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Clinical applications of circulating tumour cells in breast cancer patients – based on a case report

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Orientado por:

Prof. Dr. Luís António Marques da Costa

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Abstract

Worldwide, breast cancer (BC) is considered to be the most commonly diagnosed and deadly cancer in women, having the majority of deaths resulted from metastatic disease. In order to tackle this global problem, it is mandatory to strengthen its preventive, diagnostic and therapeutic programs.

Circulating tumour cells (CTC) have been proposed as a possible approach to depict both heterogeneity and dynamics of BC, contributing to a more personalized and holistic patient management. CTC are tumour cells shed from both primary tumour and metastasis that can be isolated from the bloodstream and analysed by laboratory techniques.

This work intends to suggest the use of serial evaluation of the quantification and qualification of CTC in the management of BC patients, based on a case report of a 40-year old woman diagnosed with stage IV luminal B-like BC, managed according to the contemporary European guidelines and reaching a 5-year survival.

On one hand, regarding overall prognosis estimation, CTC analysis have shown its potential in better stratifying the already established stages. On the other hand, for follow-up assessment, imaging and tumour markers could be upgraded by the addition of serial CTC quantification.

Regarding treatment lines, spatial and temporal cancer cells' differences and kinetics could be better detected and explained by the molecular and numerical analysis of CTC. This would contribute not only to understanding general resistance mechanisms, but also to tailoring ameliorated therapies.

To sum up, CTC integrate crucial information of tumour evolution, recurrence and response to treatments. In the future, they might play a huge role as adjuvants or substitutes of the current methods for BC evaluation. This work expresses the authors' opinion, not the faculty's one.

Key-words

Circulating tumour cells; Breast cancer; Tumour heterogeneity.

Abstract

Mundialmente, o cancro da mama (CM) é considerado o cancro mais diagnosticado e mortal, sendo a maioria desta mortalidade resultado de doença metastática. Para combater este problema global, é essencial reforçar os programas de prevenção, diagnóstico e tratamento.

As células tumorais circulantes (CTC) têm sido propostas como uma possível abordagem para retratar a heterogeneidade e dinâmica do CM, contribuindo para uma gestão do doente mais personalizada e holística. As CTC são células tumorais provenientes tanto do tumor primário como das metástases, que se encontram no sangue periférico e podem ser isoladas e analisadas por técnicas laboratoriais.

Este trabalho pretende sugerir o uso da avaliação seriada da quantidade e qualidade das CTC na gestão dos doentes com CM, baseando-se num caso clínico de uma mulher de 40 anos diagnosticada com CM, luminal B-like, estádio IV, orientado de acordo com as orientações clínicas europeias contemporâneas, atingindo uma sobrevivência total de 5 anos.

Por um lado, tendo em conta a estimativa de prognóstico, a análise das CTC tem demonstrado o seu potencial na melhoria da estratificação dos doentes. Por outro lado, para follow-up, os métodos imagiológicos e os marcadores tumorais poderão ser melhorados pela adição da quantificação seriada de CTC.

Relativamente às linhas de tratamento, as diferenças e cinéticas espaciais e temporais das células tumorais podem ser melhor detetadas e explicadas pela análise molecular e numérica das CTC. Tal contribuirá não só para a compreensão dos mecanismos gerais de resistência, como também para o aperfeiçoamento das terapêuticas.

Resumindo, as CTC integram informação crucial da evolução tumoral, recorrências e respostas terapêuticas. No futuro, as CTC poderão vir a ter um papel importante como adjuvantes ou substitutos dos métodos correntes de avaliação do CM. O Trabalho Final exprime a opinião do autor e não da FML.

Palavras-chave

Células tumorais circulantes; Cancro da mama; Heterogeneidade tumoral.

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Introduction

Cancer is one of the leading causes of morbidity and mortality worldwide, accounting for 9.6 million deaths in 2018 [1]. In 2016, it was responsible for 22% of all non-communicable diseases' deaths, only being exceeded by the cardiovascular group [2]. In addition, breast cancer (BC) was found to be the most commonly diagnosed (24,2%) and deadly (15%) cancer in women, in 2018, having the majority of deaths resulted from metastatic disease [1, 3]. In order to tackle this global problem, it is mandatory to strengthen preventive, diagnostic and therapeutic programs of BC, based on its pathophysiology and peculiarities. Nowadays, early detection and appropriate patient management (prognosis, treatment and follow-up) are two main approaches of oncologic research, where biomarkers are indispensable [4].

