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Tiago Miguel Machado Ferreira

Innovations and new approaches regarding the detection of measurable residual disease in lymphomas - a systematic review

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E sob a

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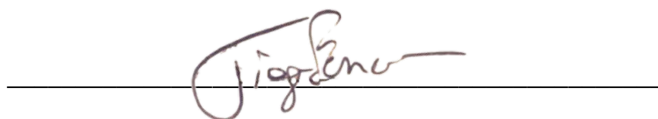
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UC Dissertação/Projeto (6º Ano) – DECLARAÇÃO DE REPRODUÇÃO

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Innovations and new approaches regarding the detection of measurable residual disease in lymphomas - a systematic review

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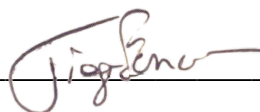

Aos meus pais e irmão, por sempre acreditarem em mim nesta longa jornada de 6 anos,
por me terem incutido valores e princípios
sem os quais nunca chegaria a este parágrafo.

Aos meus amigos, por terem mostrado que a vida não é
nem nunca pode ser só Medicina.

Ao Grupo de Fados de Medicina do Porto,
por me terem proporcionado uma aventura inimaginável ao qual serei eternamente grato,
pelos momentos de companheirismo e amizade que levarei comigo.

Por fim, a quem vai chorar sempre que ler isto, à Rita,

por ser a melhor companheira de vida que eu poderia pedir,
por me ensinar que a vida é melhor quando partilhada,
por festejar as minhas vitórias como se fossem suas.

E esta, é vossa!

Abstract

Background: lymphomas are neoplastic diseases arising from cells of the lymphatic tissue and can affect almost any organ in the body. They can arise from B or T cells. B lymphomas are histologically subclassified into Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL). Classical Hodgkin lymphoma (cHL) is the most frequent subtype of HL, composed of Reed-Steinberg cells. Diffuse large B cell lymphoma (DLBCL) is the most common type of NHL, followed by follicular lymphoma (FL) and mantle cell lymphoma (MCL). The treatment options for these lymphomas include chemotherapy, radiotherapy, immunotherapy, or a combination. The existence of lymphoma clonal cells prior to clinical presentation is known as measurable residual disease (MRD). PET/CT is commonly used to evaluate treatment response and identify disease recurrence, but its use during disease surveillance has not led to improved outcomes. **Objectives**: this article emphasizes the limitations of current MRD monitoring techniques and the need for newer, more sensitive methods. **Data sources:** systematic search in PUBMED and MEDLINE databases, using the following query: "lymphoma + minimal residual disease + liquid biopsy" and "lymphoma + minimal residual disease + imaging" **Study criteria:** cHL, DLBCL, FL, and MCL adult patients whose MRD was assessed using novel liquid biopsy-based methodologies and newer approaches in imaging techniques **Results:** the use of circulating-tumor DNA, next-generation sequencing, and digital-droplet PCR are considered as potential alternatives for MRD assessment. **Limitations**: the articles that were included in the analysis are mainly observational studies, and patient characteristics differ between studies, within the same technique. **Implications of key findings:** these methods have been extensively researched for their potential to detect early disease relapse and open the possibility to start proactive salvage therapy or effectively change treatment strategy.
Keywords: "lymphomas", "measurable residual disease", "liquid biopsy", "next-generation sequencing", "circulating-tumor DNA", "digital-droplet PCR"

Introduction

Lymphomas are a group of neoplastic diseases arising from cells of the lymphatic tissue it can affect nearly any organ in our system. Ranging from a variety of symptoms, they are histologically subclassified into Hodgkin lymphoma and non-Hodgkin lymphoma.

State of the art in classification, diagnosis, and treatment of classical Hodgkin lymphomas

Hodgkin lymphoma (HL) is a malignancy of the lymphatic system with an incidence of 2-3 cases per 100.000 individuals per year in developed countries[1]. HL is currently classified in classical Hodgkin lymphoma (cHL), the most frequent subtype (95%), which is composed of scarce Reed-Steinberg cells with a binucleated/bilobed nucleus, which expresses CD30, CD15, PAX5 and may express CD20[2]. The rare nodular lymphocyte-predominant HL lacks Reed-Steinberg cells[3]. Diagnosis is based on lymph node excisional biopsy. cHL follows a bimodal incidence pattern. The first peak occurs in individuals aged 20-30 years, whereas the second peak appears around the age of 50-70 years. cHL typically presents with enlarged, painless cervical nodes or a mass in the chest, weight loss, and fever[1]. According to the European Society for Medical Oncology (ESMO) guidelines chest x-ray and a contrast-enhanced computed tomography (CT) scan of the neck, chest, and abdomen are recommended for a first approach, and a whole-body 2'-fluorodeoxyglucose positron emission tomography (FDG-PET) scan for staging and response assessment[4]. To evaluate the extent of disease [5], risk stratification, and assess early-response and end-of-treatment response outcomes [6] only CT or PET-CT scans are recommended. The modified Ann Arbor classification system is used to descript extension of the disease (Table 1.). Early-stage disease is staged as I/II, while advanced-stage disease refers to stage III/IV[5]. It has been shown that various imaging techniques do not detect most relapses before clinical signs and symptoms[7]. Standard treatment is based on a case-by-case evaluation of risks and benefits, and it includes chemotherapy (ABVD - doxorubicin, bleomycin, vinblastine, dacarbazine - or BEACOPP - bleomycin, etoposide, doxorubicin), cyclophosphamide, vincristine, procarbazine, prednisone) and involved-site radiotherapy (20 to 30 Gy)[4]. Approximately 10-25% of patients with cHL will have refractory disease or will relapse after achieving a complete remission[8].

