EUROPEAN UROLOGY 80 (2021) 456–466

available at www.sciencedirect.com
journal homepage: www.europeanurology.com

European Association of Urology

Review – Testis Cancer

Circulating MicroRNAs, the Next-Generation Serum Biomarkers in Testicular Germ Cell Tumours: A Systematic Review

Ricardo Leão [a], *Maarten Albersen* [b], *Leendert H.J. Looijenga* [c], *Torgrim Tandstad* [d], *Christian Kollmannsberger* [e], *Matthew J. Murray* [f,g], *Stephane Culine* [h,i], *Nicholas Coleman* [f,j], *Gazanfer Belge* [k], *Robert J. Hamilton* [l], *Klaus-Peter Dieckmann* [m,*]

[a] *Department of Urology, Hospital de Braga, Hospitais CUF, Faculty of Medicine, University of Coimbra, Coimbra, Portugal;* [b] *Department of Urology, University Hospitals Leuven, Leuven, Belgium;* [c] *Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands;* [d] *The Cancer Clinic, St. Olav's University Hospital, Trondheim, Norway;* [e] *Department of Medicine, Medical Oncology Division, BC Cancer, Vancouver Centre, University of British Columbia, Vancouver, Canada;* [f] *Department of Pathology, University of Cambridge, Cambridge, UK;* [g] *Department of Paediatric Haematology and Oncology, Cambridge University Hospitals NHS Foundation Trust, University of Cambridge, Cambridge, UK;* [h] *Department of Medical Oncology, Hôpital Saint-Louis, AP-HP, Paris, France;* [i] *Paris-Diderot University, Paris, France;* [j] *Department of Histopathology, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK;* [k] *Faculty of Biology and Chemistry, University of Bremen, Bremen, Germany;* [l] *Department of Surgical Oncology, Princess Margaret Cancer Centre, Toronto, Canada;* [m] *Department of Urology, Asklepios Klinik Altona, Hamburg, Germany*

Article info

Article history:
Accepted June 9, 2021

Associate Editor:
Giacomo Novara

Keywords:
Biomarkers
MicroRNA
Testicular germ cell tumours

Abstract

Context: Clinical management of testicular germ cell tumours (GCTs) is hampered by low sensitivity and specificity of the biomarkers currently in use. Circulating microRNAs (miRs) might offer the potential to address areas of unmet clinical need.
Objective: To systematically evaluate the evidence for clinical applications of serum levels of miR302/367 and miR371-3 in adult testicular GCTs in terms of primary diagnosis, various clinical scenarios, and the costs of clinical implementation.
Evidence acquisition: We performed a critical review of PubMed/Medline, Embase and the Cochrane Library in January 2021 in accordance with Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) statement.
Evidence synthesis: Thirty-one manuscripts addressed miR performance and potential clinical use in testicular GCT. Of these, 23 evaluated the utility in primary diagnosis, seven in early-stage disease, and 13 in metastatic disease, and two addressed the costs of clinical implementation. Of the various miRs studied, miR-371a-3p appears the most useful and potentially the only one that needs to be assayed, with an area under the receiver operating characteristic curve >0.90, sensitivity of 89–96%, and specificity of >90% for both seminoma and nonseminoma, surpassing the classic serum tumour markers. The miRs studied to date are not elevated in cases with teratoma only. Levels of miR-371a-3p correlate with primary tumour mass, clinical stage, and International Germ Cell Cancer Collaborative Group risk groups. Serial measurements mirror treatment efficacy in all clinical stages.
Conclusions: Circulating miRNA levels, particularly of miR-371a-3p, have potential for incorporation in clinical practice and may aid in clinical decision-making in various clinical scenarios in GCT.

* Corresponding author. Department of Urology, Asklepios Klinik Altona, Paul Ehrlich Strasse 1, D-22763 Hamburg, Germany. Tel. +49 40 1818811661; Fax: +49 40 1818814910.
E-mail address: dieckmannkp@t-online.de (K.-P. Dieckmann).

https://doi.org/10.1016/j.eururo.2021.06.006

Patient summary: We analysed the current evidence on the usefulness of blood levels of molecules called microRNAs in the management of testicular tumours. The microRNA-371a-3p molecule has better sensitivity and specificity than the markers currently being measured. This new biomarker may soon have a place in clinical practice.