Currently, prognosis estimation and treatment regimens for BC are tailored to its phenotypical characteristics - hormonal receptors (HR), human epidermal growth factor receptor (HER-2/neu) and tumour-node-metastasis (TNM) stage - which are determined at diagnosis, through a primary tumour biopsy and imaging [5, 6]. However, BC is a spatial and temporal dynamic disease, evolving through the Darwinian theory of natural selection, and thus originating a wide genomic heterogeneity inter- and intra-tumour site. This selection is enforced by the tumour microenvironments and drugs, originating resistance and disease recurrence [7]. Therefore, the initial evaluation of a neoplasia might underestimate its complexity and instability, inducing wrong therapeutic decisions and predictions.

In this context, circulating tumour cells (CTC) have been proposed as a possible approach to face this heterogeneity and dynamics, and so, to offer a more targeted and personalized patient management. CTC are tumour cells (single or in clusters) found in the bloodstream, shed from both primary tumours or metastases. CTC play a huge role in tumour progression, metastatic spread and the interaction between diverse cancerous sites along the body [8].

Numerous techniques have been developed in order to isolate CTC from venous blood samples and analyse their clinical worthiness. Nowadays, CellSearch technique is the only Food and Drug Administration (FDA) approved [9, 10]. CTC are integrated in the so-called "liquid biopsy", alongside other circulating tumour products (CTP) such as circulating cell-free tumour DNA (ctDNA) and RNA [11]. Overall, they offer a minimally invasive and real-time method of evaluating heterogeneity, throughout tumour evolution and treatment [7]. In fact, the superiority of these "liquid biopsies" over solid biopsies, radiological imaging and serum tumour markers – carcinoembryonic antigen (CEA) and cancer antigen 15-3 (CA 15-3) – is being investigated [10, 12, 13].

Moreover, CTC have demonstrated their potential in the clinical management of both early and metastatic BC patients, concerning not only their quantity, but also their quality. In general, the prognostic value of CTC at baseline and follow-up seems independent of cancer subtype, except for the amplificated HER-2/neu subtype where a selective action of anti-HER-2/neu therapies against CTC has been reported [14, 15].

Firstly, in early BC, the presence of CTC was significantly associated with decreased disease-free survival (DFS) and overall survival (OS) [16]. Also, their persistence after adjuvant chemotherapy significantly reduced DFS [17].

Secondly, in metastatic BC, a baseline ≥5 CTC/7.5mL of blood was significantly associated with an increased number of metastatic sites and shorter progression-free survival (PFS) and OS [18, 19]. Additionally, after chemotherapy, patients with ≥5 CTC/7.5mL still had significantly worse clinical benefit rate, PFS and OS. Further, if a baseline ≥5 CTC/7.5mL dropped to <5 CTC/7.5mL after chemotherapy, OS would become similar to those who already had <5 CTC/7.5mL [20].

Furthermore, in order to metastasise, the primary tumour cells must undergo a type 3 epithelial-to-mesenchymal transition (EMT), leading to motile and aggressive tumour cells that enter circulation [21]. Modifications are induced by reactive stroma and hematogenous factors, and reversed in the micro-metastatic places [11, 22]. Thereby, CTC's features change overtime, originating a continuum of phenotypes from epithelial to epithelial-mesenchymal, mesenchymal and stem-like [23]. In fact, EMT markers (vimentin, TWIST1) occur more frequently in metastatic than early BC and the mesenchymal and stem-like phenotypes have been correlated with non-responders to therapy [24, 25].

In the future, molecular analysis of CTC may allow the detection of tumour advantageous characteristics and heterogeneity, associated with tumour aggressiveness, worse prognosis, treatment resistance and disease recurrence. This would contribute not only to research and the understanding of cancer, but also to identify crucial biomarkers throughout cancer progression, which could be quantified and targeted and, consequently, ameliorate patient evaluation and management.

Finally, this work intends to discuss the usefulness of CTC in BC patients, based on a case report of a 40-year old woman diagnosed with stage IV luminal B-like breast cancer and managed according to the contemporary European guidelines, where the addition of serial liquid biopsies could have been preponderant to its final course.