Stage	Involvement	Extranodal status
I	One node or a group of adjacent nodes	Single extranodal lesions without nodal involvement
II	Two or more nodal groups on the same side of the diaphragm	Stage I or II by nodal extent with limited contiguous extranodal involvement
II "bulky"	As II with "bulky" disease	Not applicable
III	Nodes on both sides of the diaphragm; nodes above the diaphragm with spleen involvement	Not applicable
IV	Additional noncontiguous extralymphatic involvement	Not applicable

Table 1: *Revised Ann Arbor staging system (Lugano Classification)*[5]

State of the art in classification, diagnosis, and treatment of most frequent non-Hodgkin B-cell lymphomas

Diffuse large B cell lymphoma (DLBCL), the most common type of non-Hodgkin lymphomas (NHL), had an incidence rate of 6.3% in the United States, in 2016[9]. It frequently affects people in their seventh decade and manifests as a rapidly growing mass in one or more lymph nodes or extranodal sites, coupled with B symptoms such as fever, weight loss and night sweats[10]. Although there are variations of DLBCL, the hallmark is a diffuse arrangement of large lymphoma cells that disrupts nodal/extranodal structure[11]. An essential element in DLBCL diagnosis is evaluation of the immunophenotype. These neoplastic lymphoma cells express B-cell antigens and transcription factors, including CD19, CD20 and CD22, PAX5, BOB.1 and OCT2[12]. FDG-PET/CT scan is the gold standard for staging DLBCL patients[13]. The established treatment is 4 cycles of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) regimen followed by involved-site radiotherapy for early-stage disease, while 6 cycles plus involved-site radiotherapy, if necessary, are reserved for advanced-stage or bulky disease[14]. Around 30-40% of patients will relapse or fail to respond to this therapy. Salvage therapy is available to younger patients via autologous stem cell transplantation[10] preceded by platinum-based chemotherapy regimens and polychemotherapy in older or non-transplantable patients[13].
Follicular cell lymphoma (FL) is the second most frequent lymphoid malignancy in western countries[15] characterized by its growth in a follicular pattern[16], and is typically found in abdominal and supradiaphragmatic lymph nodes. The founding event of FL physiopathology is believed to be t(14;18), which involves BCL2/IGH translocation as this translocation occurs in 90% patients with FL[17]. Diagnosis of FL should be based on accessible excisional lymph node biopsy. Bone marrow biopsy, CT scan or PET/CT are fundamental for staging of the disease, which is carried out according to Ann Arbor classification. Treatment options depends on the stage of the disease with radiotherapy with/without rituximab being recommended for stages I-II and systemic therapy reserved for selected cases. Unfortunately, there is no curative therapy for stage III-IV, and treatment should only be given to symptomatic patients. Immunochemoradiotherapy regimens are available for patients with high tumor burden[18].
Mantle cell lymphoma (MCL) is a rare B-cell lymphoma characterized by the translocation (11;14) (q13;q32) that causes the overexpression of cyclin D1. It comprises approximately 3-10% of NHL cases in western countries[9]. Clinical presentation can range from asymptomatic monoclonal disease to progressive lymphadenopathy, cytopenia, splenomegaly, and symptoms of extra-nodal disease. To diagnose MCL, immunohistochemical analysis of nodal/extra-nodal sites is performed, showing strong nuclear staining for cyclin D1 expression, alongside karyotype confirmation using FISH. Staging is done with the use of PET-CT and CT scans, as well as Ki-67%[19]. In low-grade MCL with a good prognosis, a wait-and-see strategy may be employed[20]. The established treatment for MCL is a regimen consisting of polychemotherapy regimens (R-HCVAD - cyclophosphamide, vincristine, doxorubicin, dexamethasone, R-DHAP – dexamethasone and cytarabine, R-CHOP) which may be followed by autologous stem cell transplantation with or without maintenance therapy[19].

