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Research Paper

PERIACINAR RETRACTION CLEFTING AND D2-40 EXPRESSION IN PROSTATIC ADENOCARCINOMA

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Abstract

Retraction clefting is known to appear in various types of tumors, but it has only recently been recognized as a specific histological phenomenon. Previously, it was considered merely a laboratory procedure artifact, but lately, there have been some assumptions that peritumoral retractions actually represent lymphatic spaces. In our study, we analyzed neoplastic glands in 52 specimens of prostatic adenocarcinoma. Immunohistochemical analysis was performed using D2-40 antibody, to highlight lymphatic endothelium and thereby differentiate actual lymph vessels or lymphovascular invasion from periacinar retractions. Our results showed that the number of lymph vessels was significantly lower in tumorous tissue compared to adjacent normal prostatic tissue. On the other hand, the number of lymph vessels in tumorous tissue was significantly higher than the number of lymph vessels mimicking periacinar retractions. Overall, the number of lymph vessels mimicking periacinar clefts was particularly low. These results are in accordance with our previous studies, which had shown that periacinar clefting appears due to lack of basal cells and stromal changes around tumorous acini. Also, these results support our hypothesis that retractions do not represent lymph vessels but should be considered a distinct entity, which is proven to be helpful both as diagnostic and predictive factor.

Key words: D2 40; lymphovascular invasion; prostatic adenocarcinoma; retraction clefting

Introduction

Clefting is a well known histological phenomenon described in various carcinoma types beside prostatic carcinoma, mainly in basal cell carcinoma and breast carcinoma. The neoplastic cells of prostatic cancer often appear pulled away from the surrounding stroma, leaving empty spaces that completely or partially encircle the acini; these are called retraction clefts or retraction artifacts and were described for the first time in 1960s in autopsy studies by Halpert and co-workers [1, 2]. Periacinar retraction clefts in prostatic carcinoma serve as a helpful additional criterion in setting of pathohistological diagnosis, particularly in differentiating it from some benign conditions (atrophy, postatrophic hyperplasia, atypical adenomatous hyperplasia), which may mimic prostatic carcinoma [3-8]. They are mostly seen in Gleason grade 3, occasionally appear in grades 2 and 4 but are uncommon in comedo type Gleason grade 5 carcinoma [6]. The origin of clefting is still not clear. At first considered a consequence of inadequate laboratory procedure, it has also been suggested that clefts actually represent pre-lymphatic spaces or lymph vessel compartments [9]. However, in our opinion they probably result from lack of basal cells and/or stromal changes [10, 11]. Some authors suggest that the presence and extent of clefts around tumorous tissue, not only in prostatic adenocarcinoma but also in some other tumors, especially breast carcinoma, can predict nodal metastasis and patients' outcome [12-14]. Moreover, the presence of extensive retraction clefting in prostatic carcinoma is associated with more aggressive tumor phenotype and a shorter biochemical recurrence-free interval [15].

A few years ago D2-40, a novel monoclonal antibody directed against mucin-type transmembrane glycoprotein podoplanin, specifically expressed by lymphatic endothelial cells, was introduced [16]. D2-40 was originally raised against M2A antigen, a surface sialoglycoprotein first detected in association with germ cell neoplasia and fetal testicular gonocytes [16]. The main significance of D2-40 lies in the fact that it is a selective marker of

lymphatic endothelium, it is unreactive with vascular endothelium and can be used to identify lymphatic invasion of primary tumors [17, 18]. In our study, we used D2-40 to detect lymph vessels in prostatic cancer and compare their presence and distribution with periacinar retraction clefts. Aim was to confirm our hypothesis that clefting is a distinct entity unrelated to lymphatic system.

Materials and methods

Neoplastic glands were analyzed in 52 consecutive specimens obtained from patients who underwent radical prostatectomy due to prostatic adenocarcinoma in the period from January 1st to June 30th 2006. Specimens were taken from the archive at the Ljudevit Jurak Department of Pathology, Sestre milosrdnice University Hospital Centre, Zagreb. Overall, 13 of 52 (25%) patients had extraprostatic extension and/or seminal vesicle invasion. Six (11.5%) patients had lymph node metastases.

