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Typical and Atypical Circulating Tumour Cells in Bladder Cancer. Why Improve our Knowledge?

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Abstract

Liquid biopsy is the new frontier in cancer biology: easy to perform at every stage of disease course, low invasive, and not painful, it looks amazing. In this field can recognize two main characters: circulating tumour cells (CTCs) and circulating tumor DNA (ctDNA). They can be considered as a patient's individual tumour fingerprint, promising an easiest way to follow cancer progression. Taken together they all seem bright and sparkle, in reality they find low application in clinics, especially in bladder cancer where only the CTC count has been correlated to clinical outcomes. Since stage bladder cancer is difficult to manage due to chemotherapy resistance, recurrences and few therapeutics protocols, we need to go over simply count of CTCs, and use them to improve the patient's management therapy and find potential new biomarkers for innovative pharmacological treatments. Here we provide an overview about the potential employment of CTCs in clinics highlighting the relevance of non-clinically detectable CTCs, named "atypical CTCs", which may be responsible for recurrences and chemotherapy resistance.

Liquid biopsies in bladder cancer, why so important?

The first time the presence of CTCs has been reported was in 1869, 150 years ago, and now they are considered the newest frontier in cancer biology. CTCs are cells originated from either a primary or a metastatic site, which could develop metastatic foci in a distant area or district. To achieve this, they must both survive to sheer stresses and escape to the host immune system, and then they have to extravasate and adapt themselves to a new local microenvironment. Liquid biopsy relying on analysis of such cells, together with ctDNA, has increased hopes to finally decrypt the metastatic process. So both CTCs and ctDNA could play a pivotal role in precision and personalized medicine, providing important information as cancer biomarkers, prognostic data and for therapy design [1,2].

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Table 1: Tests approved by FDA for detection of bladder cancer [6,7].

Test	Protein	Assay type	Sensitivity	Specificity
BTA stat	Complement factor H – related protein and complement factor H	Immunoassay or point-of-care	57 – 83%	60-92%
BTA TRAK	Complement factor H – related protein and complement factor H	Sandwich ELISA	66%	65%
NMP22 Bladder Chek	Nuclear mitotic apparatus protein	Sandwich ELISA or point-of-care	47-100%	69-79%
uCyt+	Carcinoembryonic antigen, two bladder tumorcell-associated mucins	Immunocytochemistry	50-100%	69-79%
UroVision	Alterations in chromosomes 3, 7, 17, and 9p21	FISH	41-70%	80%

Bladder cancer is the most common malignancy of the urinary tract and the ninth most common cancer worldwide, and in advance stages is difficult to treat and manage because of its high recurrence rates, rapid progression, poor response to chemotherapy, and lack of novel targeted therapeutics [3-5].

Diagnostic protocol for bladder cancer begins with cystoscopy and computed tomography (CT) images. The first is considered the golden standard radical treatment, whereas the latter one is recommended as surveillance method. Indeed, 50% of recurrence cases will occur within 5 years, possible answers can be found in undetected micro metastatic diseases after cystectomy. Although unsettle, it is tempting to hypothesize that micrometastatic disease is responsible for recurrences, so their implementation in surveillance can be more effective than CT imaging alone, optimizing patient's management. Scientists are now involved in urinary biomarkers research in order to strengthen standard bladder cancer detection protocols, however, few of them are used in clinics and have received Food and Drug Administration (FDA) approval (Table 1) [6-8,10]. The same for blood-based tests, or "liquid biopsies", for micrometastasis or metastasis detection in post-cystectomy bladder cancer, where they could provide important evidences and information about possible residual disease, before conventional image detection, and even more sensitive and precise than them.

Even if there are still no valid demonstrations in clinical practices, implementing bladder cancer with novel liquid biopsies analysis can improve patient's life condition and expectancy. Unfortunately, CTCs applied to bladder cancer reported conflicting results [3,11-16]. CTCs have been detected in patients with localized muscle invasive bladder cancer, but also in patients with non-muscle invasive bladder cancer [12-14]. No association between CTC count and extravesical disease has been demonstrated [12]. However, there are findings regarding CTCs and bladder cancers demonstrating that the presence of CTCs is correlated with advance tumour stage, histological grade, metastasis and regional lymph node metastasis [3,11-14,17-23]. The presence of even only one CTC in cancer patient's blood sample is correlated with low survival and worse prognosis [13-15].