Assessing measurable residual disease

Measurable residual disease (MRD) is the presence of a minimal burden of clonal lymphoma cells after initial treatment, without detection of signs or symptoms of disease[21]. It is measured by serial sample collection during pre-treatment, interim evaluation, and post-treatment. MRD serves as a reliable prognostic marker since it can predict relapse or refractory disease in lymphomas.
Disease monitoring in cHL involves physical examination and laboratory tests for a minimum of two years after treatment[22]. Interim PET monitoring is available after 2 cycles of chemotherapy. Result of interim PET,

positive or negative with the Deauville criteria, is used to increase or decrease intensity of the therapy[4]. Surveillance PET scans are not recommended once remission has been confirmed and CT scans should only be used if clinically indicated[23].
In DLBCL, MRD relies on FDG-PET/CT, using the Deauville criteria, which can take place mid-treatment or post-treatment. Clear progression in the interim evaluation can be decisive in switching treatment. However, there is currently no evidence that routine imaging after achieving complete remission may improve outcomes[13].
Few reports on MCL have demonstrated that achieving MRD-negative status can significantly improve outcomes, emphasizing the need for maintenance therapy after stem cell transplant. However, there is still no consensus on the optimal method assessing MRD[24]. Various techniques are employed for MRD evaluation, including immunoglobulin heavy chain (IgH) rearrangement and Bcl1-IgH derived from t(11;14)(q13;q32) rearrangement, flow cytometry methods, PCR-based methods, and next-generation sequencing (NGS)-based methods[25]. Radiological exams are also available mid- and post-treatment. Nonetheless, it is still uncertain what options can be offered to MCL patients who are MRD positive[26].
In FL, PET/CT is recommended following induction chemotherapy. Follow-up includes regular clinical history, physical examinations, blood work, LDH and IgG tests, and may necessitate abdominal ultrasound and CT scan. There is currently no established role for PET/CT in interim response evaluation[27].
Considering these limitations, the aim for this systematic review is to search for evidence of newer, more sensitive techniques that could be employed in the measurement of MRD in cHL, DLBCL, MCL and FL.

Searching methods

We conducted a systematic search in the PUBMED and MEDLINE databases, during the month of January 2023, using the following query: "Lymphomas + minimal residual disease + liquid biopsy" and "Lymphoma + minimal residual disease + imaging" to identify articles published between January 2013 and January 2023 involving MRD assessment in cHL, DLBCL, FL and MCL adult (>18-year-old) patients. Publications were included if MRD was evaluated using novel liquid biopsy-based methodologies and newer approaches in imaging techniques, such as tumor total lesion glycolysis and metabolic tumor volume. We excluded reviews, book chapters, and opinion articles. Two researchers assessed each eligible manuscript independently. The following information was extracted from each study: sample size, technique used in MRD assessment, outcome related to disease progression. Risk of individual bias was assessed using the ROBINS criteria[28]. The search yielded 34 results, of which 10 were excluded based on the criteria outlined in the flowchart (Fig 1). A list of studies included in this review is presented by Table 2.

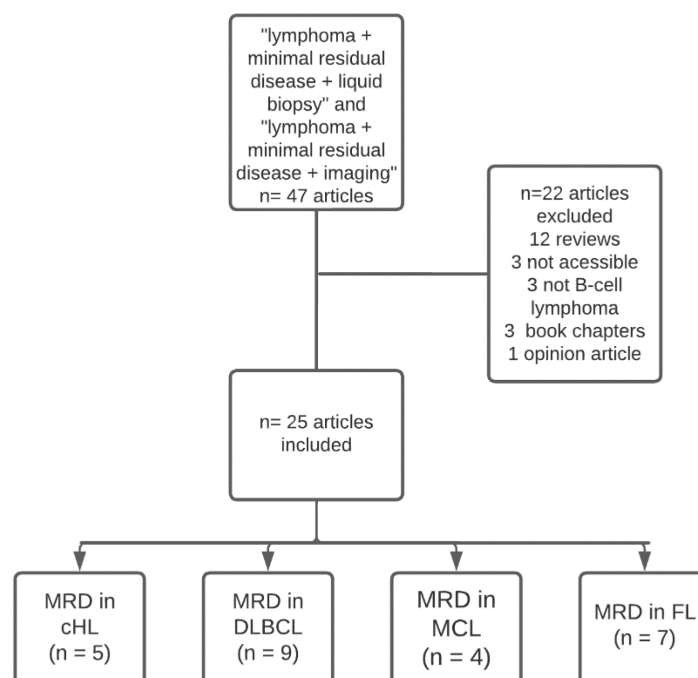

Figure 1 Flowchart. DLBCL: diffuse large B-cell lymphoma; cHL: classical Hodgkin lymphoma; FL: follicular lymphoma; MCL: mantle cell lymphoma; MRD: measurable residual disease

Results

2’-fluorodeoxyglucose positron emission tomography

2’-fluorodeoxyglucose positron emission tomography (FDG-PET) identifies increased glycolytic activity found in malignant cells as in proinflammatory cells. Glucose uptake and utilization is quantified using the standardized uptake value (SUV). SUV is a ratio of the relative increase of the FDG metabolism in a region of the body. It is equal to 1 if FDG distribution is equal throughout the body. In lymphoma, SUV is increased, and maximum SUV correlates with lymphoma aggressiveness. Metabolic tumor volume (MTV) refers to the cumulative 3D measurements of the volume of all lesions exhibiting FDG uptake[29]. Progression-free survival (PFS) can be predicted by the baseline MTV in both primary and relapsed cases of HL[30]. Total lesion glycolysis (TLG) is a metric that captures both the size of the tumor and the extent of FDG uptake, by multiplying the average SUV of the complete tumor with its corresponding MTV. This metric is a comprehensive measure of the metabolic activity across the entire tumor, encompassing the inflammatory microenvironment. Several studies have explored the prognostic value of it in DLBCL[31,32]. Deauville score (DS) was developed for better standardization and interobserver reproducibility. It employs a 5-point scale, where residual uptake is contrasted to a fixed reference background, with the liver being the preferable visual reference. A score of 4 indicates moderate uptake increase over the liver, and score of 5 significantly increased uptake. Score 1-3 is considered complete metabolic remission, while scores 4 and 5 are considered positive FDG-PET for residual disease[33]. There was a single study that found that SUV3 and SUV6 were the best predictors of response in aggressive B-lymphomas patients. TLG volumes were also associated with residual disease at the end of treatment. It was also implemented a tumor segmentation method that allowed for a semiautomated evaluation that corresponded to morphological dimensions of the tumor[34].