All available sections were examined on both hematoxylin and eosin (HE) and immunostained slides. Lymphatic endothelial lining was highlighted using D2-40 antibody, enabling the distinction between lymphovascular invasion, defined as prostate adenocarcinoma cells within D2-40 positive compartments, and retracted periacinar stroma. Therefore, to evaluate the number of lymph vessels among periacinar clefts, each section was first examined under low magnification field (x100) on HE stained slide, than the same section was examined on D2-40 immunostained slide.

The area with highest number of D2-40 positive compartments was selected under lower magnification (x100) and than lymph vessels were counted under high magnification (x400) on 10 high power fields (HPF), in both tumor and surrounding normal prostatic tissue.

Similarly, thirty glands with most extensive clefts were selected on HE slides under low power magnification, marked, and than lymph vessels were counted in the same area on immunostained slides.

The number of lymph vessels mimicking periacinar retraction clefts was counted on the whole mount section under high magnification (x400), on immunostained slides. That referred to lymph vessels appearing to be periglandular retractions on HE slides, but as demonstrated by D2-40, represented lymphovascular invasion.

Immunostained sections were examined by three independent pathologists (U.M, T.D. and K.B.) and the final score was determined as a mean value counted by individuals.

Tissue samples were fixed in 10% buffered formaldehyde for 24 hours after surgery. Following fixation, prostate samples were cross sectioned through regions and placed into tissue containers. Samples were fixed for another 24 hours, embedded in paraffin, cut at 5 μm and routinely stained with hematoxylin and eosin. Each sample contained at least 5% of tumorous tissue and normal prostatic tissue that was used as an internal control. Lymph vessels were demonstrated with D2-40 antibody (Novocastra, mouse antibody, clone 49, 1:100).

Immunohistochemical staining was performed using standard procedures on DAKO TechMate Horizon automated immunostainer using antibodies to D2-40.

Briefly, 5 μm tissue sections were deparaffinized. Antigen retrieval for D2-40 was performed in a steamer using citrate buffer (pH 6.0) for 30 minutes. After blocking with hydrogen peroxide and normal goat serum, the sections were incubated with primary monoclonal mouse anti-human antibodies against D2-40 (Novocastra, mouse antibody, clone 49, 1:100) for 30 minutes at 37°C. The sections were incubated sequentially with biotinylated goat anti-mouse immunoglobulin and peroxidase-conjugated streptavidin. Primary antibody was replaced by phosphate-buffered saline in negative control sections. Color was developed by incubation

with 3, 3'-diaminobenzidine tetrahydrochloride and slides were counterstained by hematoxylin.

Statistical analysis was performed using Mann-Whitney U test. Pearson X^2 test was used to compare Gleason score with the number of lymph vessels mimicking periacinar retractions and number of lymph vessels in the area with most extensive periacinar retractions. The level of significance was set at $p<0.001$.

Results

The number of lymph vessels ranged between 2 and 8 (median value 4) in tumorous tissue and between 3 and 14 (median 6) in surrounding, normal prostatic tissue, counted on 10 HPF. The number of lymph vessels mimicking periacinar clefts ranged between 0 and 6 on whole cross section (median 0) (Figure 1A). In 30 tumorous glands with most extensive retractions, the number of lymph vessels ranged between 0 and 5 (median 1) (Figure 1B). The results are summarized in Table 1.

The number of lymph vessels in tumorous tissue was significantly lower than in surrounding, normal prostatic tissue ($p<0.001$) (Figure 2A). On the other hand, the number of lymph vessels in tumorous tissue was significantly higher than the number of lymph vessels mimicking periacinar retractions in the same area ($p<0.001$) (Figure 2B). There was also a statistically significant difference between the number of lymph vessels in the area with 30 glands with most extensive retractions and lymph vessels mimicking retractions, the latter being less frequent ($p<0.001$). (Figure 2C).