Clinical Case

The following clinical case was described based on the consultation of the patient's medical process, from the Oncology Department of Hospital Santa Maria, Lisbon, Portugal.

Female, 40 years old, Caucasian, administrative worker, autonomous on her daily life and apparently healthy. She had taken oral contraceptives for 10 years and never gave birth or breastfed. On a familial note, her aunt suffered from BC. More importantly, she was being investigated for cutaneous thickening and nipple retraction on her left breast, not yet diagnosed.

In December 2011, the patient presented to her general practitioner complaining of a continuous low back pain, with no accompanying symptoms. A lumbosacral spine X-Ray showed no evidence of lesions. However, a lumbar CT scan from January 2012 detected multiple lithic lesions on the vertebral bodies of D12, L1, L2, L3, L4, sacrum and both iliac bones, not affecting the surrounding soft tissues nor the spinal canal. Being most likely of secondary nature, the patient was referred to an oncologist.

Oncologic Clinical Examination (January 2012)

Patient presented with difficult and painful mobilization, with no evidence of medullary compression. Left breast showed cutaneous thickening, nipple retraction and hardness behind the nipple. Left axilla had a fixed and hard adenopathy (level 1). No other alterations regarding right breast, other lymph nodes, abdominal and thoracic examination.

Mammography, Ultrasonography and Biopsies (February 2012)

Left breast showed an individualized dense region behind the nipple, of 7,7 mm, heterogenous and stellate, conditioning invasion, thickening and retraction of it, without clear constitution of nodules nor microcalcifications. Histologically, the tissue was classified as an invasive ductal carcinoma (IDC), grade II, with positive oestrogen receptor (ER), positive progesterone receptor (PR), high ki-67, negative p53 and no HER-2/neu amplification (BI-RADS 6). Right breast showed only diverse microcystic formations (BI-RADS 2). Left axilla showed an irregular and capsulated nodal conglomerate, histologically confirmed as a BC metastasis.

Thoracic, Abdominal and Pelvic (TAP) CT Scan (February 2012)

Lithic bone lesions were found in all vertebral spine and iliac bones, with aggressiveness evidenced by cortical ruptures. There was a pathological fracture of a left medium rib. Normal sized liver showed a 2 cm hypodense nodule in the left lobe. No other abnormalities.

Bone Scan (February 2012)

There was focal hyperfixation of the radioactive drug at L1-L2. Less intensity was seen at D11, D12, two right ribs, one left rib, left iliac bone and right trochanteric region. Alterations corresponded to multiple metastases.

To sum up, in February, patient was diagnosed with stage IV, luminal B-like, IDC on her left breast, which had already metastasized to her left axillary lymph nodes and axial and appendicular skeletons.

Endocrine therapy (ET) with Tamoxifen [20 mg *per os* (PO) daily] and Goserelin Acetate [10,8 mg subcutaneous (SC) every 12 weeks] were proposed. In addition, Zoledronic Acid [5 mg intravenous (IV) monthly], palliative Radiotherapy (RT) [30Gy/10fr regimen] on the affected bone areas and an Orthopaedic vest were prescribed. Every treatment was well tolerated, with only mild and non-pharmacologically treated gastrointestinal toxicity.

After RT (March), patient presented better, with less pain and no analgesics needed. Analytically, CA 15-3 had decreased from 273 (February) to 213 U/mL (March). TAP CT Scan confirmed no new metastasis.

In August, patient was submitted to a successful radical left Mastectomy with lymph node dissection. Surgical management of bone metastasis was not considered adequate.

Until December, patient felt good [performance status (PS) 0-1]. Regarding tumour markers: CA 15-3 decreased from 273 (February) to 45,5 U/mL (October) and CEA had been stable, between 0,5-1,6 ng/mL. However, follow-up imaging showed disease progression.

Bone Scan (December 2012)

Heterogeneity was found, with focal hyperfixation on D5, D6, D8, D10, right and left ribs, both scapulae-humeral joints, both ischia, pubic symphysis and right trochanteric region. This represented discrete progression with new lesions.

Lumbar CT Scan (December 2012)

Mixed (lithic and blastic) lesions were seen in almost all vertebrae, sacrum and iliac bones, associated with cortical ruptures and soft tissue components. There were pathological collapses of C7, D5 and L2, with discrete posterior and intracanal deviation. No evidence of spinal compression.