Liquid biopsy, circulating-free DNA and circulating-tumor DNA

Circulating-free DNA (cfDNA) are fragments of DNA that has been released in the plasma due to cell death through apoptosis or necrosis, as well as actively secreted by tumor cells. It first appeared in a study conducted by Leon et al in 1977, where they discovered cfDNA in serum to be increased in cancer patients[35]. A portion of cfDNA, known as circulating-tumor DNA (ctDNA) is derived from tumor cells and makes up for a minor part of total cfDNA[36]. Therefore, liquid biopsy refers to the ability to detect and examine ctDNA from the available cfDNA. While samples for liquid biopsy can be obtained from several body fluids including urine, saliva and cerebrospinal fluid, plasma is presently the widely accepted choice for cfDNA extraction[37,38]. Multiples studies have indicated that the genetic profile of ctDNA accurately reflects that of the original lymphoma[39–41]. Next-generation sequencing-based methods and digital droplet or quantitative PCR can be used to identify acquired mutations that are specific to tumors in cfDNA. These methods will be discussed in further detail.

Regarding cHL, Spina et al. identified a relationship between >2-logarithm-fold decline of ctDNA load (relative to pre-treatment level) after 2 cycles of ABVD and complete metabolic response and cure in 24 advanced stage cHL patients. <2-logarithm-fold of ctDNA load was associated with progression disease. They also observed that patients with interim PET/CT positive and a >2-logarithm-fold decrease of ctDNA load were cured, whereas those with interim PET/CT negative and <2-logarithm-fold decrease experienced relapse[42]. Sobesky et al. devised a ctDNA sequencing platform to address MRD in 121 cHL patient samples prior to, during, and after treatment. They concluded that patients who had negative MRD evaluation based on ctDNA, and negative PET-CT had an excellent prognosis, with no relapses. However, their primary concern was a small sample size[43]. In a study of 60 cHL patients, Camus et al. found no significant difference in ctDNA concentration between low-grade interim PET score (DS 1-3) and high-grade interim PET score (DS 4-5)[44].

Early detection of treatment failure and disease relapse made possible by liquid biopsy has the potential to improve the cure rate of aggressive B lymphomas[45]. In DLBCL patients, ctDNA was detected in 96% and an increase of this biomarker by day 15 was linked to lack of response to treatment [46]. On the other hand, long-term remission led to clearance of ctDNA[41], indicating its utility as a biomarker. Roscheswki et al. also demonstrated higher sensitivity of ctDNA surveillance using IgNGS compared to CT imaging through a detection lead-time 3.5 (median) months and 7.4 months for all patients who relapsed and late recurrences, respectively[41]. A smaller study on DLBCL found detectable ctDNA by NGS in all 11 patients at the time of recurrence and 7 patients before imaging translation with a median lead-time of 2 months, which supports the previous data. However, this study doesn’t mention the stage of disease.[47]. In chemo-free regimens such as CAR-T therapy, Frank et al. conducted a prospective trial to explore ctDNA as tool for MRD assessment. They found ctDNA to be detectable either at the time of relapse or before it occurred in 29 of 30 (94%) patients[48]. In a previous study by Hossain et al., ctDNA levels were found to increase before the progression of disease in 4 patients (80%) who received CAR-T cell therapy. These elevated levels persisted at the time of PET-CT progression disease confirmation[49].

Delfau-Larue and colleagues conducted a retrospective study analyzing peripheral blood samples from 133 FL patients and discovered a correlation between cfDNA and total metabolic tumor volume (TMTV). They found that when cfDNA exceeded 2550 equivalent-genome/ML, PFS was significantly lower[50]. Similarly, Sarkozy et al. identified ctDNA in 86% of FL patients, establishing it as a significant biomarker in MRD.

Their study also revealed that high levels of ctDNA at diagnosis were considered an independent prognostic factor for PFS[51].

Next-generation sequencing-based assays

The high-throughput screening for mutations that aids in MRD assessment is made possible through next-generation sequencing (NGS) assays. By providing a high number of reads across a genomic area of interest, this method facilitates the sequencing of the immunoglobulin gene (IgNGS), evaluation of VDJ rearrangements, and detection of somatic mutations by sequencing a specific panel of frequently mutated genes through the Cancer Personalized Profiling by Deep Sequencing (CAPP-Seq)[52]. The VDJ serve as a distinctive identifier for an individual tumor and may work as an early indicator of disease recurrence. The primary characteristic that sets it apart from PCR is its ability to identify unknown variants during screening [53].

