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Íris Joana da Costa Ascenção

Liquid Biospy in Rectal Cancer: Applications in Neoadjuvant Therapy Monitoring

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Íris Joana da Costa Ascenção

Liquid Biopsy in Rectal Cancer: Applications in Neoadjuvant Therapy Monitoring

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Doutora Laura Elisabete Ribeiro Barbosa

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Dr. João Paulo Meireles Araújo Teixeira

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UC Dissertação/Projeto (6º Ano) – DECLARAÇÃO DE REPRODUÇÃO

NOME

Íris Joana da Costa Ascenção

NÚMERO DE ESTUDANTE | E-MAIL

201406292 | iris.joana13@hotmail.com

DESIGNAÇÃO DA ÁREA DO PROJECTO

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Liquid Biopsy in Rectal Cancer: Applications in Neoadjuvant Therapy Monitoring

ORIENTADOR

Laura Elisabete Ribeiro Barbosa

COORIENTADOR (se aplicável)

João Paulo Meireles Araújo Teixeira

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Faculdade de Medicina da Universidade do Porto, 20 / 03 / 2020

Assinatura conforme cartão de identificação: Íris Joana da Costa Ascenção

Para os meus pais, por me ensinarem a amar incondicionalmente, por todas as gargalhadas sonoras, pela confiança inabalável – por serem o alicerce.

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Liquid Biopsy in Rectal Cancer: Applications in Neoadjuvant Therapy Monitoring

Biópsias Líquidas no Cancro do Reto: Aplicabilidade na Monitorização da Resposta à Terapêutica Neoadjuvante

Íris Joana da Costa Ascenção, Masters Student, Faculdade de Medicina da Universidade do Porto (FMUP, Porto, Portugal), https://orcid.org/0000-0002-3181-1442.

Laura Elisabete Ribeiro Barbosa, Professor/Researcher, Faculdade de Medicina da Universidade do Porto (FMUP, Porto, Portugal), MD, Head of Department of General Surgery, Centro Hospitalar Universitário São João (CHUSJ, Porto, Portugal), https://orcid.org/0000-0003-1181-9217.

João Paulo Meireles Araújo Teixeira, Doctorate, Faculdade de Medicina da Universidade do Porto (FMUP, Porto, Portugal), Senior MD, Department of General Surgery, Centro Hospitalar Universitário São João (CHUSJ, Porto, Portugal), https://orcid.org/0000-0001-6065-0193.

Institution: Faculty of Medicine of the University of Porto

Author Information:

Name: Íris Joana da Costa Ascenção

Address: Alameda Professor Hernâni Monteiro, 4200-319, Porto Portugal

E-mail: iris.joana13@hotmail.com

Abstract

Introduction: Currently, regarding locally-advanced rectal cancer, a complete pathological response after neoadjuvant therapy is poorly predicted by conventional imaging modalities. Methods of response assessment are now starting to have a pivotal role in improving treatment strategies. However, the clinical potential of ctDNA analysis in non-metastatic rectal cancer remains unsettled. Thus, this work aims to review the established evidence on the role of liquid biopsies as a method of assessing the response to neoadjuvant therapy and refining the current treatment strategies of locally-advanced rectal tumors.

Methods: A literature search was conducted in the MEDLINE database, using PubMed search engine, the Web of Science and Scopus databases. A total of 45 articles were comprised in this literature review.

Results/Discussion: The results evidenced in this work suggest that, although no predictive molecular biomarker for response to nCRT has been proved to be sufficiently robust to have clinical utility on its own, significant differences between response and nonresponse could be found during treatment. Biomarkers such as DNA level variations, DNA integrity index, methylation or mutation status of specific genes, in the context of a multidisciplinary approach to treatment, have proven to be a valuable tool in assessing treatment response and refining therapy strategies.

Conclusion: The inclusion of liquid biopsies in the monitorization of neoadjuvant treatment response can improve the accuracy of the clinical and radiological assessment of rectal cancer patients and offer, in the near future, a concrete opportunity for a more personalized treatment approach.

Keywords: Liquid biopsy; rectal cancer; neoadjuvant therapy;

Resumo

Introdução: No que concerne ao carcinoma do reto localmente avançado, uma resposta clínica completa à terapia neoadjuvante é, atualmente, prevista com dificuldade pelas modalidades de imagem convencionais. Os métodos de avaliação da resposta começam agora a assumir um papel central no desenvolvimento das estratégias de tratamento. Todavia, o potencial clínico da análise do ctDNA no contexto do cancro do reto não metastático continua por esclarecer. Assim, este trabalho visa proceder à revisão da evidência estabelecida sobre o papel das biópsias líquidas como método de avaliação da resposta à terapêutica neoadjuvante e no progresso do tratamento dos tumores localmente avançados do reto.