There was no statistically significant correlation between lymph vessels mimicking periacinar retractions and Gleason score (P= 0,728), or between lymph vessels in area with 30 glands with the most extensive retractions and Gleason score (P=0,690).

Discussion

While periacinar retraction clefts are a common finding in formalin-fixed, paraffin-embedded tissue samples, they are usually absent on frozen section material, therefore considered a laboratory procedure artifact. Frequently, periacinar retractions are difficult to distinguish from lymphovascular invasion. Moreover, Irie J et al [9], suggest that clefts actually represent lymph vessel compartments, based on the analysis of breast carcinoma [9]. According to our results, lymph vessels can mimic periacinar clefts inside tumor, but most periacinar retraction clefts do not represent lymphatic spaces. In our study, in areas containing 30 glands with most extensive retractions, the number of lymph vessels was low and ranged from 0 to 5 (median 1) and although some clefts represented lymphovascular invasion, none of these lymphatic spaces were in the area with most prominent clefts.

In our previous study, we analyzed periacinar retractions in p63 immunostained material of prostatic intraepithelial neoplasia (PIN) and prostatic carcinoma [19]. We found that retractions are significantly more prominent in prostatic carcinoma, compared to PIN and non-neoplastic glands, in which they only occur sporadically [6,19]. These findings are consistent with previous assumption that clefting is associated with lack of basal cells and stromal changes. Neoplastic glands lack basal cell layer, hence are always p63 negative, PINs stain positive to p63 in the whole circumference of the gland or discontinuously, while normal glands are positive in the whole circumference [20,21]. Stromal change preceding invasive stage of cancer is the appearance of myofibroblasts/fibroblasts which replace smooth muscles, the normal constituent of prostatic stroma. Our previous studies confirmed the presence of myofibroblasts in prostate cancer; especially in Gleason pattern 3 tumors, which is also the pattern with most prominent periacinar retractions [6], but myofibroblasts were not found in surrounding normal tissue [11]. Similar studies have been made in breast carcinoma. The

study by Acs et al [8] showed the presence of retractions in 60% of invasive carcinomas, and their rare occurence around in situ carcinomas or benign ducts and acini.

Recent studies have also focused on potential prognostic significance of retraction clefting in various types of tumors, including prostatic adenocarcinoma. Acs and co-workers found that breast carcinomas associated with lymphovascular invasion and lymph node metastasis had significantly higher percentage of retraction artifacts compared to tumors without these features. Furthermore, extensive retractions were significantly associated with both poor overall prognosis and disease-free interval [12]. Recently, same authors have confirmed these findings in core needle biopsy material of invasive ductal carcinoma [13]. Similar studies showed statistically significant correlation between extensive peritumoral retractions in esophageal squamous cell carcinoma and lymph node metastasis [14]. In our previous study, we examined whether extensive retractions could predict biochemical reccurence-free survival in prostatic carcinoma. The extent of retraction showed a statistically significant positive correlation with preoperative PSA and negative correlation with biochemical disease free survival [15]. Also, tumors associated with seminal vesicle invasion and/or extracapsular extension showed significantly higher percentage of retraction artifact than tumors without these features [15].

Lymphangiogenesis has been associated with poor prognosis in a number of human cancers. Its prognostic significance in prostate cancer is uncertain. Some studies suggest that lymph vessel density inside intratumoral compartment of prostatic carcinoma is reduced [22,23] as it is in our study. Roma et al [22] associated higher peritumoral lymphatic vessel density with higher Gleason score and peritumoral invasion with frequent lymph node metastasis. Cheng et al [23] did not correlate these findings and concluded that quantification of lymphangiogenesis in prostate adenocarcinoma does not offer useful prognostic information. According to our study, the number of lymph vessels is significantly lower in tumorous tissue

compared to peritumoral compartment and it does not correlate with Gleason score. We have not found any statistically significant correlation between lymph vessel density and nodal metastasis, but any possible conclusions in this regard are hampered by the fact that only six patients in our study group had nodal metastasis, which is insufficient number for reliable statistical analysis. Number of lymph vessels mimicking clefts (lymphovascular invasion) is particularly low. These results support our hypothesis that clefts appear due to lack of basal cells and stromal changes around tumorous acini and that clefts do not represent pre-lymphatic or lymphatic spaces but should be considered a distinct entity.