Oki et al. searched tumor-specific clonotypes using IgNGS, in 17 cHL patients, and reported an increased sensitivity in finding lymphoma-specific sequences in serum vs peripheral blood mononuclear cells[54].

Kurtz et at. conducted a comparison between serum lactate dehydrogenase (LDH) and ctDNA detected by IgNGS as a blood biomarker for disease burden in DLBCL. The study found that IgNGS had significant superior sensitivity (88% 30/34) compared to LDH (59% 20/34) and superior specificity (100% 38/38) compared to surveillance PET/CT (56% 18/32)[55]. In subsequent study, Kurtz et al. developed a model that translated the relationship between changes in ctDNA using CAPP-seq assay, clinical outcome after two cycles of chemotherapy, and ultimately radiographical relapse[39]. Rossi et al. also employed CAPP-seq to assess clonal dynamics after R-CHOP treatment and persistence of mutations in cfDNA of refractory patients[40].

In their study, Pott et al. utilized NGS techniques to evaluate MRD in 113 FL patients. They discovered that identifying IgNGS clonotypes was a reliable MRD monitoring technique, like qPCR, which can complement established MRD techniques[56]. Additionally, Ubieto et al. found in 29 FL patients that MRD positivity during interim assessment or at end of therapy was associated with significantly lower PFS[57].

Digital-droplet PCR and quantitative PCR

Quantitative PCR (qPCR) and Digital-droplet PCR (ddPCR) are two PCR-based MRD detection techniques. Both work by detecting tumor-specific DNA at a known locus. qPCR quantifies DNA by measuring the number of amplification cycles needed for detection above background levels[58]. On the other hand, ddPCR is a modified version of the original PCR that dilutes DNA into thousands of small water-in-oil droplets for quantification of low variant allele frequency mutations. This means that each droplet functions as an individual qPCR reaction, enhancing its sensibility[59].

Camus et al conducted a retrospective study to examine the mutational profile of cHL in frozen ctDNA samples of 28 patients at the end of treatment, using ddPCR analysis, confirmed by NGS. They identified 7 patients with a recurrent XPO1 E571 mutation, and 4 relapsed during follow-up. Interestingly, only 1 patient had positive PET-CT scan, which shows that XPO1 E571 may be a more sensitive biomarker for disease relapse than PET-CT.[60]

Liu et al. utilized qPCR in MCL and discovered that 12 of 21 MRD positive patients progressed within 3 years of follow-up, whereas only 4 of 18 that were MRD negative progressed. The detection of MRD in this case predicted disease progression with hazard ratio of 3.7[61]. In contrast, Klener et al. studied 67 patients, and did not observe any correlation between MRD status and PFS after induction therapy[62]. Szostakowska et al. found that qPCR detection of SOX11 expression was more specific and useful than t(11;14), and patients with higher SOX11 expression had shorter PFS than those with low SOX11 expression[63]. Finally, Drandi et al. compared various MRD techniques and determined ddPCR to be more sensitive than qPCR at positivity below quantitative range[64].

Delfau-Larue et al. utilized ddPCR to evaluate MRD and subsequent complete molecular remission in FL patients at the end of therapy. They found these to relate to improved 3-year PFS[65]. In another study, Cavalli et al. compared qPCR and ddPCR in detecting BCL2 rearrangement in 67 FL patients and discovered that ddPCR was comparable to qPCR in assessing MRD and promisingly more accurate[66]. Galimberti et al. evaluated MRD through BCL2/IGH assessment using qPCR technique. They reported that patients without MRD or low MRD had a higher complete remission rate and improved PFS at 12 and 24 months after the end of treatment[67].

Authors	Lymphoma	Sample size	Timing of sampling	Technique	Results
Spina et al[42]	cHL	N=349 samples	Untreated and relapsed/refractory after ASCT	ctDNA NGS quantified.	>2-log decline of ctDNA after 2 cycles associated to complete metabolic response and cure. Better than PET/CT.
Sobesky et al[43]	I-IV cHL	N=121 baseline plasma N=77 follow-up samples	Before, during and after different chemotherapy regimens	ctDNA sequencing platform.	MRD^- and PET/CT^- group without relapse.

