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European Association of Urology

Letter to the Editor

Re: Gillian Vandekerkhove, Werner J. Struss, Matti Annala, et al. Circulating Tumor DNA Abundance and Potential Utility in De Novo Metastatic Prostate Cancer. Eur Urol 2019;75:667–75

How Does Circulating DNA Reach the Blood Stream?

We have read with great interest the contribution published in the current issue of *European Urology* by Vandekerkhove et al. [1]. The authors observed that "tumor DNA shed into the bloodstream can be measured via a blood test. The information from this test provides complementary information to a prostate needle biopsy and could be used to guide management strategies" [1]. Other publications, including reviews, have also appeared in recent times, showing, more in general, the clinical significance and role of liquid biopsy in patients with prostate cancer (PCa) [2–5].

This has led us, a transnational group of closely collaborating pathologists, urologists, and oncologists, to consider the ways tumor DNA can reach the blood circulation [4,5]. One way could be related to blood and/or lymphatic vessels located in the tumor stroma or tumor vascular invasion. The lymphatic way, at a certain point, reaches the blood stream, thus allowing the circulating tumor DNA (ctDNA), and more in general cell-free DNA (cfDNA), to become detectable. In the last few years, our group has examined thousands of radical prostatectomy (RP) specimens with the complete sampling procedure and processed these samples with the whole mount technique. Based on such material, lymphatic vessel tumor invasion (Fig. 1A) have been observed in a minority of cases, the proportion being similar to that reported in a recent paper by Wilczak et al. [6], that is, 14%. The observation of tumor invasion of blood vessels (Fig. 1B) is exceedingly rare. Since ctDNA is detectable in most of the PCa patients, there should be another way cfDNA and therefore ctDNA can reach the blood stream.

For this reason, we have re-examined recent RP cases of our series, having in mind the idea of finding an additional relationship between the tumor in the stroma and the contents in the lumen of the blood vessels. In several prostates, we have found dilated "empty spaces" in different anatomical locations. The shape of these "empty spaces" varies from round to oblong and slit like. Usually, they are not isolated but remain in groups. The most frequent locations are the lateral (Fig. 2) and apical regions of the prostate, and around the urethra (Fig. 3A), ejaculatory ducts, and seminal vesicles. To confirm their blood vessel nature, selected cases have been sectioned serially. This approach has shown groups of erythrocytes in the lumen (Fig. 3B). Such a finding has not been seen in an additional series of cystoprostatectomy specimens, with urothelial carcinoma in the bladder but no cancer in the prostate gland.

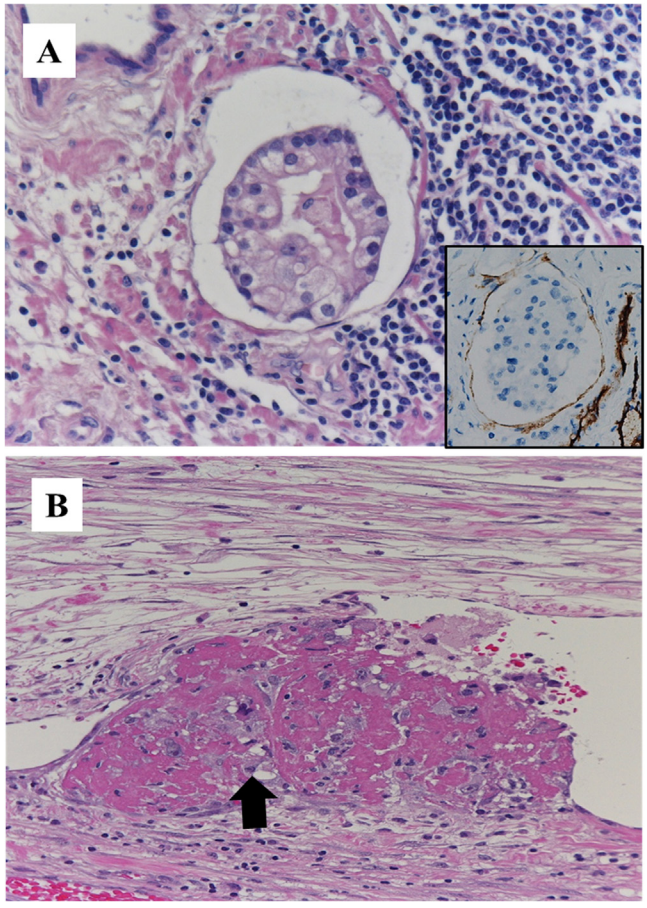

Fig. 1 – (A) Lymphatic tumor invasion (insert: lined by D2-40-positive endothelial cells; see text) and (B) blood vessel tumor invasion with neoplastic cells (arrow).