Consequently, in January 2013, Tamoxifen was replaced by Letrozole (2,5 mg PO daily).

Until April, there was stabilization of both tumour markers: CEA between 0,5-0,7 ng/mL and CA 15-3 between 45,1-50,2 U/mL; followed by a decrease in CA 15-3 from 45,9 (April) to 18,8 U/mL (February 2014).

Clinically, patient was asymptomatic (PS 0) and waiting for breast reconstruction surgery. Mild and reversible paraesthesia on both arms was described but not translated into imaging medullary compression.

Spinal MRI (July 2013)

Diffuse and heterogenous metastatic lesions were found, being larger in C7, D1, D5, D8 and D12, and diffuse in the lumbar segment, sacrum and iliac bones. They were more expressed in D5 and L2, with reduction of the vertebral bodies' height and anterior involvement of the dural sac. Incipient anterolisthesis of L5 over S1 and degenerative alterations in the cervical vertebral discs were identified. No other abnormalities.

Until April 2014, patient remained asymptomatic. Follow-up exams were incoherent and tumour markers were not increasing. Thereby, no change in endocrine therapy was described. Only Zoledronic Acid posology was altered to 5 mg every 3 months in February.

Bone Scan (January 2014)

There was maintenance in the distribution and metabolism of bone involvement in D5, D6, D8, D10, right and left ribs, both scapulae-humeral joints, both ischia, pubic symphysis and right trochanteric region. There was a discrete and focal increase of intensity in L1, L2, left sacral wing and right femoral head, which was attributed to new secondary lesions.

Pelvic CT Scan (April 2014)

Multiple and diffuse metastatic lesions were seen in the same areas as before, having central osteolysis and peripheral sclerosis, being heterogenous and with unclear borders, namely in the

left acetabular and supra-acetabular areas. These changes were considered inherent to treatment, which hindered comparative studies. No new lesions.

In October, patient restarted the low back pain, which was controlled by analgesics. Clinical examination was clear. However, CA 15-3 had been slightly increasing: 21,6 (March) to 28,9 (July) to 34,6 (November) to 52,4 U/mL (February 2015).

Chest X-Ray (November 2014), superior abdominal Ultrasound (December 2014), Bone Scan (December 2014), spinal MRI (March 2015) and pelvic CT Scan (March 2015) showed no new lesions. In addition, no deleterious mutations were detected in BRCA 1 or 2 by sequencing and quantitative PCR analysis (March 2015).

Consequently, therapy was maintained.

In February 2015, patient successfully finished all left breast reconstructive surgeries.

In May, patient complained of sporadic mechanical pain which was extinguished by analgesics. Clinical examination was unremarkable. However, CA 15-3 (61 U/mL) and total bilirubin (value not shown) had been increasing, with no changes in the liver enzymes.

Superior Abdominal Ultrasound (June 2015)

Liver was normal sized, regular and homogenous. There were some hypoechogenic and solid nodules, the biggest one of 2,7 cm and localized in the right lobe periphery. Smaller nodules were seen in the right (two) and left (one) lobes. Changes were suspicious of hepatic metastasis. In addition, cystic formations remained (maximum 2 cm). No other abnormalities.

Thereby, Letrozole was replaced by Fulvestrant [500 mg intramuscular (IM) monthly].

Patient remained asymptomatic and thoracic CT (July 2015) and Bone Scan (August 2015) showed no significant changes. However, CA 15-3 was increasing (225 U/mL in November).

Superior Abdominal Ultrasound (November 2015)

Diffuse hepatic lesions had become greater in number and dimension. The greater one was central (segments IV-V) with 5x4,3 cm and the previous 2,7 cm lesion had 3,8 cm. New multiple nodules varied between 1,5-2,0 cm. Cystic formations seemed stable. No other abnormalities.

ET was discontinued. A Doxorubicin/Cyclophosphamide 5-cycles regimen was prescribed, from November 2015 to March 2016. Initially, patient suffered from grade 1 toxicities

(neutropenia, mild infections, fever), so, concomitant Filgrastim (G-CSF) was prescribed, achieving tolerance. After the last cycle, patient still complained of anorexia, asthenia and abdominal discomfort, having been diagnosed with febrile neutropenia, admitted to the hospital and completely recovered.