Camus et al[44]	I-IV cHL	N=60 patients	After chemotherapy	ctDNA NGS quantified.	No difference between ctDNA and PET DS 1-3 and DS 4-5. Moderate correlation between ctDNA and MTV.
Oki et al[54]	cHL	N=17 patients	Time of diagnosis or recurrence	IgNGS	More sensitive than PBMC (8 of 9 vs 3 of 9).
Camus et al[60]	cHL	N=94 patients	Time of diagnosis and at end of chemo/radiotherapy	ddPCR	XPO1 E571K detected in 25% of patients. Tendency for shorter PFS.
Parvez et al[34]	I-IV DLBCL	N = 82 patients	Not applicable	PET/CT	SUV3 and SUV6 predictors of response. TLG volume associated with residual disease at the end of treatment
Assouline et al[46]	I-IV DLBCL	N=40 patients	Immunotherapy	ctDNA ddPCR + NGS quantified	ctDNA detected in 96% of patients. ctDNA increase linked to lack of response to treatment.
Roscheswki et al[41]	II-IV DLBCL	N = 980 + 578 serum samples (surveillance + interim)	During chemotherapy	ctDNA NGS quantified	998 of 1000 serum samples from disease-free patients had negative ctDNA; ctDNA also had a median detection lead-time 3.5- and 7.4-months vs CT scan (relapse and late recurrences).
Scherer et al[47]	DLBCL	N = 116 patients	Before treatment	ctDNA NGS quantified	ctDNA correlated with LDH and MTV and independent factor associated with PFS; ctDNA detected prior to clinical relapse in 8 of 11 patients, with a median lead-time of 2 months; ctDNA undetectable in healthy and disease-free patients.
Frank et al[48]	DLBCL	N = 72 patients	Before treatment	ctDNA PCR quantified	ctDNA detected at or before relapse in 29 of 30 (94%) patients; 15 of 17 patients who had ctDNA detected on day 28 after treatment relapsed.
Hossain et al[49]	DLBCL	N = 6 patients	Before and after immunotherapy	ctDNA NGS quantified	4 of 5 patients with increasing ctDNA before progressive disease and PET/CT recognition.
Kurtz et al[55]	DLBCL	N = 75 patients	Before treatment and after 4 cycles of chemotherapy	IgNGS	Superior sensitivity and specificity vs LDH and PET/CT (88% vs 59% and 100% vs 56%).
Kurtz et al[39]	DLBCL	N = 10 patients	Before, during and after immunochemotherapy	ctDNA NGS quantified	PR and CR associated with 2.9 log decrease in ctDNA concentration. SD and PD patients reported an increase of 0.3 log in ctDNA concentration.
Rossi et al[40]	Untreated DLBCL	N = 50 patients	Before, during and after chemotherapy	NGS	Rapid clearance of DLBCL mutations in cfDNA after R-CHOP in responding patients.
Liu et al[61]	MCL	N = 39 patients	Post-induction and post-ASCT	qPCR	12 of 21 (57%) MRD^{+} progressed in the 3-year follow-up vs 4 of 18 (18%) MRD^{-}.
Klener et al[62]	MCL	N = 67 patients	After 3- and 6-cycles of induction therapy	qPCR	MRD not correlated with PFS after induction treatment.
Szostakowska et al[63]	MCL	N = 34 patients	Diagnosis and during treatment	SOX11 qPCR detected	High SOX11 expression associated with shorter PFS vs low SOX11 expression (p=0.04)
Drandi et al[64]	MCL	N = 416 samples	Not mentioned	ddPCR	ddPCR more sensitive vs qPCR at MRD^{+} BQR (10^{-4} and 10^{-5})
Delfau-Larue et al[50]	I-III FL	N = 133 patients	After chemotherapy	cfDNA ddPCR quantified	cfDNA > 2550 equivalent-genome/mL correlated with TMTV and < PFS
Sarkozy et al[51]	FL	N = 34 patients	At diagnosis	ctDNA NGS quantified	High levels of ctDNA found in 86% of patients, and found to be independent factor for PFS
Pott et al[56]	FL	N = 113 patients	At end of induction	NGS vs qPCR	NGS comparable to qPCR in assessing MRD
Ubieto et al[57]	FL	N = 29 patients	After 4- and 6- cycles of treatment	NGS	MRD significantly lower values at CR vs AD. MRD^{+} at interim or end of treatment resulted in inferior PFS.
Delfau-Larue et al[65]	I-IV FL	N = 222 patients	At screening and at week 24 after end of treatment	ddPCR	MRD^{+} at end of treatment associated with significantly lower PFS.
Cavalli et al[66]	I-II FL	N = 67 patients	At diagnosis	ddPCR vs qPCR	ddPCR potentially more accurate than qPCR. Tumor burden significantly correlated with PFS when quantified by ddPCR.
Galimberti et	II-IV FL	N = 415	At diagnosis, end of, 12	qPCR	MRD^{-} status correlated with

al[67]	patients	and 24 months after treatment		improved PFS in CR and PR.

Table 2. List of studies assessing measurable residual disease. cHL: classic lymphoma Hodgkin; ASCT: autologous stem-cell transplant; ctDNA: circulating tumor DNA; NGS: next generation sequencing; MRD: measurable residual disease; DS: Deauville score; MTV: metabolic tumor volume; PBMC: peripheral blood mononuclear cells; ddPCR: digital droplet PCR; MTV: metabolic tumor volume; DLBCL: diffuse large B cell lymphoma; PFS: progression free survival; LDH: lactate dehydrogenase; PR: partial response; CR: complete response; SD: stable disease; PD: progression disease; MCL: mantle cell lymphoma; qPCR: quantitative PCR; TMTV: total metabolic tumor volume; BQR: below quantitative range; AD: active disease;

Discussion

The evaluation of MRD in lymphomas has become a crucial topic of clinical investigation due to the delay in diagnosing clinical relapse and poorer prognosis associated to disease recurrence. The ability to identify low-level disease before clinical manifestation is critical for enhancing chances of survival and it may allow for a response evaluation that can translate in a timely implementation of salvage therapy, escalation/de-escalation of therapy or change of treatment regimen.