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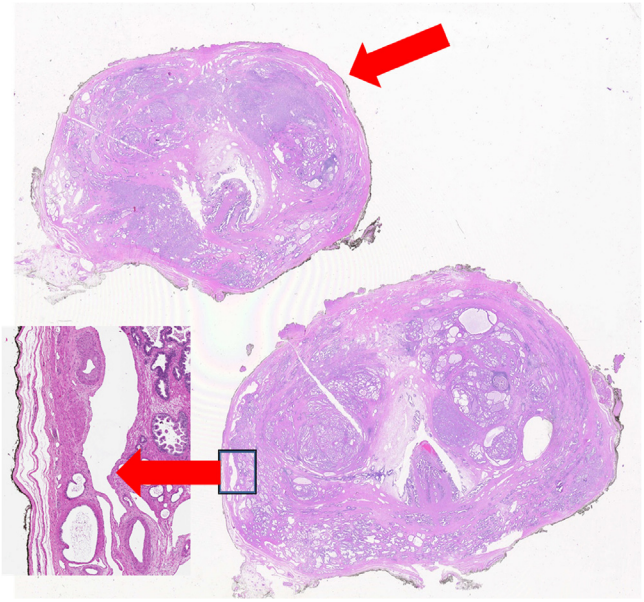

Fig. 2 – Slit-like "empty spaces" in the lateral aspect of the body of the prostate (arrows and enlarged).

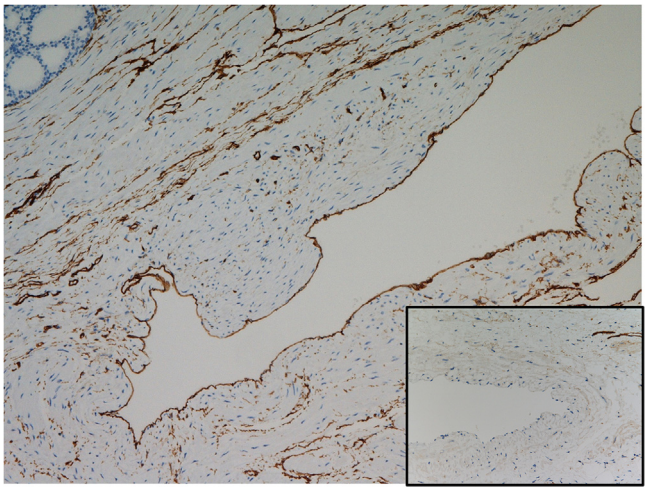

Fig. 4 – "Empty spaces" are lined by C31-positive cells (ie, endothelial cells; insert: the same cells are D2-40 negative).

These empty spaces are lined by a very thin layer of flattened cells. Based on their morphology, such cells could be endothelial cells, epithelial cells (as in cystically dilated ducts and acini), or mesothelial cells (as in adenomatoid tumors, for instance). To answer this question, immunohistochemical investigations have been conducted. The cells are strongly and diffusely positive for CD31, CD34, and ERG (markers for endothelial cells in blood vessels and lymphatics; Fig. 4), and negative for D2-40 (a marker for endothelial cells in lymphatics; Fig. 4, insert), cytokeratins, and mesothelial markers, including calretinin. Based on