Overall, CA 15-3 decreased from 263 (December) to 147 (January) to 97 U/mL (February). However, imaging showed only partial favourable response.

Superior Abdominal Ultrasound (March 2016)

Previous solid nodular images were no longer identified. In the right lobe, a calcified formation of 1,3x1,2 cm and another with an hypoechogenic periphery and a fibrotic zone were seen. In the left lobe, cystic images of 2 and 1,5 cm were described. No other abnormalities.

In April 2016, 8-cycle Paclitaxel regimen was started. However, grade 1 toxicity (afebrile neutropenia) was seen, forcing treatment with G-CSF and postponement of the following cycles, finishing only in July.

Clinically, the patient was asthenic and complained of haemorrhoids, that were not thrombosed nor infected. Symptomatic measures were taken. In May, patient showed facial asymmetry and slower speech which spontaneously reversed.

Nevertheless, CA 15-3 increased from 135 (April) to 152 U/mL (May), demanding new imaging studies. Superior abdominal Ultrasound (June/2016) was similar to previous one.

Bone Scan (July 2016)

There was heterogeneity along the spine, individualizing multiple hyperfixations in D3, D4, D5, D10, D11, D12, L3, bilateral ribs, sternum, both iliac bones and right trochanteric region. Heterogenous distribution in the humeral heads showed incipient metastasis. Comparatively, there were no new lesions and metabolic activity was diminished, meaning improvement.

TAP CT Scan (July 2016)

Multiple macronodular images were seen in the hepatic parenchyma, meaning multiple secondary lesions involving diverse liver segments. The dominant nodules rested in segments IV B (3 cm) and VI/V (6,2 cm), with coarse calcifications. No other abnormalities.

A new line of chemotherapy was proposed: a 4-cycle Capecitabin regimen from August to November. The first two cycles were badly-tolerated with grade 1 haematological (neutropenia) and grade 2 gastrointestinal (colitis) toxicities. Thereby, the last two cycles were performed with dose reduction.

Superior Abdominal CT Scan (November 2016)

In the right hepatic lobe, previous solid and hypodense images with intralesional calcifications showed discrete reduction.

Even tough, last CT scan showed slight progress, in December, CA 15-3 and CEA increased to 113 U/mL and 10,2 ng/mL, respectively.

Concomitantly, patient showed up with a painful and oedematous trajectory of her right external jugular vein, which was examined by the vascular surgery department, being diagnosed as a superficial thrombosis. No other venous thrombosis was detected. Patient was successfully treated with Tinzaparin (during 18 days).

Superior Abdominal CT Scan (February 2017)

There had been a growth of the previous hepatic nodules. The most predominant one was localized in the right lobe, having 5,6 x 6,5 cm.

Furthermore, CA 15-3 and CEA increased to 251 U/mL and 15 ng/mL, respectively.

Thereby, treatment was changed to Vinorelbine, every 2-3 weeks, until May.

In March, Bone Scan overlapped the previous one.

In April, patient's CTC were isolated from a whole blood sample (7,5 mL) by being processed through a microfluidic device, immunostained and analysed as follow:

- Total: 1091 CTC/7,5 mL;
- Epithelial (DAPI+/CD45-/CK+/Vim-): 679 CTC/7,5 mL;
- Epithelial-mesenchymal (DAPI+/CD45-/CK+/Vim+): 310 CTC/7,5 mL;
- Mesenchymal (DAPI+/CD45-/CK-/Vim+): 102 CTC/7,5 mL.

At this time, patient complained of lumbar pain, irradiating to the abdomen and inferior limbs and conditioning body rotation and mobilization. No other complaints. Patient was symptomatically treated, achieving PS 1.

Analytically, LDH was high (328 UI/L), GGT was increasing (120 to 174 U/L, in one week) and the tumour markers were also increasing (CA 15-3 from 549 to 694 U/mL and CEA from 17,5 to 21 ng/mL, in one week).

Lumbar MRI (April 2017)

Diffuse secondary infiltration of bone marrow with expansive lesion on L2, affecting the cauda equina roots and conditioning hypersignalling of the medullary cone. There was another lesion conditioning a bulging of the posterior wall, with soft tissue component but no evident involvement of the spinal cord.