1. Introduction

Testicular germ cell tumours (TGCTs) represent the most common malignancy among young males aged 20–40 yr [1]. For clinical purposes, two major histological subgroups of GCT are used: pure seminoma, accounting for 60% of all GCTs, and nonseminomas, encompassing all other types including combined forms [2].

Clinical management of TGCTs greatly relies on measurement of the serum tumour markers (STMs) α-fetoprotein (AFP), β-human chorionic gonadotropin (βhCG), and lactate dehydrogenase (LDH). However, the utility of these markers is limited by low sensitivity and specificity for tumour detection [3] because of their heterogeneous expression across GCT subtypes and stages. Specifically, βhCG and LDH are elevated in only 28% and 29.1% of pure seminomas, while βhCG, AFP, and LDH are elevated in 53%, 60.1%, and 38.7% of nonseminomas, respectively [4].

In light of the limited clinical utility, there has been an ongoing search for novel biomarkers since the introduction of these protein-based markers in the 1970s. During the past decade, nucleic acid–based markers, specifically microRNAs (miRs), have garnered attention [5]. miRs are small noncoding RNA molecules of approximately 22 nucleotides. They are involved in epigenetic regulation of mRNA translation and thereby influence cellular differentiation and other physiological processes, as well as carcinogenesis, for which they can operate as oncogenes or tumour suppressors [6].

TGCTs originate from undifferentiated embryonic germ cells and accordingly may develop a wide variety of morphological features mimicking embryonic and extra-embryonic developmental stages while retaining biological and biochemical features of embryonic germ cells [7]. A number of miRs are specifically expressed in embryonic stem cells, particularly the clusters miR-371-3 and miR-302-367, which are among others involved in cell pluripotency control [8–10]. Because of the biological similarities between embryonic stem cells and germ cell neoplasms, it was suspected that these miRs characteristic for embryonic stem cells might also play a role in the biology of TGCTs. Three findings formed the rationale for exploring miRs as biomarkers in TGCT: (1) detection of the two main miR clusters characteristic of embryonic stem cells in TGCT tissues; (2) the discovery that these tissue-based biomarkers could be detected in serum; and (3) the great stability of circulating miRs in body fluids [11].

The present review provides a systematic summary of current knowledge regarding the utility of circulating miRs for clinical management of adult (type II) TGCT.

2. Evidence acquisition

Evaluation of records retrieved from the literature search followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) approach (Fig. 1) [12] and the search results were reviewed according to Standards for Reporting Diagnostic Accuracy (STARD) [13]. The Supplementary material provides further information on evidence acquisition.

2.1. Search strategy

On January 4, 2021, we conducted a systematic search in the PubMed/Medline, Embase, Web of Science, and Cochrane Library databases to identify all relevant studies in the English language. The search algorithms, criteria for inclusion, and methods for data extraction are described in detail in the Supplementary material.

3. Evidence synthesis

Our search identified 31 manuscripts. The primary outcomes were miR roles in primary diagnosis (n = 23), early-stage disease (n = 7), and metastatic disease (n = 13) and the costs of clinical implementation (n = 2), as shown in Figure 1 and Supplementary Table 1.

3.1. Risk of bias and quality assessment

The risk of bias of the studies included was independently analysed by two authors (R.L., K.-P.D.) using 11 signalling questions related to four domains (Supplementary Fig. 1). Important heterogeneity was found among the studies, with low to intermediate risk of bias in 11 studies. The highest risk of bias was identified in the population domain, for which 19 studies had intermediate to high risk of bias.