Métodos: Foi conduzida uma revisão da literatura nas bases de dados da MEDLINE (através do motor de busca da PubMed), Web of Science e Scopus. Um total de 45 artigos foram incluídos neste trabalho.

Resultados/Discussão: Os resultados explanados neste trabalho sugerem que, apesar de nenhum biomarcador preditivo se ter revelado suficientemente robusto para ter utilidade clínica por si só, foram encontradas diferenças significativas entre os doentes com boa e escassa resposta ao tratamento neoadjuvante. Parâmetros como a variação dos níveis de DNA, o índice de integridade do DNA ou o estado de metilação e mutação de genes específicos, integrados numa abordagem multidisciplinar ao tratamento, provaram ser uma ferramenta valiosa na avaliação da resposta terapêutica e uma oportunidade para aprimorar as estratégias de tratamento.

Conclusão: A inclusão das biópsias líquidas na monitorização da resposta ao tratamento neoadjuvante pode melhorar a acuidade da avaliação clínica e radiológica dos doentes com cancro do reto e providenciar, num futuro próximo, uma oportunidade concreta para um tratamento mais personalizado destes doentes.

Palavras-chave: Biópsias líquidas; cancro do reto; tratamento neoadjuvante;

Introduction

Colorectal cancer is one of the most frequently diagnosed cancers in the world, with over 1.8 million new cases estimated to have occurred in 2018[1]. In fact, in the European Union, about 35% of the total colorectal cancer incidence is attributed to rectal cancer, reflecting 15–25 cases/100 000 population per year, with a mortality of 4–10/100 000 population per year. Given that its incidence is predicted to increase further in both genders[2], it's clear that rectal carcinoma continues to be an important global health challenge.

The treatment of primary rectal cancer differs from colon cancer. One of the cornerstones in the approach to rectal cancer, with positive impact on five-year patient survival, is the multimodal therapy strategy[2,3], as is the case in the management of locally advanced rectal cancer patients. These are frequently defined as clinical stage M0, and T3 or T4 or any T stage with nodal involvement[4]. Pelvic magnetic resonance imaging (MRI) and endoscopic ultrasound (EUS) are the modalities of choice to define locoregional clinical staging[2], and they are able to identify those with a threatened circumferential resection margin and node positive disease, who have higher rates of recurrence[5,6]. The standard of care for these patients consists in a trimodality treatment: preoperative chemoradiation therapy (CRT) or short-course preoperative radiotherapy (SCPRT), surgery with total mesorectal excision (TME), followed by adjuvant chemotherapy when recommended[2]. Currently, capecitabine is the standard chemotherapeutic agent used for neoadjuvant chemoradiation (nCRT) of locally advanced rectal cancer, although additional chemotherapy agents such as 5-Fluorouracil (5-FU) have shown promising results in increasing the pathologic complete response (pCR) to therapy[2,7,8].

Preoperative RT or CRT reduces the rate of local recurrence, although with no improvement on overall survival, for middle/low stage II/III rectal cancers[2,9]. About 8%–20% of patients achieve a pCR at the time of surgery, defined as the absence of tumor on pathological examination of the surgical specimen after nCRT, which appears to improve local disease control and confer a survival benefit[7,10,11]. However, a subset of tumors (about 20%) demonstrate either tumoral progression or only minimal regression to stable disease, exhibiting resistance to nCRT[8,12]. Therefore, methods of predicting which patients would have a superior benefit could facilitate a better patient stratification, improving therapy targeting to those who are likely to achieve a good response to nCRT[6],

and, on the other hand, reducing the toxicity of ineffective nCRT and providing an adequate treatment option to those who show a worse response[8].