Most of these data are provided by using the CellSearch® technology (Janssen Diagnostics). CellSearch® is, until now, the only CTC assay approved by the FDA for breast [24], prostate [25] and colorectal cancers [26]. Mainly, CellSearch® relies on immune-magnetic positive selection of cancer cells using antibodies against EpCAM antigen. As CellSearch® there are other tests as AdnaTest (AdnaGen AG) [27], which works in the same way as CellSearch® with further RNA evaluation. These assays allow detection and the analysis of only EpCAM positive cells. EpCAM protein is strongly expressed in epithelia and most carcinomas, thus it is involved in several cellular processes such as proliferation, migration and differentiation [27-30]. In addition to EpCAM expression CTCs are also cytokeratin (CK) positive and CD45 negative responding to "CTC-criteria" [31]. Nevertheless, there are several evidences coming not only from urogenital cancers field, but also from breast, lung, esophageal and colorectal cancers, which have demonstrated that there are other "atypical" CTCs which express heterogenous markers panelon their surface and could be correlated with worse outcomes (Table 2) [16,32-42]. Not all metastatic cells express EpCAM resulting in potential false negative, decreasing CellSearch® predictive value. Chalfin

Table 2: Atypical CTC markers.

Marker	Cancer type	Clinical impact	References
CK+, CD45+, EpCAM+/−	Breast cancer	Prognostic and predictive value for OS	[32,33]
	Pancreatic cancer	Correlated with advance stages of the disease	
CK+, EpCAM+, CD14+, CD11c+, CD45+/−	Breast Pancreatic Prostate	Increased risk of disease progression, number affected by treatment	[34–36]
CD14+, CD68+, CK+, EpCAM+, CD163+, CD204/6+	Melanoma	Not determined	[37]
CSV+, CD14+, CD68+, CD45−	Sarcoma (including gastrointestinal stromal tumors)	Predict metastasis	[38]
CD14+, CD68+, CD163+	Colon Breast Ovarian Colorectal cancer	Not determined	[39]
CD133+, N-cadherin+,CK-	Renal	Shortened progression free survival	[40]
CK +/-, PD-L 1	Bladder	Decrease OS	[16]

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and co-workers demonstrated the presence of EpCAM negative CTCs in patients with muscle invasive, non-muscle invasive and metastatic bladder cancer [43]. Also, CTCs negative for CK have been detected in patients with muscle-invasive and metastatic bladder cancer [44].

So, what about the negative or "atypical" circulating cell population?

In breast cancer, evidences show that EpCAM and CD45 negative cells are associated with significantly decreased overall survival (OS) [32], EpCAM negative and CK/CD45 positive cells are suggested to be correlated to tumour-associated macrophages with worse outcome [32]. Taken together, many experimental sets and clinical investigations have described the presence of EpCAM-negative and undetectable CTCs, but few were able to address their presence with clinical relevance. Moreover, several findings have demonstrated that CTC clusters have increased metastatic potential compared to a single cell, with shortened survival [41,45,46]. This may be correlated with epithelial-to-mesenchymal transition (EMT), in which the cell loses epithelial characteristics gaining mesenchymal and invasive features. EMT is assumed not only to increase tumour invasion, but also it contributes directly to therapy resistance, enable cell to escape from death acquiring stem cell capabilities and increase the aggressiveness of the tumour [41]. In addition to that, it could be useful in some cases the molecular marker expression analysis using digital droplet PCR (ddPCR) and next generation sequencing (NGS) in order to better classify CTCs and distinguish them from tumour-associated hematopoietic cells, as cancer associated macrophages-like cells or tumour-associated neutrophils [34,47,48].

In order to detect these "atypical" CTCs, it is required an improvement in clinical and laboratory techniques aimed to improve the sensitivity of the method, enriching CTC pools allowing the detection of possible useful new biomarkers through molecular biology assays by genetic, epigenetic and transcriptomic approaches.