3.2. miRNAs in TGCTs

3.2.1. miRNA in TGCT tissue

It has been shown that specific miRs are associated with TGCT, namely the miR-371-3 cluster (miR-371a-3p, miR-372-3p, and miR-373-3p) located on chromosome 19, and miR-367-3p and the miR-302 cluster (miR-302a-3p, miR-302b-3p, and miR-302c-3p) located on chromosome 4 [14–19]. The miR clusters characteristic of embryonic germ and stem cells were first documented in GCT tissue and in GCT cell lines by Voorhoeve et al in 2006 [20]. A high-throughput analysis of 156 miRs in 69 TGCT tissue samples

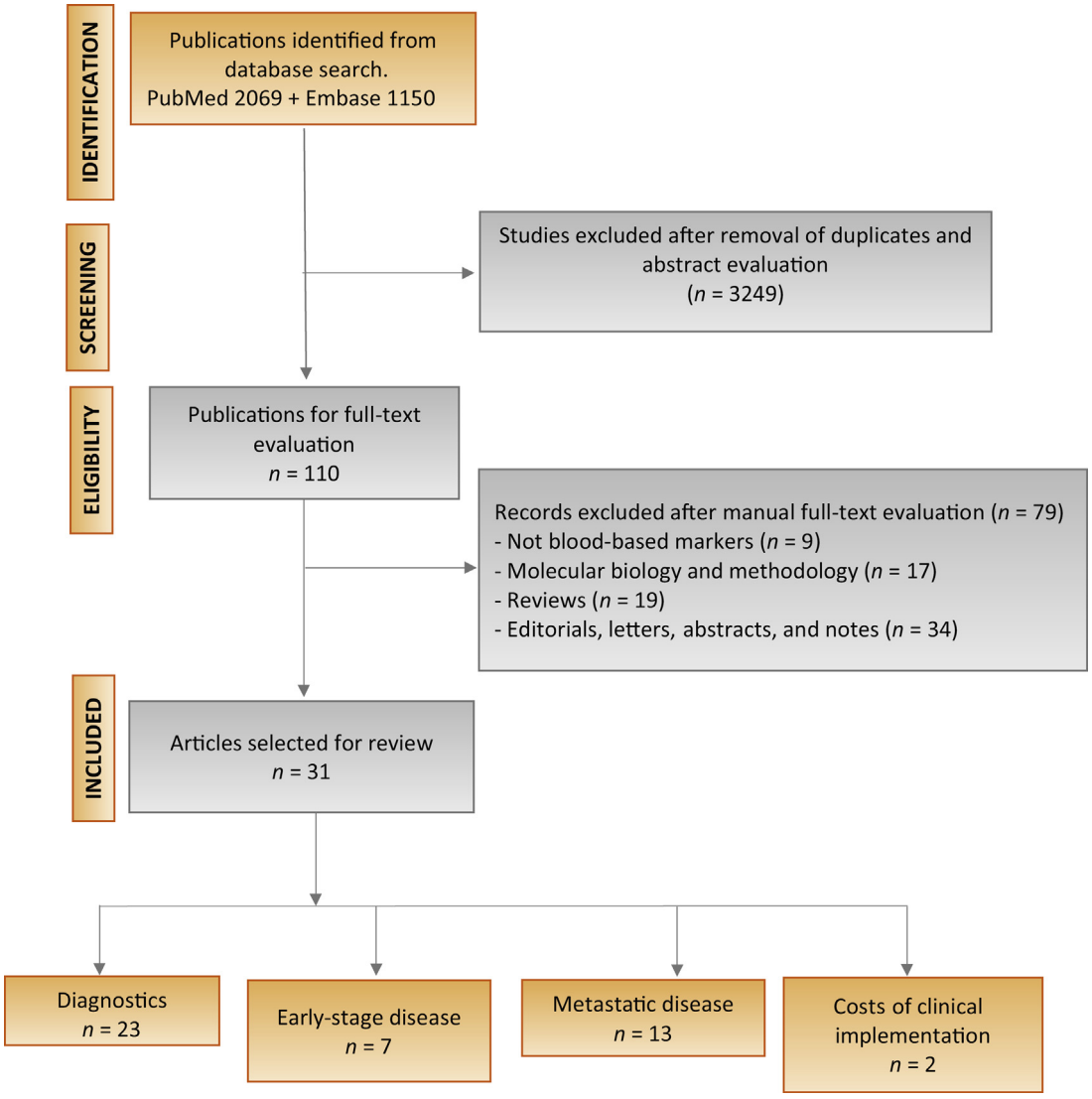

Fig. 1 – Flow diagram of evidence acquisition for the systematic review of miRNAs as biomarkers for testicular germ cell tumors. The last group of boxes show the number of studies addressing diagnostic properties, the decision-making value for early-stage and metastatic disease, and the costs of clinical implementation.

revealed that expression of miRs from clusters miR-371-3 and miR-302a-d was significantly higher in GCT tissue in comparison to normal testicular tissue [14]. These results were confirmed with polymerase chain reaction (PCR) and in situ hybridisation techniques [15–19].