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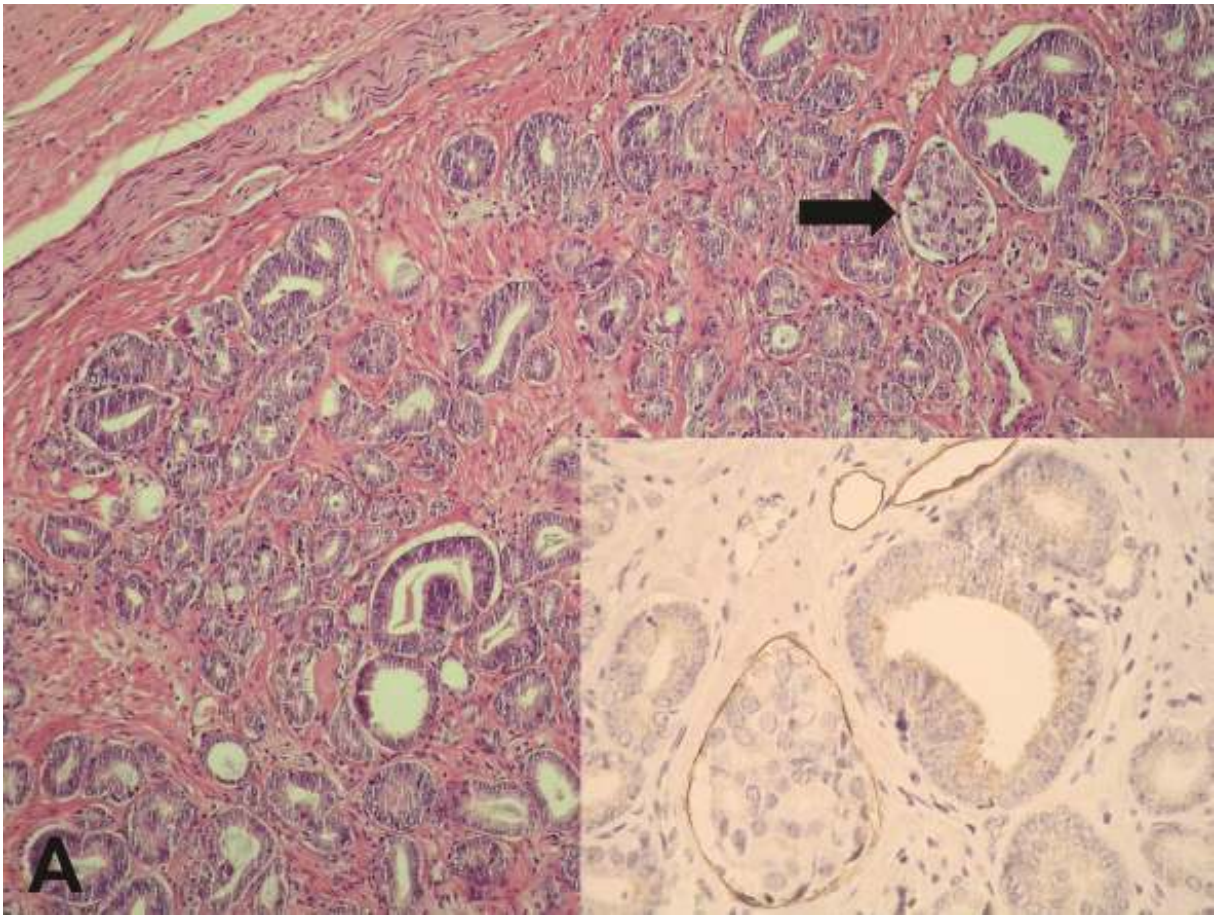
Figure 1. A, Lymphovascular invasion, detected with D2-40 immunostaining (inserted slide, 400x), appearing to be periglandular clefting on HE slide (200x). B, Expressed periglandular clefting on HE slide (200x) near lymph vessels evident on D2-40 immunostained slide (inserted slide, 400x).