Superior Abdominal CT Scan (May 2017)

Liver had multiple focal lesions (cystic, necrotic and calcified), that grew in number and dimension. The dominant lesions were in segment V (7,1 cm), IV B (4,6 cm) and VIII (4,2 cm). Bone metastasis were also seen in all vertebral bodies, having important blastic expression and less lithic one, with deformation of L1-L2; and in both iliac bones, being sclerotic and more developed than the previous exam.

In May, patient referred episodes of paraesthesia on both inferior limbs and pain on her right hip. Thus, palliative Radiotherapy (20Gy/5fr regimen) was prescribed on L1-L3, right femoral head and neck. Only abdominal discomfort was stated, not demanding treatment.

On physical examination, a hard and posterior cervical lymph node was found.

Cervical Ultrasound and Biopsies (June 2017)

Thyroid parenchyma showed multiple nodules on both lobes, namely one in the inferior right lobe, of 14 mm, heterogenous, hypoechogenic and irregular, suggesting a need for histological characterization. On the right cervical level V, there was a solid heterogenous nodule of 17x15x20 mm, between the muscles, possibly a secondary lesion. No other abnormalities. Biopsies confirmed metastasis on the inferior right thyroid and right level V lymph node.

Moreover, CA 15-3 was evaluated at 1700 U/mL.

Therefore, a 4-cycle Docetaxel/Gemcitabine regiment was initiated in June, lasting until August. First cycle conferred grade 1 toxicities, forcing dose reduction and addition of Filgrastim to the next cycles.

Clinically, patient complained of asthenia, anorexia, general discomfort, myalgias and headaches. Patient was, also, diagnosed and treated for several respiratory infections.

In June, a 1-week dull epigastric pain, without irradiation, associated with microcytic anaemia (Hb 8,1 g/dL) and elevated liver enzymes (GGT 752 U/L), was diagnosed as an upper gastrointestinal haemorrhage and treated appropriately.

Until September, patient came repeatedly to the hospital with several complains of obstipation, abdominal pain, abdominal distension, difficulty breathing and fever. Clinical examination confirmed ascites, inferior limbs' oedema and diminished respiratory sounds. Analytically, patient remained pancytopenic, PCR and CK were elevated and albumin was low. Patient got septic, being admitted for stabilization, but dying in September 2017.

Discussion

The previous case report represents a typical and adequately managed patient with stage IV luminal B-like BC, at diagnosis. Overcoming the median overall survival (3 years), the patient integrated the 25% that reach a 5-year survival [26].

Regarding treatment lines and follow-up assessment, most ESO-ESMO recommendations were respected. Firstly, haematology, biochemistry, tumour markers and imaging of the chest, abdomen and bone were serially performed in order to evaluate general status, response to treatment and disease progression. Painful and neurological symptoms were constantly deeply analysed. Secondly, removal of the primary tumour, ovarian function suppression, ET, chemotherapy and bisphosphonate regimens resembled the ESO-ESMO guidelines, being always adapted to the patient and to the primary tumour biopsy. In addition, never did a tumour marker alteration alone drive a change in treatment [26, 27]. Overall, patient management is summarised in **Chart 1**, easing comprehension.