All TGCT histological subtypes, including the preinvasive germ cell neoplasia in situ (GCNIS), showed expression of the germ-cell– and stem-cell–specific miRs, except for teratoma [21,22]. It is noteworthy that normal testicular tissue also contained miR-371a-3p at levels that were low but significantly higher than those in nontesticular tissues [23–25]. Studies on the expression of stem-cell–specific miRs in TGCT tissues greatly increased our understanding of the biology of GCT as developmental cancers and provided a rationale for exploring these miRs as biomarkers in body fluids [26].

3.2.2. miRNA detection in TGCTs biofluids: proof of principle as liquid biopsy

Murray et al [27] were the first to document circulating miR molecules of the miR-371-3 cluster in a paediatric patient with a yolk sac tumour. In adult GCTs, serum miR 371-3 expression was confirmed in 11 patients and further verified in several small case series [18,19,28–30]. Importantly, miR-371a-3p was not detectable in 24 non-GCT malignancies, suggesting the GCT specificity of this biomarker [31]. Taken together, these studies revealed four important results. First, levels of the two miR germ-cell– and stem-cell–specific clusters were elevated in serum of adult GCT patients but not in healthy controls. Second, there was variation in expression levels across the various miRs, and expression levels of the miRs varied across the five GCT histological subtypes. Third, serial