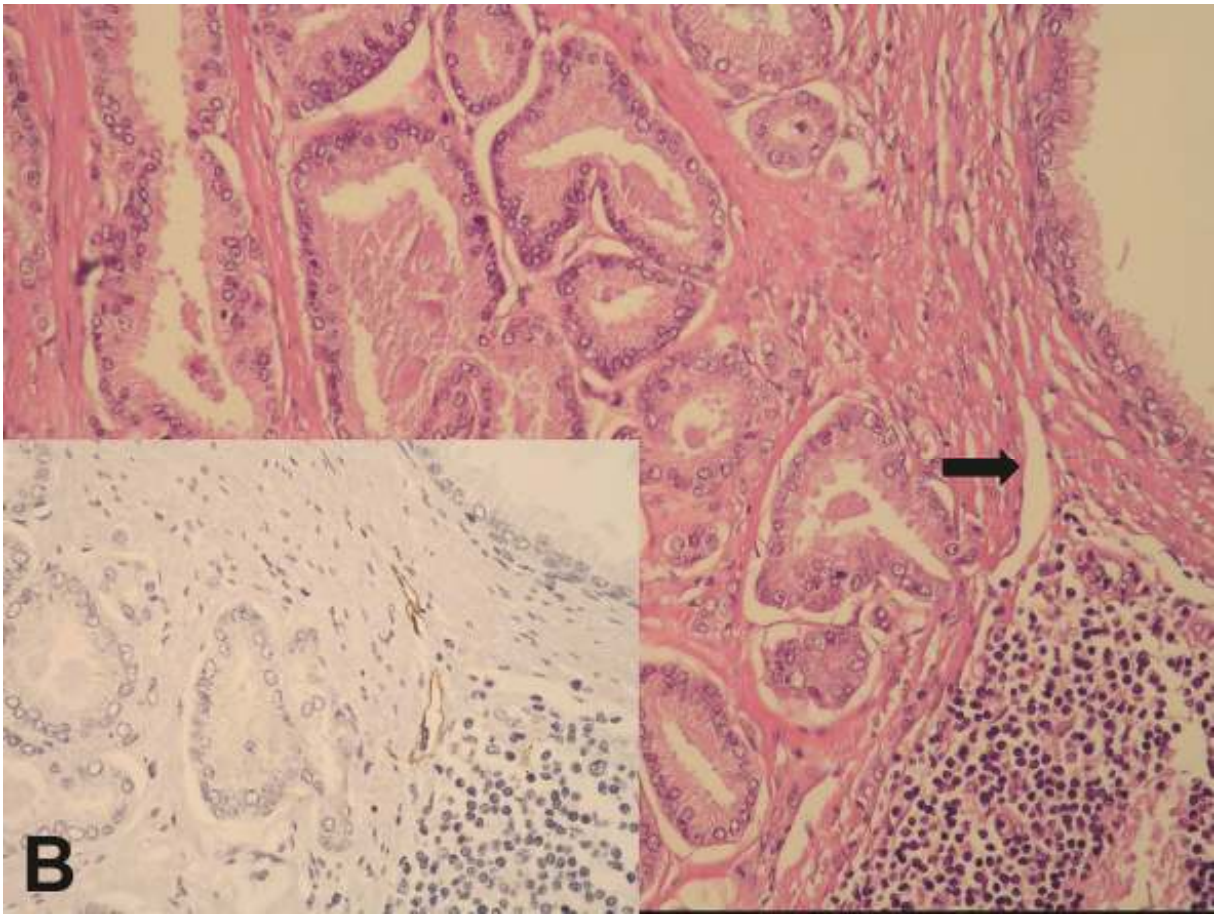
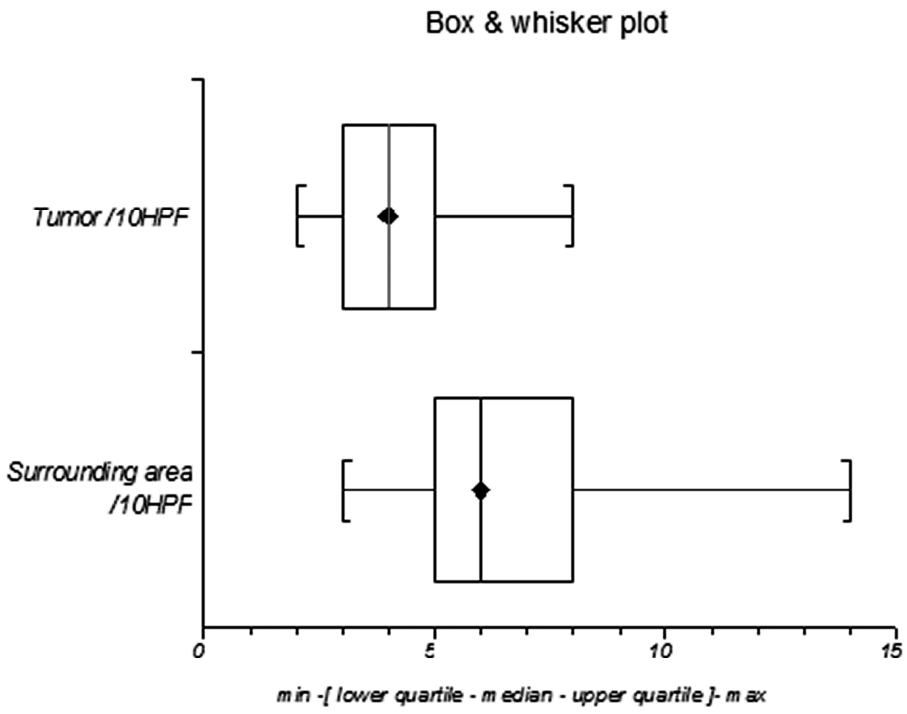