There are many available techniques, which provide the isolation of all CTCs in patient's blood, independently on their marker expression. Today the scene is currently dominated by the density-based gradient tools as the FicollParque® and the novel OncoQuick®. This could overcome heterogenous marker expression problems, but findings demonstrated that there is effectively cell loss in the blood sample, maybe because of differentiae's in CTCs densities. Other label-free separation technologies are based on the biophysical properties of CTCs such as deformability and electrical properties (Parsortix system, ANGLE). Therefore, there are several CTCs assays, which allow studying from a different point of view these cells making possible future CTC applications in clinical medicine. Improvement in CTCs approaches could be useful to better classify and stratify patients and to divide them for pharmacological treat which may have more neoadjuvant chemotherapy benefit respect systemic chemotherapy treatment guiding for individual targeted therapies [3,17,49,50].

Conclusion

Since CTCs are detected in 50% of metastatic urothelial cancer, CTCs approaches has to be implemented with possible further prognostic role in recurrences detection, promising also preclinical, clinical, pharmaceutical and real-time surveillance data [12,13,19,41,51,52]. There is the need to determine an appropriate study designs within clinical evaluations of CTCs. However, an interventional study regarding CTCs, the "gold standard" for evaluating a new discovery and its causal impact on an outcome, as the randomized controlled trials, has some disavantages. Among them, the applicability of the study results to real-world situations may be limited by the study population characteristics, procedures implemented, outcomes measured and above all the time needed to arrive at an answer [53]. Undoubtedly it can be useful to include liquid biopsies in bladder cancer patients' management alongside standard diagnostic protocols. There is the necessity to improve the research and detection of typical CTCs, mesenchymal-like CTCs and in general atypical CTCs, which could have lost some epithelial cell features. Addition of extra markers specific for CTCs can aid to discriminate tumor cells from other events and creates a narrower definition, which will decrease the intra-reader variation and improve the identification of true CTCs. Indeed, as highlighted by Tibbe et al., when researchers exploit the number of CTCs analyzing the standard volume of liquid biopsy (7.5 ml) to stratify patients, the Poisson distribution has to be applied and the probability of over/underestimation is very high [54]. For all these reasons CTCs' enumeration itself should be avoided giving way to a new perspective. After detection CTCs can be further analyzed at the DNA, RNA and protein level to obtain global information on tumor biology and targets relevant to cancer therapy [54].

In conclusion, the analysis of CTCs complementary to other liquid biopsy biomarkers such as ctDNA or exosomes has the potential to improve the management of individual cancer patients and contribute to the vision of personalized medicine decreasing healthcare cost, ameliorating patient management and therapeutic design care.

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References

1. Gorin MA, Verdone JE, Toom E Van Der, Bivalacqua TJ, Allaf ME, Pienta KJ. Circulating tumour cells as biomarkers of prostate, bladder, and kidney cancer. Nat Rev Urol. 2017; 14: 90-97.

2. Batth IS, Mitra A, Manier S, Ghobrial IM, Menter D, Kopetz S, et al. Circulating tumor markers: Harmonizing the yin and yang of CTCs and ctDNA for precision medicine. Ann Oncol. 2017; 28: 468-477.

3. Zhang Z, Fan W, Deng Q, Tang S, Wang P, Xu P. The prognostic and diagnostic value of circulating tumor cells in bladder cancer and upper tract urothelial carcinoma : a meta- analysis of 30 published studies. Oncotarget. 2017; 8: 59527-59538.

4. Azevedo R, Peixoto A, Gaiteiro C, Fernandes E, Neves M, Lima L, et al. Over forty years of bladder cancer glycobiology: Where do glycans stand facing precision oncology? Oncotarget. 2017; 8: 91734-91764.

Citation: Maggi F, Amantini C, Nabissi M, Marinelli O, Santoni G and Morelli MB. Typical and Atypical Circulating Tumour Cells in Bladder Cancer. Why Improve our Knowledge? Clin Oncol. 2019; 2(2): 1008.

5. Chen C, Qi XJ, Cao YW, Wang YH, Yang XC, Shao SX, et al. Bladder Tumor Heterogeneity: The Impact on Clinical Treatment. Urol Int. 2015; 95: 1-8.