Also, knowing that a pathologic complete response has been observed in a significant proportion of these patients, alternative treatment strategies to radical surgery are starting to be taken into consideration, based on tumor response to nCRT[13]. In fact, patients with no clinical or radiological evidence of residual disease (complete clinical response) have been offered less aggressive treatment strategies, including close surveillance without any immediate surgery (Watch and Wait Strategy) or transanal local excision (in case of lower rectal cancers)[14]. This conservative strategy would have the advantages of an organ-sparing approach, by avoiding significant postoperative morbidity, functional disorders associated with surgery (fecal incontinence, sexual, and urinary dysfunctions) and the need for intestinal stomas, but it would require the precise identification of patients with pCR after nCRT and strict follow-up for early detection of local and systemic recurrences[15]. Conversely, more successful treatment approaches should be contemplated for patients that exhibit resistance to nCRT earlier in their management. Thus, the intricate approach to these patients urges the need for validation of accurate biomarkers that are able to monitor response to nCRT early during the treatment course[8].

In line with the research progress on this subject, it is now established that various cells within a tumor constantly release many biomolecules such as DNA, RNA, and proteins into body fluids. Circulating tumor DNA (ctDNA) is fragmented DNA derived from a tumor into the bloodstream that is not associated with cells, and because it carries genetic information from the primary tumor, it is often referred to as a liquid biopsy[16]. As such, it allows the characterization of the tumor by a non-invasive procedure and likewise resolves many of the complexities of traditional tissue biopsies[17]. The detection of ctDNA in the plasma is the most promising modality of liquid biopsies in the identification of minimal residual diseases, assessment of treatment response and prognosis, and identification of resistance mechanisms[18], due to its minimally invasive nature, possibility to represent a background from multiple tumor sites and facility to repeat tests during treatment[19].

Nevertheless, the feasibility and clinical potential of ctDNA analysis in non-metastatic rectal cancer remains unsettled[20]. Thus, this work aims to review the established evidence on the role of liquid biopsies as a method of assessing rectal cancer's response to

neoadjuvant therapy and its ability to refine the current treatment strategies of locally-advanced rectal tumors.

Materials and Methods

A literature search was conducted in the MEDLINE database, using PubMed search engine, in September 2019 using the following MeSH terms: “liquid biopsy” or “cfDNA” or “ctDNA” and “rectal cancer” and “therapy”. A language filter was used to select only articles in English or Portuguese and the date of publication was limited to studies published from September 2015 until September 2019. The same search was conducted in the Web of Science and Scopus databases. A total of 36 articles were retrieved. The exclusion criteria were: colon carcinoma; different study objectives; full article availability. Thus, 33 potentially relevant articles were selected in this primary search by reviewing the title and abstract.

Posteriorly, specific research on each subtopic and older studies was led, in order to obtain a better understanding on the subject and allow for a critical analysis of the retrieved articles.

As such, a total of 45 articles were comprised in this literature review.

Results

Different groups have studied the role of liquid biopsies as a tool to monitor treatment response after CRT in locally-advanced rectal cancer (LARC)[21]. The results of these studies are difficult to interpret and compare, given the small numbers, the significant heterogeneity regarding the methods used for the assessment of ctDNA - total circulating cell-free DNA (cfDNA), DNA integrity index or tumor-specific molecular alterations - and the outcome measures selected for the evaluation of tumor response to treatment (either pathological downstaging or tumor regression grading scores)[20].

Initially, Zitt et al.[22] conducted a proof of principle study, analyzing the levels of circulating cell-free DNA in 26 patients who were treated with pre-operative chemoradiotherapy for cT3–4 mid-low tumors, using a real time PCR technique. A reduction of DNA levels was observed after completion of chemoradiotherapy in all patients, but neither baseline nor post-treatment DNA concentrations were predictive of pathological downstaging. A significant difference between responders and non-responders was found only after surgery. As such, they observed for the first time a correlation between the concentration of cfDNA and clinical response after surgery, but not after neoadjuvant CRT.

Another work by Carpinetti et al.[23] used two patient-specific chromosomal rearrangements to trace the ctDNA of 4 LARC patients in serial blood samples, in order to study the value of early changes in ctDNA in the assessment of clinical response after neoadjuvant CRT. It showed that personalized biomarkers and liquid biopsies were successful in monitoring early treatment response to nCRT, but ctDNA detection proved insufficiently sensitive to rule out the presence of microscopic residual disease after nCRT completion. Still, they concluded its use would complement clinical and radiological evaluation and that changes in ctDNA levels after surgery appeared to predict tumor recurrence[24].

In line with these results, a study of 30 patients subsequently concluded that ctDNA level variations - somatic mutations identified by Next Generation Sequencing (NGS) of 61-gene panels - can be indicative of tumor response to neoadjuvant CRT, with a predictive accuracy of pretreatment ctDNA profiling of 70%, suggesting that ctDNA mutation profiling may be a powerful tool for predicting tumor regression in LARC patients undergoing CRT[25].