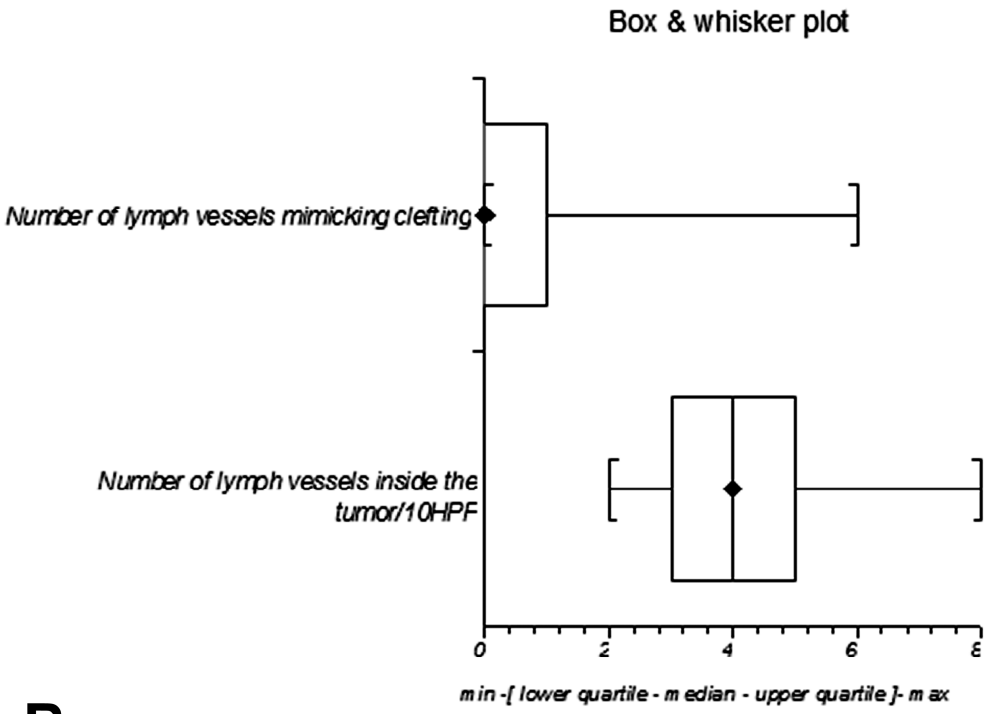