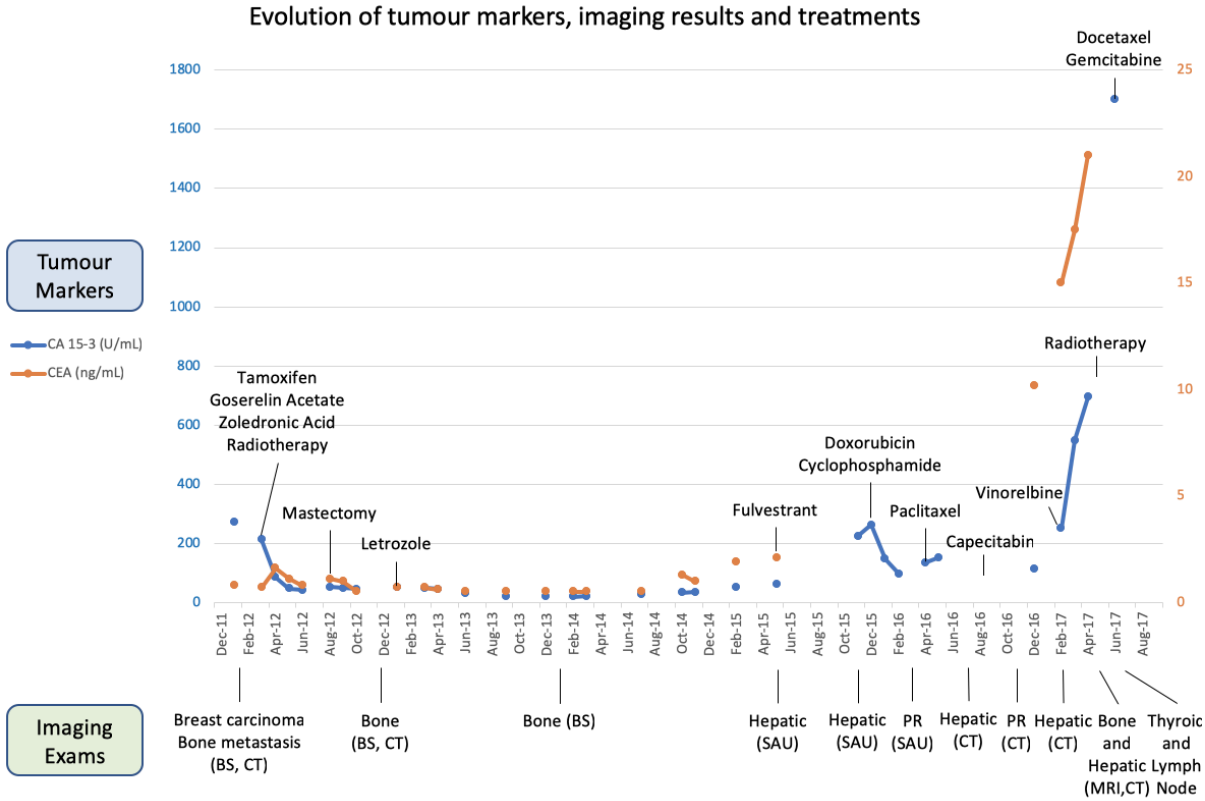

Chart 1. Tumour markers, imaging results and treatments. BS = bone scan, CT = CT scan, SAU = superior abdominal ultrasound, MRI = magnetic resonance imaging, PR = partial response. Bone, Hepatic, Thyroid and Lymph Node represent disease progression in those regions.

In this case report, initial prognosis estimation was based on TNM stage (IV) and biopsy phenotype (IDC, luminal B-like, malignancy grade II), which could have been complemented by the quantification and qualification of baseline CTC, contributing to a better stratification and evaluation of the patient inside the metastatic group [18, 19].

Nevertheless, follow-up assessments were based on imaging (TAP CT scan, bone scan and ultrasound) supplemented by the progression of BC tumour markers (CA 15-3 and CEA). However, CTC have appeared to not only correlate with radiographic determinations of disease progression, but also to be more accurate and reproducible, having an inter-reader variability of 1% versus 15%, a short half-life (1-2.4h) and no radiation exposure – for a standard adult, each TAP CT scan and bone scan equals to 10 and 4 mSv of radiation, respectively [11, 12, 28, 29, 30]. CellSearch has shown to be associated with high intra- and inter-observer as well as inter-instrument accordance [31]. Also, even though the superiority of CTC over serum tumour markers (CEA and CA 15-3) has not yet been reported, it is known that the later carry not only therapy-related surges, but also limited specificity and sensibility (80% when combined, at first progressive disease), possibly skewing management [13, 32, 33]. Thereby, comparative and cost-effective studies between current methods and CTC must be carried on.

Nowadays, metastases' biopsies might be proposed, as to reassess biomarkers (ER, PR and HER-2/neu), embrace tumour dynamics and heterogeneity and, possibly, guide alterations of the targeted therapy [26]. In fact, discordances between the primary breast tumour and its metastasis have been shown to be as frequent as up to 40% for the HR and 10% for HER-2/neu, leading to worse outcomes and changes in treatment in 14% of patients [34]. However, solid biopsies are invasive and not always possible, while liquid biopsies offer an easier alternative, which overcome the problem of scarce tumours and allow serial monitorization [7]. Yet, CTC are rare, so their detection is still challenging, and they might fail to detect co-existing intra-tumour clones, deviating and lessening therapy [8].