Also, another work presented at the 2018 American Society of Clinical Oncology (ASCO) meeting studied 88 rectal cancer patients and reported that ctDNA levels became undetectable during neoadjuvant chemoradiotherapy in 65.5% of the patients, which was consistent to their radiological and pathological changes, reinforcing the potential of this strategy to guide patient selection for Watch and Wait strategy or adjuvant chemotherapy[26,27]. In the following year, other two Chinese prospective cohort studies were presented: Yang et al. reported that the detection of a mutation of TP53 and APC gene in pre-treatment samples was negatively correlated with patients' response to nCRT, as the detectability of pre-treatment mutations during nCRT was significantly decreased in patients with a bad response to treatment (TRG3 vs. TRG0 groups)[28]; Zhou et al. concluded that detectable ctDNA after the completion of nCRT can be significantly predictive of an unsatisfactory curative effect on patients with LARC, given that its persistency was linked with pathological N+, whereas an undetectable preoperative ctDNA correlated with pathological downstaging[21,29]. This was also the conclusion of another study by McDuff et al., that found that undetectable preoperative ctDNA was associated with a R0-NN surgical outcome[30].

On the other hand, research on the possible applications of liquid biopsies has also led to less consistent results concerning neoadjuvant therapy monitoring in rectal cancer. Schou et al. quantified the total level of cfDNA by fluorescence assay, using 40 μL of plasma of 123 LARC patients[31], and observed no differences either between baseline and post-treatment levels of cfDNA or between patients who achieved a pathological complete response (pCR) and poor responders. However, their results confirmed its prognostic value by demonstrating an association between a high baseline plasma level of cfDNA and increased risk of recurrence, shorter time to recurrence, and shorter DFS in patients with LARC[32]. Also, another group sought out to assess the feasibility and potential clinical implications of using KRAS/BRAF mutations as markers for ctDNA detection in a relatively large prospective series of LARC patients – while they failed to predict prognosis or refine patient selection, the results confirmed that KRAS/BRAF mutations in ctDNA can be detected with high sensitivity and provided further support for the initiation of prospective trials investigating the potential clinical applications of ctDNA analysis in this setting[20]. In a 2019 study, Tie et al. also analyzed tissue and multiple plasma samples of 159 LARC patients treated with neoadjuvant CRT using NGS

techniques, but found no statistically meaningful association between reduction or negativization of ctDNA after CRT and pCR[33].

Other studies focused on different aspects of cfDNA analysis, such as the ratio between the long and short cfDNA fragments (cfDNA integrity index), and reached valuable conclusions. Firstly, Agostini et al. concluded that the plasma levels of the longer fragments of cfDNA and the cfDNA integrity index are promising markers to predict tumor response after preoperative CRT for rectal cancer, by showing that the cfDNA integrity index was statistically significantly decreased in responders compared to non-responders only after completion of fluoropyrimidine-based CRT, but not at baseline. The DNA integrity index at this time point was also found to be the only independent predictive factor of response to neoadjuvant treatment in a multivariate analysis[34,35].

Sun et al.[36] confirmed the potential of the DNA integrity index to serve as a biomarker for treatment response, as they observed an association between this parameter (both at the baseline and after neoadjuvant treatment) and tumor regression grading according to the Dworak's score, in rectal cancer patients who received an oxaliplatin-based CRT for cT3-4 and/or N+ rectal tumors. Simultaneously, they studied the methylation status of MGMT and the mutational status of KRAS, concluding that, although the rate of KRAS codon 12 mutation decreased with chemoradiotherapy in all patients, with no difference between responders and non-responders, a higher rate of MGMT methylation at baseline was predictive of pathological response[8,18].

In fact, another study described the same correlation between methylated status of the promoter of MGMT, and also ERCC1 genes, with response to CRT[37]. A higher methylation status was found to be associated with a better tumor response after preoperative CRT, suggesting that the methylation of genes involved in DNA repair mechanisms may be indicators for response to therapy. More recently, Chen et al.[38] identified ctDNA by investigating the methylation status of two specific to colorectal cancer genes, BCAT1 and IKZF1, in 9 LARC patients. This analysis proved to be sensitive to the effect of the chemotherapy regimen, and found that a partial/complete response to their treatment regimen and/or reduction in tumor burden was reflected in the loss of detection of these two genes[21].