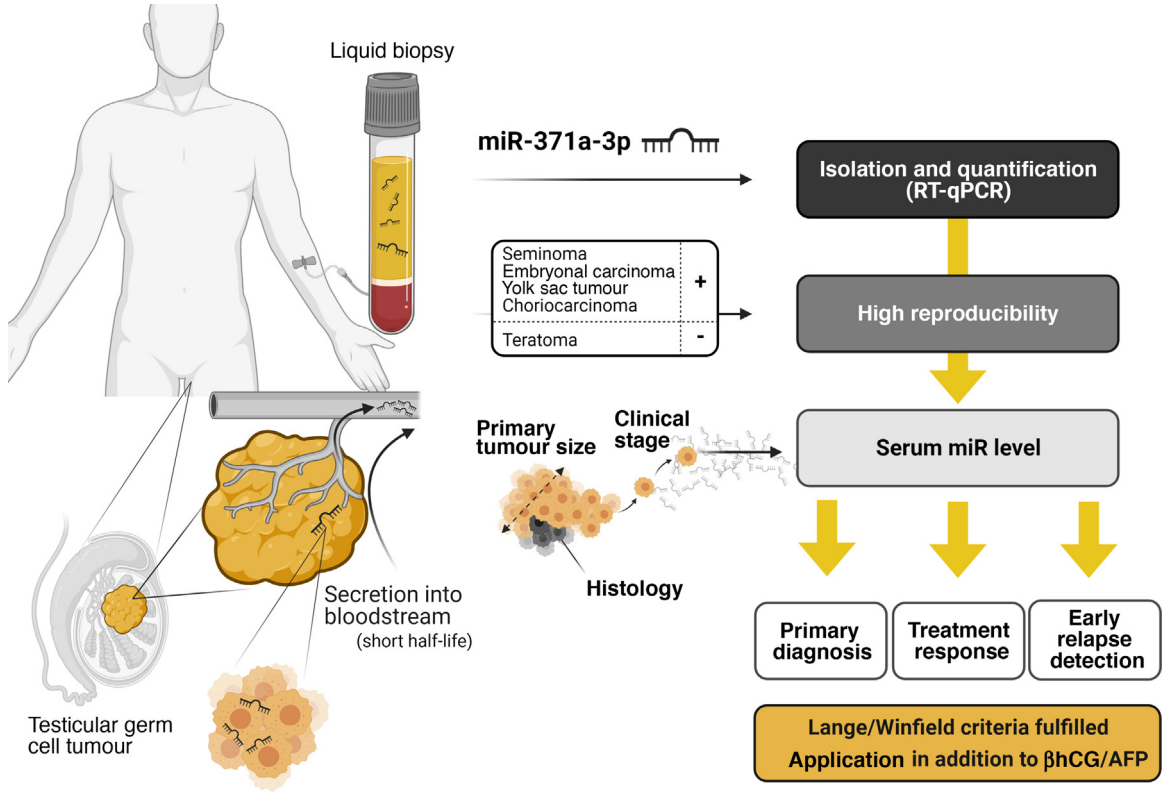

Fig. 2 – Serum miR-371a-3p in testicular germ cell tumours. Biology, detection, and clinical application as a blood-based biomarker in compliance with the Lange-Winfield criteria for biomarkers. (1) The candidate substance is produced only by the malignancy itself. (2) It is secreted into body fluids. (3) It can be measured in reproducible fashion. (4) Levels in body fluids correlate with the amount of tumour present. (5) The substance can be detected even in early disease. (6) Measured levels correlate with response to treatment. (7) The half-life of the substance is short. Created in Biorender (www.biorender.com). CSI = clinical stage I; CSIIA = clinical stage IIA; RPLND = retroperitoneal pelvic lymph-node dissection.

measurements showed close correlation of miR levels with treatment response. Fourth, circulating miR levels appeared to be stable in serum and could reproducibly be measured using PCR techniques. Overall, these studies provided the first evidence of blood-based miR markers in GCTs.

3.3. *Clinical applications in TGCTs*

3.3.1. *Diagnosis*

Subsequent studies performed a systematic evaluation of the sensitivity and specificity of several candidate serum miRs for primary diagnosis of TGCT (Fig. 2). The highest accuracy was observed for miR-371a-3p, with three studies reporting an area under the receiver operating characteristic curve (AUC) of 0.929, 0.943, and 0.951, sensitivity of 84.7%, 88.7%, and 89%, and specificity of 99%, 93.4%, and 90%, respectively [30,32,33] (Table 1). In view of the outstanding performance of serum miR-371a-3p, it became clear that this miR is as sensitive for TGCT diagnosis as a panel of miRs originally deemed necessary for clinical testing [30,32–35] (Table 2).

In 2019, a prospective study of serum miR-371a-3p levels in 616 GCT patients and 258 control subjects confirmed the diagnostic accuracy, with AUC of 0.958 (95% confidence interval [CI] 0.942–0.974) for clinical stage I (CSI) and 0.998 (95% CI 0.995–1.0) for CSII/III, sensitivity of 89.8% and specificity of 96.1% for seminoma, sensitivity of 95% and specificity of 96.1% for nonseminoma, and an overall positive predictive value (PPV) of 97.2% and negative predictive value (NPV) of 82.7% [36]. Another study evaluating plasma miR-371a-3p levels reported specificity and PPV of 100% for the presence of GCT [37]. It was demonstrated that miR-371a-3p is helpful in identifying GCT in marker-negative testicular masses suitable for testicle-sparing surgery [38].

3.3.2. *miR-371a-3p expression is associated with primary lesion size, clinical stage, and histology*

A positive correlation has been found between primary lesion diameter and levels of miR-371a-3p, miR-373-3p, and miR-367-3p for both seminoma and nonseminoma in CSI lesions [26,28,32,33,38]. In pure seminoma, miR-371 sensitivity was only 59% and 77% for lesions sized <10 mm and <20 mm, respectively, while sensitivity remained at 100% for nonseminoma lesions <10 mm [36]. Expression of miR-371a-3p seems to correlate with tumour bulk, since significantly higher levels are observed in CSIII disease than in CSI and CSII [4,26,28,30,31,33,34,36,38–40]. miR-371a-3p is also associated with International Germ Cell Cancer Collaborative Group (IGCCCG) risk groups [36,40,41].

