-1



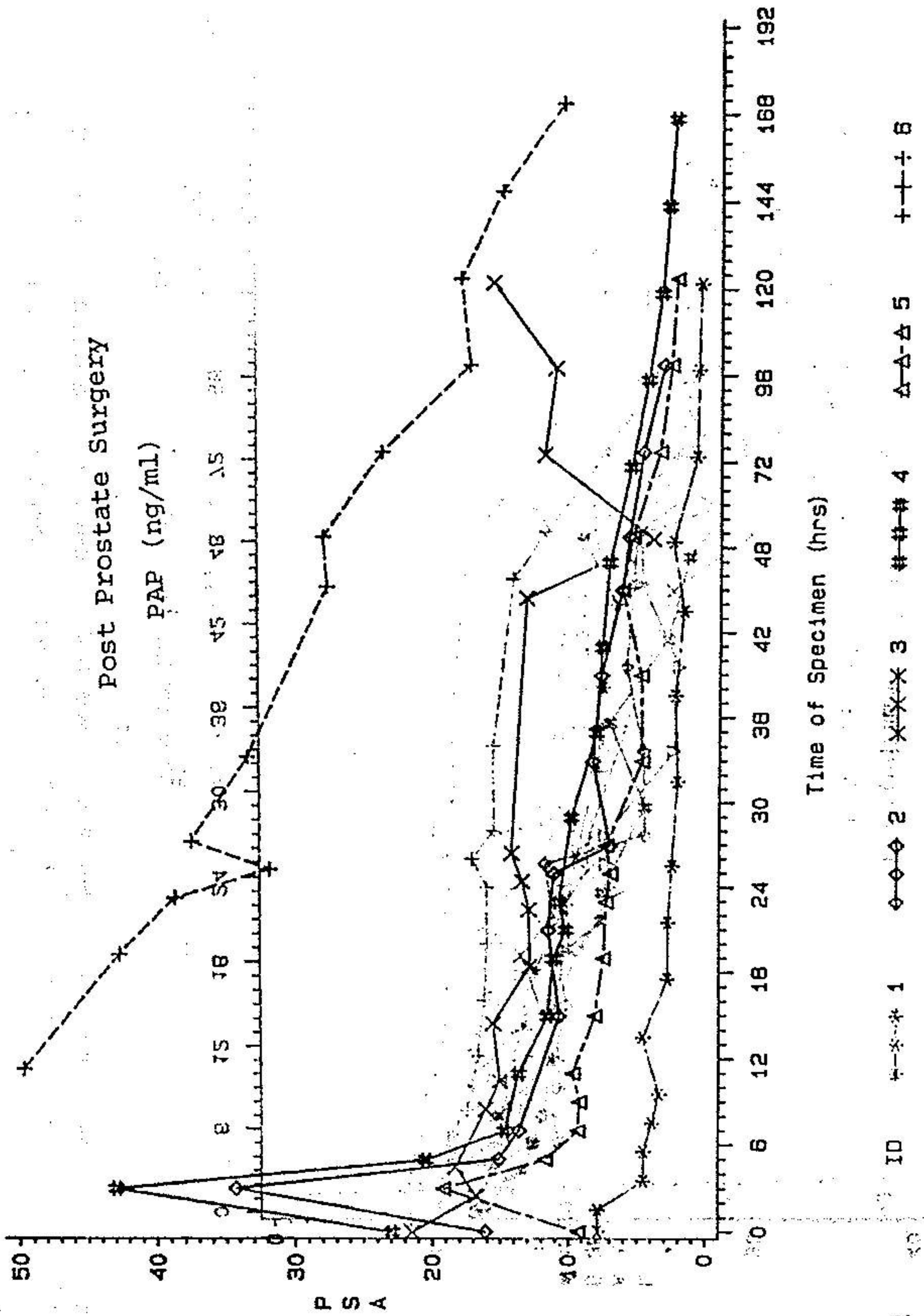


Fig-2

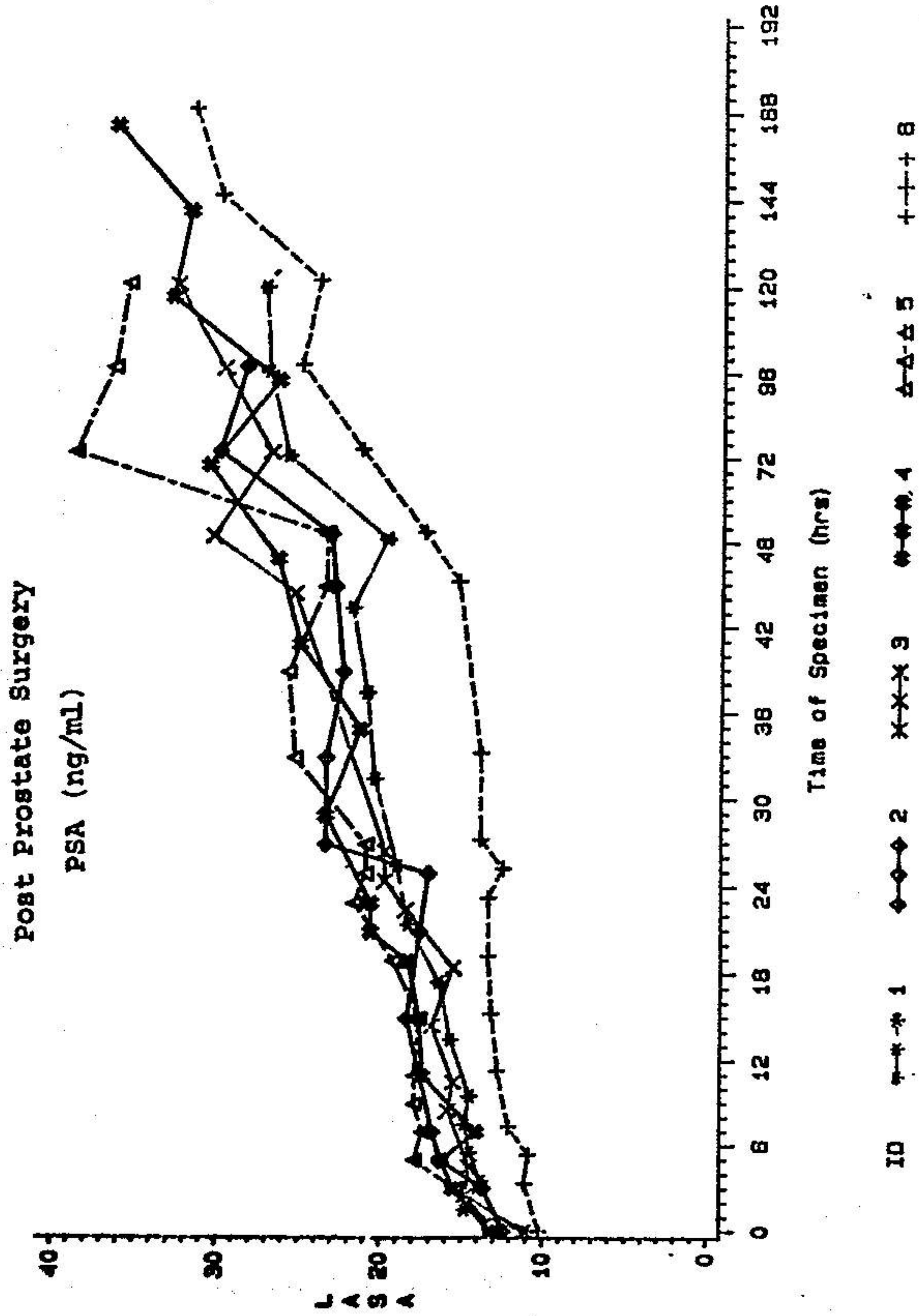


Fig-3



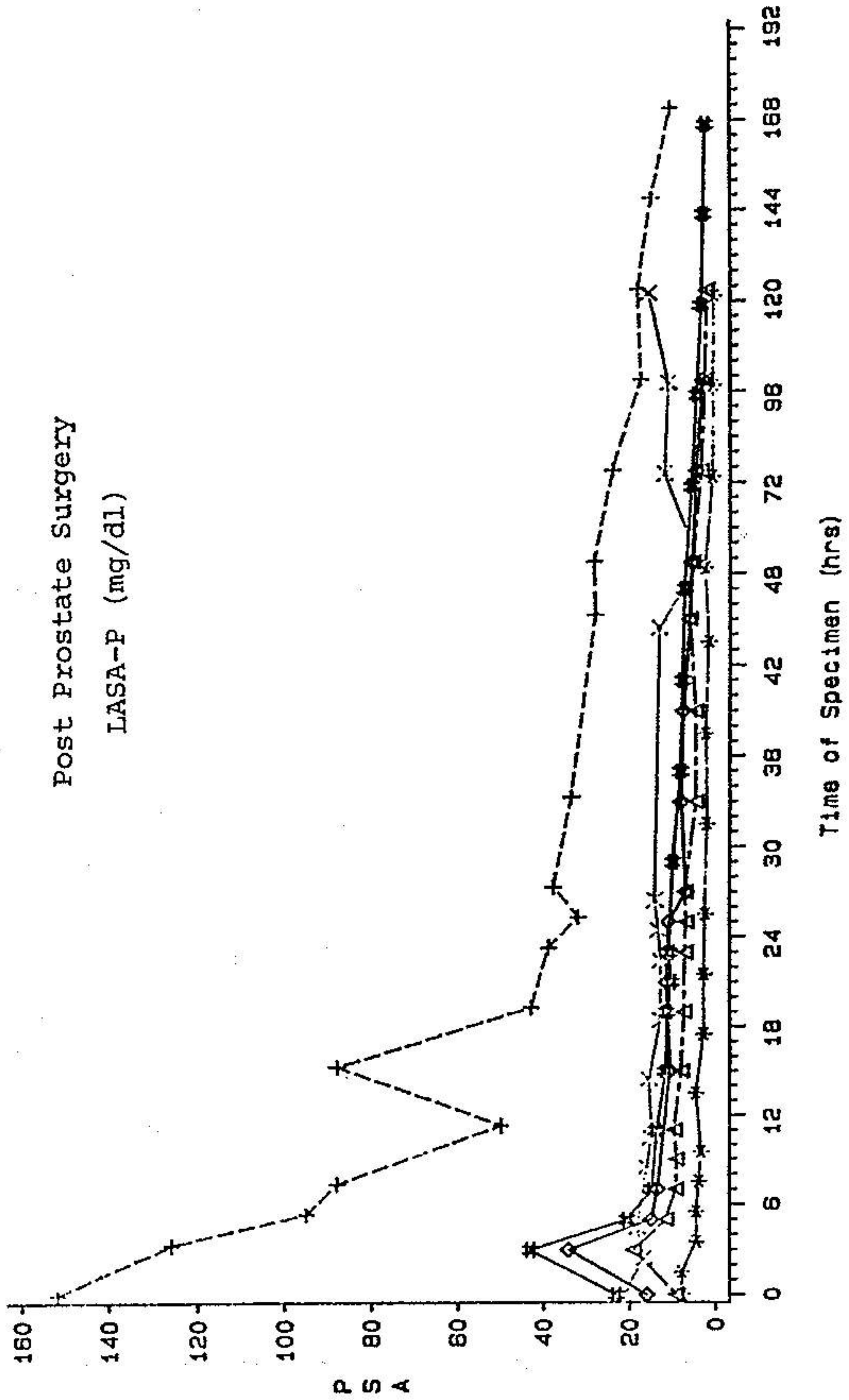


Fig-4 ID \*-\*-\* 1    ◊-◊-◊ 2    x-x-x 3    #-#-# 4    Δ-Δ-Δ 5    +--+ 8

bilities to monitor the evolution of this disease.(9)  
It appears by several studies that PSA is a more sensitive and specific marker than PAP for prostate cancer.(9)  
In order to define the rate of disappearance and the prognostic significance of 3 markers (2 specific for prostate and 1 commonly elevated in malignancies) we undertook the present studies. It is evident that SPSA is highly sensitive since it was elevated in 4 out of 6 patients with clinically localized prostatic cancer. The elevation was not noted on SPAP. As observed, prostatic manipulation during surgery greatly elevated both prostatic specific markers as well as LASA. Prostatic acid phosphatase returned to normal within 12 hours and remained normal in all patients. Serum PSA however took longer to return to normal (36-72 hours) and in 2 patients remained elevated throughout the study. These two patients, 1 had extensive disease involving the bladder neck on pathological examination. and the other has developed metastases to the ribs three months after surgery.  
From this preliminary observation it is clear that SPSA had strong prognostic significance in this group of patients. Utilizing the present information. further studies need to be done to define populations at risk of developing metastasis, and the role of SPSA in predicting the appearance of disseminated disease.

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