There is another ongoing study where baseline tumor biopsies and serum ctDNA collected at different time points were assessed for mutations in KRAS/NRAS exons 2-

3-4, BRAF exons 11-15, and PIK3CA exons 9-20. Preliminary analysis of their results conducted in 28 patients with LARC showed a promising role of low baseline value of ctDNA in predicting pCR, but still with statistically not significant results, due to the small sample size considered in this interim analysis. The conclusion on whether the suggested effect will be confirmed is pending confirmation until the final analysis[39].

Ultimately, what seems to be the consensual conclusion to the works conducted on this subject is that liquid biopsies, in the various possible methods of analyzing ct/cfDNA, could be a viable tool to pair with imaging for assessment of patient response to treatment[40]. This has actually been established in a prospective feasibility study evaluating the role of multimodality imaging and liquid biopsy for response assessment in locally advanced rectal carcinoma, that determined that a model of Computed Tomography Perfusion (CT-P) parameters plus liquid biopsy more accurately predicts tumor response than PET-MRI, CT-P alone, or liquid biopsy alone[41].

Discussion

Neoadjuvant therapy has become the standard of care for locally advanced rectal cancer patients, so as to downstage the tumor prior to surgery. In addition, it has also been brought into practice in select cases of "watchful waiting", in which disease status is monitored with close clinical imaging and endoscopic follow-up, rather than being surgically resected. Nonetheless, it remains a challenge to assess treatment response with suitable sensitivity or specificity to manage clinical decisions, given that preoperative clinicopathological features have limited ability to predict response to nCRT, and even the imaging methods routinely used for pretreatment staging, such as MRI and PET-CT, still face important limitations[42]. As such, taking into account the broad spectrum of potential response to nCRT, there is a clear unmet need for a biomarker that is able to predict response and guide personalized modifications to the treatment plan[35] – this would, on the one hand, spare patients from unnecessary CRT and its associated toxicity if the benefit is unlikely and, on the other, potentially identify patients suitable for a radical surgery sparing strategy, with upfront neoadjuvant chemotherapy alone, that is currently under investigation[43]. Therefore, prediction of complete response to nCRT has gained an increasing importance and is now a topical area of investigation[6,41], with research on liquid biopsies and its potential applications in the setting of non-metastatic rectal cancer just recently emerging, as they have been, so far, the subject of limited investigation[40].

In this work, a review was conducted on the studies examining the role of liquid biopsies in the monitorization of response to neoadjuvant therapy in locally advanced rectal cancer, in an effort to provide a better understanding on its ability to solve the difficulties still present in the clinical management of these tumors – specifically, ascertaining if the rate of change in ctDNA levels during treatment can be predictive of overall response to neoadjuvant strategies and guide subsequent decisions, if a low or undetectable ctDNA level can help identify suitable patients for de-escalation of treatment or even if a high level might suggest treatment intensification.

Firstly, the inclusion of liquid biopsies into practice, being still a relatively novel concept in colorectal carcinoma, brings about new possibilities to be noted[18]. Very often, the genetic heterogeneity of the tumor poses a challenge to its approach, hindering the efficacy of radiotherapy, chemotherapy or targeted therapy[40]. This difficulty can be

overcome by ctDNA or cfDNA analysis, given its ability to assess for specific mutations and potential to display further biological information during the disease course, with the advantage of being a cost-effective, rapid and non-invasive method to obtain this comprehensive profile of the tumor[18]. Consequently, it seems natural that liquid biopsies will assume an increasingly important role in cancer therapy monitoring.

In this setting, there is contrasting evidence on the reliability of circulant DNA as a tool to predict treatment response in locally-advanced rectal cancer patients. The consensual observation in most studies retrieved is a reduction in cf/ctDNA levels in responders, while non-responders often show an incremented circulant DNA[22,23,26,29,30,33,34,36]. However, the timing of plasma collection - at baseline, after induction chemotherapy, after neoadjuvant CRT or after surgery – appears to be critical in the comparison of the results and their adequate interpretation.

In order to have clinical utility for selecting patients to nCRT, a biomarker would need to have predictive value in the baseline sample, but the clinical relevance of baseline levels of cf/ctDNA is not clear. One small study verified a correlation between a higher DNA integrity index and treatment response[36], but this was not consistent with the conclusions of a previous broader study, that found that this variable was an independent predictive factor of response to neoadjuvant treatment only after completion of the treatment[34]. Still, it appears that a strong methylation of MGMT or ERCC1 genes at baseline might predict a better tumor response after nCRT[36,37], whereas the detection of TP53 and APC gene in ctDNA of pre-treatment samples is negatively associated with response to nCRT[38].