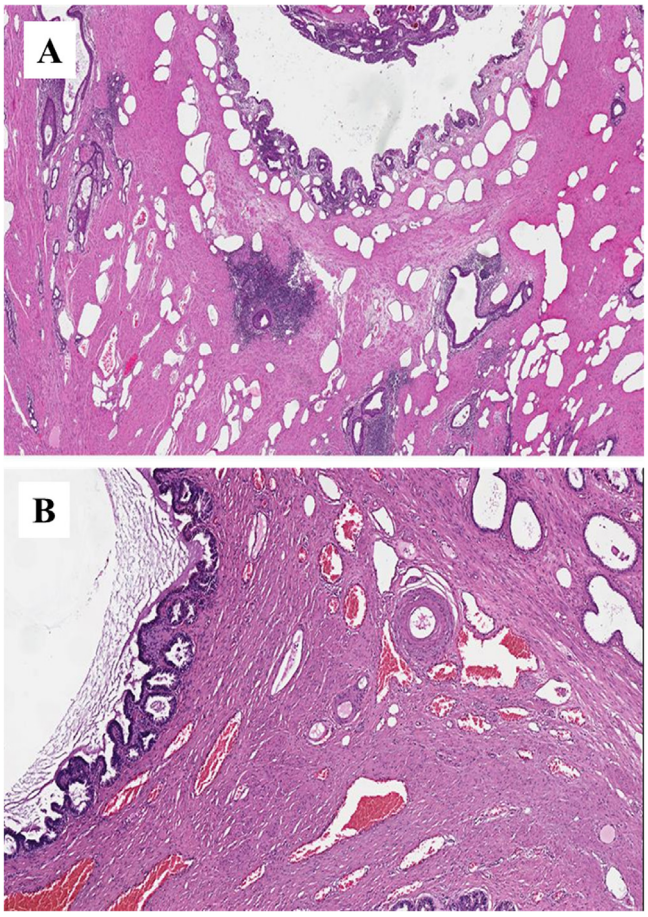

Fig. 3 – "Empty spaces" (A) around the urethra and (B) around an ejaculatory duct, in the latter location containing erythrocytes.

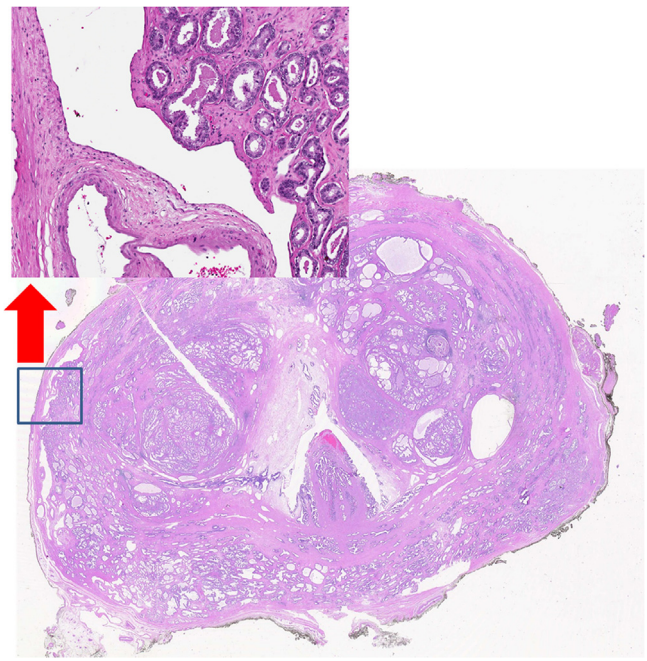

Fig. 5 – Slit-like vessels with tumor cells protruding into the lumen (enlarged).