Thereby, in this case report, serial immuno-phenotyping of CTC could have been an adequate alternative to depict both inter and intra-tumour heterogeneity, analyse *de novo* modifications of tumour targets, recognize resistance mechanisms and drive treatment lines. Decision making could have considered the possible negativation of the HR, providing ET resistance, and the possible amplification of HER-2/neu in the cancer stem cell (CSC) population, providing aggressiveness [27, 35]. These could explain the bone and hepatic disease progression concomitant to the three lines of ET (Tamoxifen, Letrozole and Fulvestrant from 2012 to 2015), suggesting ET inefficiency. In addition, adjuvant anti-HER-2/neu drugs (e.g. Transtuzumab) could have been a possible approach to tackle recurrence.

Furthermore, one possible use for CTC enumeration and kinetics is the early evaluation of chemotherapy effectiveness and patient prognosis, since, after the first cycle, a CTC status of < 5/7.5 mL of blood or its reduction to it is highly predictive of a better outcome [17, 20, 36]. Indeed, a better evaluation of chemotherapy efficiency would benefit treatment decisions, sparing patients of unnecessary cycles/regimens and their inherent side effects, such as the constitutional, haematological, infectious and gastrointestinal toxicities reported in the case report [37]. However, changing regimens based on the failure of reducing CTC did not seem to change OS, suggesting chemo-resistance and a need to opt for different approaches [10]. Therefore, an investment in future trials to identify the most adequate management decision regarding a post-chemotherapy persistence of high-level CTC should be carried on.

In April 2017, 1091 CTC/7,5mL were collected and analysed from the patient's blood. Using CellSearch, CTC have been detected in nearly 60% of metastatic BC, with a median value of 2 CTC/7.5mL [38, 39]. Higher numbers have been found in patients with positive ER, multiple bone or both bone and visceral metastasis [39, 40]. Indeed, this is concordant with the high CTC level seen in this case report, making feasible their extraction and molecular/genomic characterization. Also, at this time, the patient had already been submitted to three lines of ET and four regimens of chemotherapy, thus >5 CTC/7.5mL of blood represented a significantly worse clinical benefit rate, PFS and OS, and previous treatments' inefficiency [20]. However, the lack of serial CTC evaluations makes it impossible to make greater conclusions as a patient's CTC history and dynamics (throughout treatments) would better reflect the overall aggressiveness and prognosis of that BC than a current CTC status alone [36].

Regarding the EMT continuum, the patient had 679 epithelial, 310 epithelial-mesenchymal and 102 mesenchymal CTCs/7,5mL of blood. Indeed, luminal-like BC are mainly characterized by epithelial CTC, which have also been associated with bone and hepatic (in contrast to CNS) metastasis, as observed [41, 42]. Additionally, EMT markers are shown in 38% of the CTC collected, maybe representing a switch to a mesenchymal predominant phenotype, justifying disease progression, treatment resistance and worse outcome [8, 38]. Interestingly, drugs targeting the EMT and stemness pathways (e.g. Everolimus for the PIK3/Akt/mTOR pathway) are possible approaches to resistant BC [26]. Serial evaluations of CTC's EMT markers during patient evolution could grant crucial and beneficial information.

In this context, most CTC's isolation techniques enrich and identify cells by their expression of epithelial markers (EpCAM, CK), underestimating the mesenchymal group [10]. In fact, refinement and optimization of this detection is important for future uses.

Overall, the handicaps of CTC usage during BC management rely on their rarity in the bloodstream, markers' heterogeneity and our uncertain scientific knowledge towards their biological function, clinical utility and economic impact [8, 10, 43].

To sum up, this work proposes the usage of serial CTC enumeration and characterisation to better define prognosis, recurrence and treatment efficiency. Recognition of their utility not only in the understanding of general cancer mechanisms, but also in the optimal patient management is the first step to motivate future investments and researches. Indeed, CTC look forward to upgrade BC guidelines, as adjuvants or substitutes of the current follow-up methods in order to prolong survival and maintain the best possible quality of life.

Acknowledgments

I'd like to thank the Oncology division from Hospital Santa Maria and the Translational Oncobiology department from Instituto de Medicina Molecular, Lisbon, Portugal, for helping me bring this project to life.

Additionally, I'd like to thank my closest family and friends for always being there for me and supporting me in every step I take.

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