PET/CT is widely employed as the standard procedure for assessing MRD. By detecting metabolically active focal points throughout the body, it enables the precise identification of specific hotspots of disease on an individual level. There was a limited amount of information available for new FDG-PET parameters. SUV3, SUV6 and TLG might increase sensitivity for disease detection. Being able to outline tumor morphology can be helpful for guiding tissue biopsy. Still, this method has low sensitivity for small volume tumors. PET/CT's effectiveness is hindered by a high rate of false positives, the potential risk of radiation exposure to patients, non-specificity in elevated FDG uptake, and its inability to detect low-level tumor burden. Besides, its use in low metabolic rate lymphomas is questionable. The cutoffs used in the DS are subjective and may result in inaccuracies when identifying and measuring disease recurrence.

Liquid biopsy, specifically through the analysis of ctDNA, offers several benefits. It is a non-invasive and quantifiable tool that can be performed repeatedly over time, potentially aiding in the early detection of refractory disease or relapse. It has shown potential in improving early detection of treatment failure and disease relapse in aggressive B lymphomas, such as cHL and DLBCL. For cHL patients, ctDNA evaluation has been associated with complete metabolic response and cure, and a >2-logarithm-fold decrease of ctDNA load has been linked to successful outcomes. In DLBCL patients, ctDNA has been detected in a high percentage of cases and its clearance has been associated with long-term remission. Furthermore, ctDNA surveillance using IgNGS has demonstrated higher sensitivity compared to CT imaging, with a detection lead-time of up to 7.4 months for late recurrences. For FL patients, cfDNA has been correlated with total metabolic tumor volume and high levels of ctDNA at diagnosis have been identified as an independent prognostic factor for PFS. These results may offer the possibility to associate PET/CT with seriated ctDNA quantification. However, ctDNA constitutes only a fraction of the total cfDNA, and quantifying these small amounts, and enhancing specificity pose significant challenges for current methods. A connection has been observed between reduced levels of ctDNA and an enhanced metabolic response as well as improved PFS in all lymphoma types. Nevertheless, there is no established threshold value for this measure. Moreover, there is a pressing need to standardize ctDNA quantification and translate this novel biomarker into clinical practice.

NGS assays allows for a dynamic evaluation of genetic landscape and clonal evolution during and after treatment. IgNGS and CAPP-seq techniques have been used to detect tumor-specific clonotypes with higher sensitivity and specificity than traditional methods. Identifying disease recurrence related to a specific clonotype can provide valuable information for prompt and targeted treatment. However, it presents as a time-consuming analysis, that requires expertise in the technical and difficult interpretation of the data.

Various studies have demonstrated that ddPCR is a more sensitive technique than qPCR in identifying circulating biomarker, with the added benefits of cost-effectiveness, speed, and simplicity. Furthermore, the studies utilized ddPCR technique to emphasize the potential of emerging, selective biomarkers, such as XPO1 E571 and SOX11 expression, as sensitive predictors of disease recurrence in cHL and MCL, respectively. ddPCR is believed to be a powerful tool in somatic mutation detection, but several drawbacks have been cited, including its inability to detect MRD in instances where there is mutation-negative clone during relapse, limited capacity to identify MRD due to small amounts of cfDNA available, and a requirement for standardization to assess its sensitivity, specificity, and reproducibility.

This study has several limitations, mainly that it predominantly included observational studies, and some of them were subject to selection bias due to the unavailability to retrieve some individual samples (either before or after treatment, for example). The risk of confounding bias exists in several studies as they analyzed samples from patients with varying stages of disease and different treatment regimens. Additionally, several studies had a small sample size, which increases the risk of bias.

The mentioned studies demonstrate the potential of using these techniques in conjunction with established imaging methods for MRD assessment. Despite all technical issues linked to each approach, it remains limited understanding regarding the optimal treatment alternative for patients who present MRD. Larger, randomized controlled studies are necessary to clearly establish the superiority of these techniques over current MRD evaluation methods. Additionally, better standardization, clinical interpretation of results, and improved support for MRD-positive patients are also in need.