On the other hand, concerning the DNA levels detection after neoadjuvant CRT, some studies effectively observed a reduction after treatment, although not predictive of pathological downstaging, because a significant difference between responders and non-responders was found only in the plasma samples collected after surgery[22,31,33]. Still, different groups successfully verified a correlation between undetectable preoperative ctDNA status and pathological downstage[26,28–30], and a weaker methylation of BCAT1 or IKZF1 has been observed after CRT in good responders by Chen et al[38]. Also in this setting, a DNA integrity index (a ratio between long and short DNA fragments) has been proposed to be a useful guide to discriminate responding and non-responding patients even with plasma analysis conducted after neoadjuvant CRT[21,34,36]. More recent studies, adopting a different technique with NGS assays, have demonstrated the potential of post-

CRT ctDNA samples to predict tumor response, enhancing the confidence in ctDNA as a tool to guide patient selection for watch and wait strategy[23,25].

However, the rapid advances in this field of research in the last decade have also reflected on a high degree of heterogeneity between the laboratory approaches that has restrained the standardization of this technique and its clinical application[18], each one having different strengths and limitations. For instance, the major advantage of considering the total level of circulating DNA (cfDNA), being both mutated and non-mutated and a purely quantitative measurement, is its technical feasibility and detectability in practically all patients, not limiting to specific mutations in the blood stream. In contrast, ctDNA detection has a decreased sensitivity, especially in earlier tumoral stages, but it might yield a deeper biological insight into the tumor and provide important information into its management[40]. Thus, it's important to consider the obstacles to the implementation of liquid biopsies in clinical practice, the most significant being the lack of standard operating protocol, the requirement of sensitive and specific methods and the fact that personnel microenvironment may influence the released amount of cfDNA, given that it is released by both healthy and tumor cells[18,44]. Essentially, it is necessary to address these by setting up standardized pre-analytical methodologies, including blood collection, processing, storage and DNA extraction procedures, and quantifying and validating them in large prospective clinical studies[16,17]. Besides, a deeper understanding of the origin and biology of cfDNA and ctDNA is still lacking for liquid biopsies to be able to effectively have an influential role in the comprehension of the patients' therapy responses and prognosis.

Despite these shortcomings, ctDNA detection presents inherent advantages, so research on ctDNA is now translating from exploratory studies towards carefully designed large prospective clinical trials, in which ctDNA is acting as a decision-making tool[18,35], reflecting that it could serve an increasingly important role in tumor monitoring and oncotherapy[45]. Currently, the complete pathological response after preoperative CRT is poorly predicted by conventional imaging modalities[34], so methods of response assessment in LARC patients are now starting to have a pivotal role in refining treatment strategies to a more personalized approach, ranging from "watchful waiting" to surgical resection. It's in this setting that liquid biopsies can assume greater relevance and provide useful, but somewhat complex, biological information. Consequently, the studies conducted on this topic raise a number of key issues that should be considered in order to

enrich future investigations and deepen our knowledge on the practicability of DNA detection as a tool for therapy monitorization: there is room for further studies seeking to develop and unify a translational method to improve the timing, simplicity, time to results and cost-effectiveness of liquid biopsies techniques[18], and more prospective studies are needed to clarify the optimal approach for the patients predicted with complete response, of preference with close follow-up in centers of excellence[24]. In summary, there is now evidence to support the further development of ctDNA as a biomarker for the monitorization of neoadjuvant therapy in locally-advanced rectal cancer patients, given that, in the context of a multidisciplinary approach to treatment, the use of liquid biopsies could prove to be a relevant asset in obtaining an upgraded measure of treatment response and give light to new solutions that, in the future, may change the way we treat cancer.

Conclusion

The results evidenced in this work suggest that, although no predictive molecular biomarker for response to nCRT has been proved to be sufficiently robust to have clinical utility on its own, significant differences between response and nonresponse could be found during treatment, with the integration of various types of biomarkers – such as DNA level variations, DNA integrity index, methylation or mutation status of specific genes -, clinicopathological and imaging features in the management of locally-advanced rectal cancer patients submitted to nCRT. Therefore, the inclusion of liquid biopsies in the monitorization of neoadjuvant treatment response can improve the accuracy of their clinical and radiological assessment and offer, in the near future, a concrete opportunity for a more personalized treatment of rectal cancer patients.

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