6. Tabayoyong W, Kamat AM. Current Use and Promise of Urinary Markers for Urothelial Cancer. Curr Urol Rep. 2018; 19: 96.

7. Smith Z, Guzzo T. Urinary markers for bladder cancer. F1000Prime Rep. 2013; 5: 1-6.

8. Bokarica P, Hrkac A, Gilja I. Re: J. Alfred Witjes, Thierry Lebret, Eva M. Compérat, et al. Updated 2016 EAU Guidelines on Muscle-invasive and Metastatic Bladder Cancer. Eur Urol 2017; 71: 462-475. Eur Urol. 2017; 72: e45.

9. Santoni G, Morelli MB, Amantini C, Battelli N. Urinary Markers in Bladder Cancer: An Update. Front Oncol. 2018; 8: 362.

10. Agreda Castañeda F, Raventós Busquets CX, Morote Robles J. Marcadores urinarios en la vigilancia del tumor vesical no músculo infiltrante. Revisión de la literatura. Actas Urológicas Españolas. 2019.

11. Naoe M, Ogawa Y, Morita J, Omori K, Takeshita K, Shichijyo T, et al. Detection of circulating urothelial cancer cells in the blood using the CellSearch system. Cancer. 2007; 109: 1439-1445.

12. Gallagher DJ, Milowsky MI, Ishill N, Trout A, Boyle MG, Riches J, et al. Detection of circulating tumor cells in patients with urothelial cancer. Ann Oncol. 2009; 20: 305-308.

13. Guzzo TJ, McNeil BK, Bivalacqua TJ, Elliott DJ, Sokoll LJ, Schoenberg MP. The presence of circulating tumor cells does not predict extravesical disease in bladder cancer patients prior to radical cystectomy. Urol Oncol. 2012; 30: 44-48.

14. Rink M, Chun FK, Dahlem R, Soave A, Minner S, Hansen J, et al. Prognostic role and HER2 expression of circulating tumor cells in peripheral blood of patients prior to radical cystectomy: A prospective study. Eur Urol. 2012; 61: 810-817.

15. Alva A, Friedlander T, Clark M, Huebner T, Daignault S, Hussain M, et al. Circulating tumor cells as potential biomarkers in bladder cancer. J Urol. 2015; 194: 790-798.

16. Hugen CM, Zainfeld DE, Goldkorn A. Circulating Tumor Cells in Genitourinary Malignancies : An evolving Path to Precision Medicine. Front Oncol. 2017; 7: 1-10.

17. Soave A, Riethdorf S, Dahlem R, Minner S, Weisbach L, Engel O, et al. Detection and oncological effect of circulating tumor cells in patients with variant urothelial carcinoma histology treated with radical cystectomy. BJU Int. 2017; 119: 854-861.

18. Busetto GM, Ferro M, Del Giudice F, Antonini G, Chung BI, Sperduti I, et al. The Prognostic Role of Circulating Tumor Cells (CTC) in High-risk Non–muscle-invasive Bladder Cancer. Clin Genitourin Cancer. 2017; 15: 661-666.

19. Flaig TW, Wilson S, Van Bokhoven A, Varella-Garcia M, Wolfe P, Maroni P, et al. Detection of circulating tumor cells in metastatic and clinically localized urothelial carcinoma. Urology. 2011; 78: 863-867.

20. Rink M, Chun FKH, Minner S, Friedrich M, Mauermann O, Heinzer H, et al. Detection of circulating tumour cells in peripheral blood of patients with advanced non-metastatic bladder cancer. BJU Int. 2011; 107: 1668-1675.

21. Abrahamsson J, Aaltonen K, Engilbertsson H, Liedberg F, Patschan O, Rydén L, et al. Circulating tumor cells in patients with advanced urothelial carcinoma of the bladder: Association with tumor stage, lymph node metastases, FDG-PET findings, and survival. Urol Oncol Semin Orig Investig. 2017; 35: 606.e9-606.e16.

22. Fina E, Necchi A, Bottelli S, Reduzzi C, Pizzamiglio S, Iacona C, et al. Detection of Circulating Tumour Cells in Urothelial Cancers and Clinical Correlations : Comparison of Two Methods. Dis Markers. 2017; 11: 1-11.