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page and paragraph/ table #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both. **- MANDATÓRIO**	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number. **– SEGUIR RECOMENDAÇÕES DA REVISTA**	4,1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known. **– MANDATÓRIO** ***O rationale corresponde à justificação da importância da revisão sistemática***	5,4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS). **- MANDATÓRIO**	6,4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number. **– FACULTATIVO**	N/A uma vez que não foi usado protocolo de revisão
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale. **– MANDATÓRIO** ***É altamente recomendado, de acordo com as boas práticas da Cochrane, que não sejam aplicados critérios de exclusão baseados na língua e/ou data de publicação dos estudos.***	6,5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched. **– MANDATÓRIO** ***Em consonância com as boas práticas da Cochrane, é mandatório que se verifique pesquisa em pelo menos duas bases de pesquisa bibliográfica (idealmente, deverão ser pesquisadas duas bases generalistas e uma específica da área). No caso de revisões sistemáticas de estudos experimentais/ensaios clínicos aleatorizados, é altamente recomendado que uma das bases pesquisadas corresponda à CENTRAL ou a bases de ensaios clínicos como a ClinicalTrials.gov.*** ***Estudos de revisão da literatura em que a pesquisa decorra numa única base de dados não serão classificados como revisões sistemáticas.***	6,5

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Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated. **– MANDATÓRIO** ***A query de pesquisa deve ser obrigatoriamente disponibilizada. A utilização de filtros de pesquisa da InterTASC é altamente recomendada (https://sites.google.com/a/york.ac.uk/issg-search-filters-resource/home)***	6,5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis). **– MANDATÓRIO** ***As fases de selecção dos estudos primários devem ser descritas. Em consonância com as boas práticas da Cochrane, é mandatório que o processo de selecção envolva duas fases (fase de rastreio, em que os registos são seleccionados por título e abstract, e fase de inclusão, na qual se procede à leitura integral dos full texts). Em cada uma destas fases, o processo de selecção deve mandatoriamente envolver dois investigadores actuando de forma independente.***	6,5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators. **– MANDATÓRIO** ***Trata-se de descrever de que forma se procedeu à extracção de dados dos estudos primários. Em consonância com as boas práticas da Cochrane, tal processo deverá envolver dois investigadores de forma independente.***	6,5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made. **– MANDATÓRIO** ***Trata-se de descrever as variáveis para as quais foi obtida informação.***	6,5
Risk of bias in individual studies / Risk of bias across studies	12/ 15	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis. **– MANDATÓRIO** ***Em todas as revisões sistemáticas, deverá existir um processo de avaliação da qualidade dos estudos primários. No caso de revisões sistemáticas de estudos experimentais/ensaios clínicos aleatorizados, a aplicação dos critérios de risco de viés (Risk of Bias) da Cochrane é altamente recomendada. No caso de revisões sistemáticas de estudos observacionais, poderão ser seguidos os critérios ROBINS ou os critérios dos National Institutes of Health (https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools).***	6,5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means). **– FACULTATIVO. APENAS NECESSÁRIO SE FOR FEITA META-ANÁLISE**	N/A, uma vez que a revisão não se acompanhou de meta-análise
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis. **– FACULTATIVO. APENAS NECESSÁRIO SE FOR FEITA META-ANÁLISE**	N/A, uma vez que a revisão não

PRISMA 2009 Checklist

			se acompanhou de meta-análise
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified. **– FACULTATIVO. APLICÁVEL APENAS SE FOR FEITA META-ANÁLISE**	N/A, uma vez que a revisão não se acompanhou de meta-análise
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram. **– MANDATÓRIO**	6,5, fig 1.
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations. **– MANDATÓRIO**	8-9, table 2.
Risk of bias within and across studies	19/ 22	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12). **– MANDATÓRIO**	8-9
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot. **– FACULTATIVO. APLICÁVEL APENAS SE FOR FEITA META-ANÁLISE**	N/A, uma vez que a revisão não se acompanhou de meta-análise
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency. **– FACULTATIVO. MANDATÓRIO APENAS SE FOR FEITA META-ANÁLISE**	N/A, uma vez que a revisão não se acompanhou de meta-análise
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]). **– FACULTATIVO. APLICÁVEL APENAS SE FOR FEITA META-ANÁLISE**	N/A, uma vez que a revisão não

PRISMA 2009 Checklist

			se acompanhou de meta-análise
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers). **– MANDATÓRIO**	10,(1,2,3,4,5)
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias). **– MANDATÓRIO**	10,6
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research. **– MANDATÓRIO**	10,7
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. **– SEGUIR RECOMENDAÇÕES DA REVISTA**	N/A, uma vez que a revisão não teve fundos

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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Regras de Formatação

Revisões Sistemáticas e Meta-Análises

As revisões sistemáticas podem ou não utilizar métodos estatísticos (meta-análises) para analisar e resumir os resultados dos estudos incluídos.

As Revisões Sistemáticas podem ser apresentadas no formato Introdução, Métodos, Resultados, Discussão, (Conclusão?). O assunto deve ser claramente definido. O objetivo de uma revisão sistemática deve ser produzir uma conclusão baseada em evidência. Nos Métodos deve ser fornecida uma indicação clara da estratégia de pesquisa da literatura, extração de dados, classificação das evidências e análise. Deve ser seguida a normativa PRISMA (http://www.prisma-statement.org/) e realizado o registo do protocolo na PROSPERO (https://www.crd.york.ac.uk/prospero). É exigido resumo estruturado que espelhe fielmente o corpo do manuscrito.
Palavras: máximo 4000 palavras (excluindo resumo, figuras e tabelas).

Resumo: máximo 350 palavras.

Figuras/Tabelas: máximo 6. As figuras não deverão ser compostas por mais do que seis imagens cada uma.

Referências: máximo 100.