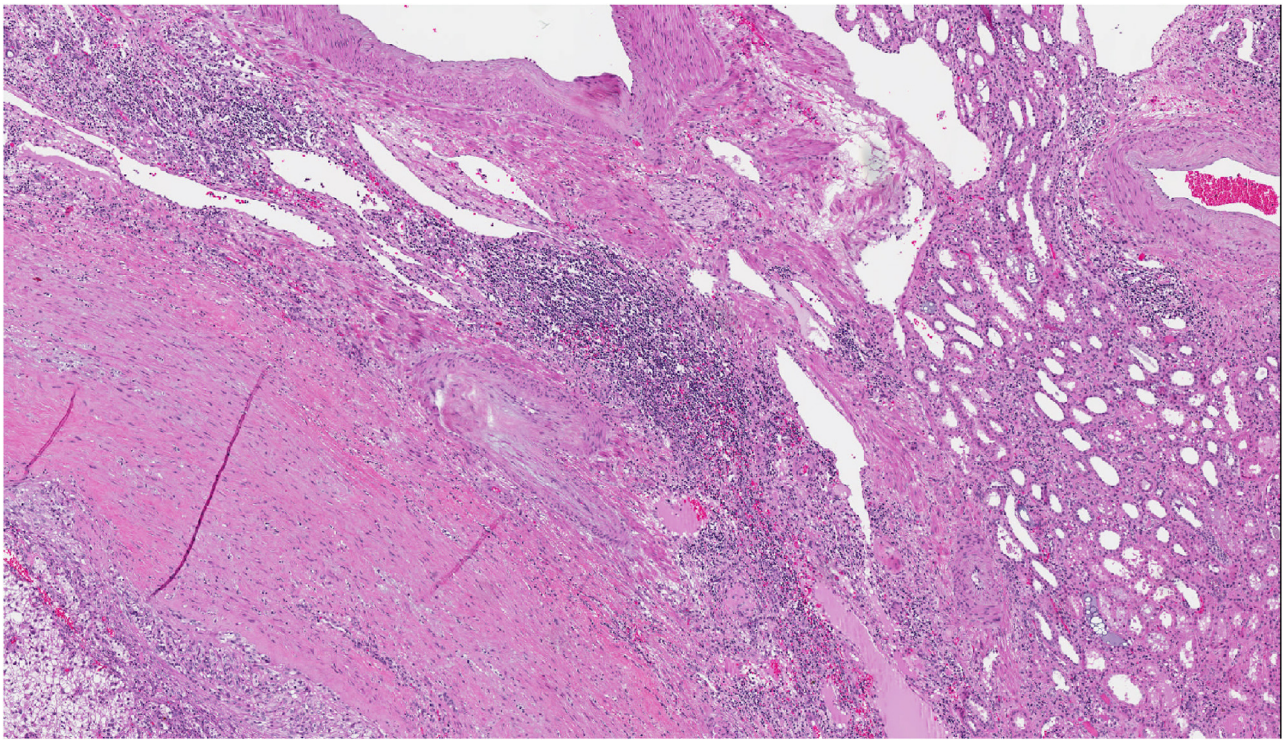

Fig. 6 – "Empty spaces," that is, small vessels with a sinusoidal pattern, in the kidney parenchyma surrounding a clear cell renal carcinoma.

such results, it has been concluded that the empty spaces are dilated blood vessels lined by endothelial cells resting on a very thin periodic acid–Schiff (PAS)-positive basement membrane, the appearance being that of blood sinusoidal spaces.

We then have tried to find a relationship with the tumor. When tumor is present in the area with such "empty spaces," these are always located at the periphery of the tumor nodule. In particular, it is not infrequent that the tumor is protruding into the lumen, however, covered with an intact endothelial barrier (Fig. 5). We serially sectioned some of our cases and have not seen the tumor ulcerating the endothelial lining, growing into the lumen, and eliciting the deposition of fibrin (see Fig. 1B for comparison). This does not exclude that our observation could represent an initial step, in some cases, in the development of vascular invasion. There is no relationship between such a feature and the grade and stage of the tumor, that is, such a feature is not seen only in high-grade and advanced-stage tumors.

To the best of our knowledge, this is a novel observation and explanation for what we see with liquid biopsy and more in general via a blood test. However, this does not exclude the role of small capillaries in tumor stroma.

In conclusion, even though the frequency and degree of the vascular changes are not quantified in the current contribution, this was not the aim of the morphologic observations, we have tried to identify the spectrum of vascular lesions associated with PCa. Based on all these, the tumor induces the formation of small vessels, or angiogenesis, in the way it has been traditionally depicted or shown. It also induces the formation of sinusoidal spaces into which ctDNA is shed by the tumor itself, through or filtered by the endothelial barrier. Such a pattern can be seen in other organs with cancer. Fig. 6 is an example of the same pattern seen in the prostate, but in the renal parenchyma adjacent to clear cell renal cell carcinoma. A criticism of our observation is that our findings described here, seen also in cases without the type of vascular invasion of Fig. 1A and B, reflect an already existing vascular pattern accentuated by the surgical or tissue-processing procures.

Conflicts of interest: The authors have nothing to disclose.

References

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