23. Yang Y, Miller CR, Lopez-Beltran A, Montironi R, Cheng M, Zhang S, et al. Liquid Biopsies in the Management of Bladder Cancer: Next-Generation Biomarkers for Diagnosis, Surveillance, and Treatment-Response Prediction. Crit Rev Oncog. 2017; 22: 389-401.

24. Alimirzaie S, Bagherzadeh M, Akbari MR. Liquid biopsy in breast cancer: A comprehensive review. Clin Genet. 2019; 95: 643-660.

25. Pantel K, Hille C, Scher HI. Circulating tumor cells in prostate cancer: From discovery to clinical utility. Clin Chem. 2019; 65: 87-99.

26. Normanno N, Cervantes A, Ciardiello F, De Luca A, Pinto C. The liquid biopsy in the management of colorectal cancer patients: Current applications and future scenarios. Cancer Treat Rev. 2018; 70: 1-8.

27. Danila DC, Samoila A, Patel C, Schreiber N, Herkal A, Anand A, et al. Clinical validity of detecting circulating tumor cells by AdnaTest assay compared with direct detection of tumor mRNA in stabilized whole blood, as a biomarker predicting overall survival for metastatic castration-resistant prostate cancer patients. Cancer J. 2016; 22: 315-320.

28. Maetzel D, Denzel S, Mack B, Canis M, Went P, Benk M, et al. Nuclear signalling by tumour-associated antigen EpCAM. Nat Cell Biol. 2009; 11: 162-171.

29. Patriarca C, Macchi RM, Marschner AK, Mellstedt H. Epithelial cell adhesion molecule expression (CD326) in cancer: A short review. Cancer Treat Rev. 2012; 38: 68-75.

30. Balzar M, Winter MJ, de Boer CJ, Litvinov SV. The biology of the 17–1A antigen (Ep-CAM). J Mol Med. 1999; 77: 699-712.

31. Plaks V, Koopman C, Werb Z. Public Access NIH Public Access. Science. 2013; 341: 1186-1188.

32. Lustberg MB, Balasubramanian P, Miller B, Garcia-villa A, Deighan C, Wu Y, et al. Heterogeneous atypical cell populations are present in blood of metastatic breast cancer patients. Breast Cancer Res. 2014; 16: 1-15.

33. Gast CE, Silk AD, Zarour L, Riegler L, Burkhart JG, Gustafson KT, et al. Cell fusion potentiates tumor heterogeneity and reveals circulating hybrid cells that correlate with stage and survival. Sci Adv. 2018; 4: eaat7828.

34. Adams DL, Martin SS, Alpaugh RK, Charpentier M, Tsai S, Bergan RC, et al. Circulating giant macrophages as a potential biomarker of solid tumors. Proc Natl Acad Sci. 2014; 111: 3514-3519.

35. Adams DL, Adams DK, Alpaugh RK, Cristofanilli M, Martin SS, Chumsri S, et al. Circulating cancer-associated macrophage-like cells differentiate malignant breast cancer and benign breast conditions. Cancer Epidemiol Biomarkers Prev. 2016; 25: 1037-1042.

36. Mu Z, Wang C, Ye Z, Rossi G, Sun C, Li L, et al. Prognostic values of cancer associated macrophage-like cells (CAML) enumeration in metastatic breast cancer. Breast Cancer Res Treat. 2017; 165: 733-741.

37. Clawson GA, Matters GL, Xin P, Imamura-Kawasawa Y, Du Z, Thiboutot DM, et al. Macrophage-tumor cell fusions from peripheral blood of melanoma patients. PLoS One. 2015; 10: e0134320.

38. Li H, Meng QH, Noh H, Somaiah N, Torres KE, Xia X, et al. Cell-surface vimentin–positive macrophage-like circulating tumor cells as a novel biomarker of metastatic gastrointestinal stromal tumors. Oncoimmunology. 2018; 7: e1420450.

39. Zhang Y, Zhou N, Yu X, Zhang X, Li S, Lei Z, et al. Tumacrophage: macrophages transformed into tumor stem-like cells by virulent genetic material from tumor cells. Oncotarget. 2017; 8: 82326-82343.