B

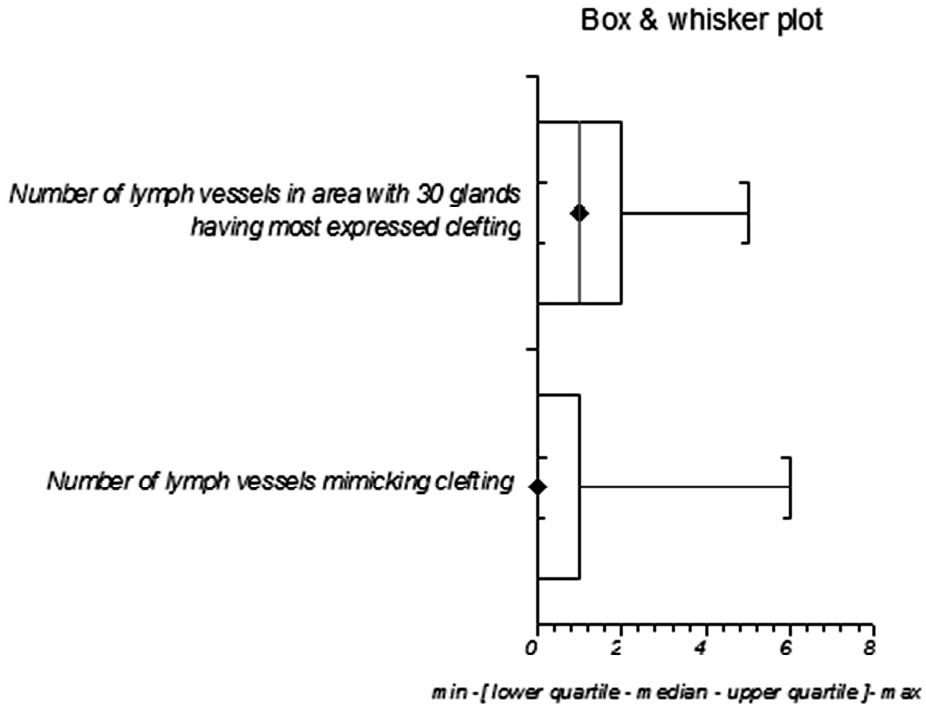
Figure 2. Correlation between the number of lymph vessels in tumor and adjacent prostatic tissue (A), between lymph vessels mimicking periacinar clefting and number of lymph vessels in tumor (B) and between the number of lymph vessels within the area with 30 neoplastic glands with most extensive retractions and lymph vessels mimicking periacinar clefting (C).



A


Box & whisker plot
Number of lymph vessels mimicking clefting
Number of lymph vessels inside the tumor/10HPF
0
2
4
6
8
min -[lower quartile - median - upper quartile]- max

B


Box & whisker plot
Number of lymph vessels in area with 30 glands having most expressed clefting
Number of lymph vessels mimicking clefting
0
2
4
6
8
min -[lower quartile - median - upper quartile]- max

C

Table 1. The number of lymph vessels in tumor tissue and surrounding prostatic parenchyma.

	Specimen number	***Max***	***Median***	***Min***
No. of lymph vessels in surrounding prostatic tissue/ 10 HPF	52	14	6	3
No. of lymph vessels in tumorous tissue/ 10HPF	52	8	4	2
No. of lymph vessels mimicking clefting (lymphovascular invasion)/ whole section surface	52	6	0	0
No. of lymph vessels in the area with 30 tumorous glands revealing most extensive retractions	52	5	1	0