40. Cegan M, Kobierzycki C, Kolostova K, Kiss I, Bobek V, Grill R. Circulating tumor cells in urological cancers. Folia Histochem Cytobiol. 2017; 55: 107-113.

41. Lampignano R, Schneck H, Neumann M, Fehm T. Enrichment, Isolation and Molecular Characterization of EpCAM-Negative Circulating Tumor Cells. In: Advances in Experimental Medicine and Biology. 2017. 181-203.

Citation: Maggi F, Amantini C, Nabissi M, Marinelli O, Santoni G and Morelli MB. Typical and Atypical Circulating Tumour Cells in Bladder Cancer. Why Improve our Knowledge? Clin Oncol. 2019; 2(2): 1008.

42. Thurm H, Ebel S, Kentenich C, Hemsen A, Riethdorf S, Coith C, et al. Rare expression of epithelial cell adhesion molecule on residual micrometastatic breast cancer cells after adjuvant chemotherapy. Clin Cancer Res. 2003; 9: 2598-2604.

43. Chalfin HJ, Kates M, van der Toom EE, Glavaris S, Verdone JE, Hahn NM, et al. Characterization of Urothelial Cancer Circulating Tumor Cells with a Novel Selection-Free Method. Urology. 2018; 115: 82-86.

44. Anantharaman A, Friedlander T, Lu D, Krupa R, Premasekharan G, Hough J, et al. Programmed death-ligand 1 (PD-L1) characterization of circulating tumor cells (CTCs) in muscle invasive and metastatic bladder cancer patients. BMC Cancer. 2016; 16: 744.

45. Mu Z, Wang C, Ye Z, Austin L, Civan J, Hyslop T, et al. Prospective assessment of the prognostic value of circulating tumor cells and their clusters in patients with advanced-stage breast cancer. Breast Cancer Res Treat. 2015; 154: 563-571.

46. Giuliano M, Shaikh A, Lo HC, Arpino G, De Placido S, Zhang XH, et al. Perspective on circulating tumor cell clusters: Why it takes a village to metastasize. Cancer Res. 2018; 78: 845-852.

47. Nicolazzo C, Colangelo L, Corsi A, Carpino G, Gradilone A, Sonato C, et al. Liquid Biopsy in Rare Cancers: Lessons from Hemangiopericytoma. Anal Cell Pathol (Amst). 2018; 2018: 9718585.

48. Zhang J, Qiao X, Shi H, Han X, Liu W, Tian X, et al. Circulating tumor-associated neutrophils (cTAN) contribute to circulating tumor cell survival by suppressing peripheral leukocyte activation. Tumor Biol. 2016; 37: 5397-5404.

49. Soave A, Riethdorf S, Pantel K, Fisch M, Rink M. Do Circulating Tumor Cells Have a Role in Deciding on Adjuvant Chemotherapy After Radical Cystectomy ? Curr Urol Rep. 2015; 16: 46.

50. Soave A, Riethdorf S, Dahlem R, Amsberg G Von, Minner S, Weisbach L, et al. A nonrandomized, prospective, clinical study on the impact of circulating tumor cells on outcomes of urothelial carcinoma of the the bladder patients treated with radical cystectomy with or without adjuvant chemotherapy. Int J Cancer. 2017; 140: 381-389.

51. Gazzaniga P, Gradilone A, De berardinis E, Busetto GM, Raimondi C, Gandini O, et al. Prognostic value of circulating tumor cells in nonmuscle invasive bladder cancer: A CellSearch analysis. Ann Oncol. 2012; 23: 2352-2356.

52. Riethdorf S, Soave A, Rink M. The current status and clinical value of circulating tumor cells and circulating cell-free tumor DNA in bladder cancer. Transl Androl Urol. 2017; 6: 1090-1110.

53. Besen J, Gan SD. A critical evaluation of clinical research study designs. J Invest Dermatol. 2014; 134: 1-4.

54. Tibbe AGJ, Miller MC, Terstappen LWMM. Statistical considerations for enumeration of circulating tumor cells. Cytometry A. 2007; 71: 154-162.

55. Pantel K, Alix-Panabières C. Liquid biopsy and minimal residual disease - latest advances and implications for cure. Nat Rev Clin Oncol. 2019.

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