Table 1 – Receiver operating characteristic analysis of candidate miRNAs for primary diagnosis of germ cell tumour

miRNA	Area under the receiver operating characteristic curve		
	Syring [30]	Dieckmann [33]	Van Agthoven [32]
miR-371a-3p	0.929	0.943	0.951
miR-372	<0.9	0.788	Not applicable
miR-373	<0.9	0.769	0.888
miR-367	<0.9	0.817	0.861

Table 2 – Performance of serum tumour markers and miRNAs for primary diagnosis of testicular germ cell tumours

Tumour marker	Sensitivity (%)		All germ cell tumours						Ref.
	SMA	NSMA	SSY (%)	SPY (%)	AUC	PPV (%)	NPV (%)	*p* value	
AFP			16.7						[18]
βhCG			8.3						
miR-371a-3p			70.8					<0.0001	
miR-372								<0.01	
miR-373								<0.001	
AFP	3	45							[34]
βhCG	62	66							
AFP + hCG	36	57							
miR-371a-3p			>90	61	0.89				
miR-372			90	81	0.91				
miR-373			90	91	0.96				
miR-367			90	81	0.94				
miR-367 + miR-371-3			98	48.3	NR				
miR-367 + miR-371-3 + miR-302			NR	8.6	NR				
AFP			19.8		0.568				[30]
βhCG			40.7		0.686				
miR-371a-3p			84.7	99	0.929			<0.001	
miR-373								<0.001	
miR-367								0.001	
miR-371-3 + 367								<0.001	
AFP	2.3	44.2	18						[33]
βhCG	31.0	40.4	34.5						
LDH	27.6	28.8	28.1						
Combined	46	57.7	50.4						
miR-371a-3p (*n* = 50)	86.2	90.4	92	84.7	0.943				
miR-371-3 + 367 (*n* = 50)			92	80					
miR-372 (*n* = 150)					0.7875				
miR-373 (*n* = 150)					0.769				
miR-367 (*n* = 150)					0.8173				
miR-371a-3p (*n* = 150)	86.3	90.4%	88.7	92.5	0.945				
miR-371a-3p			89	90	0.951	94	79		[32]
miR-373			70	89	0.888	94	58		
miR-367			79	85	0.861	92	63		
miR-371-373 + 367			92	91	0.962				
AFP			26						[36]
βhCG			43						
LDH			31						
Combined			58						
miR-371a-3p	89.8	95	91.8	96.1	0.970	97.2	82.7		
AFP			8	70	0.63				[37]
βhCG			16	89	0.75				
LDH			33	57	0.68				
miR-371a-3p			96	100	0.97	100	98		
AFP			38						[38]
βhCG			50						
LDH			32						
Combined			58	100	0.79	100	31.4		
miR-371a-3p			93.1	100	0.978	100	73.3		
AFP			28.2						[35]
βhCG			46.2						
LDH			30.8						
AFP and/or hCG	31.3	60.9	48.7						
miR-371a-3p			67.5						

Table 2 (*Continued*)

Tumour marker	Sensitivity (%)		All germ cell tumours						Ref.
	SMA	NSMA	SSY (%)	SPY (%)	AUC	PPV (%)	NPV (%)	*p* value	
miR-372-3p			55						
miR-373-3p			62.5						
miR-367-3p			20						
miR-371-373	70.6	82.6	77.5	100					
miR and/or AFP and/or hCG	75.0	91.3	84.6	100					

AFP = α-fetoprotein; hCG = human chorionic gonadotropin; LDH = lactate dehydrogenase; SMA = seminoma; NSMA = nonseminoma; SSY = sensitivity; SPY = specificity; PPV = positive predictive value; NPV = negative predictive value.

Serum levels of miR-371a-3p were found to be increased in all histological subtypes except for teratoma [26,32,33,36,38,41,42] where only trace amounts may be found in tissue and serum [17,24,32,33,36,43]. In GCNIS, conflicting results have been reported regarding the expression of miR-371a-3p and allied miRs in serum (0–51.9%) despite the well-documented evidence of intracellular miRs [21,22,31,32].

3.3.3. *miRNA profile versus classic STMs*

The classic STMs are part of the TNM classification system, but STM expression, although related to primary lesion histology, is nonspecific and yields false-negative results in 50% of cases [4]. In a comparative study evaluating disease detection, the miR-371-3 and miR-302/367 clusters had sensitivity of 98%, compared to 36% for AFP and 57% for βhCG [34]. The superior performance of the miRs was confirmed by further studies (Table 2).

3.3.4. *Early-stage disease: treatment decision-making in CSI and CSIIA*

The hypothesis that miRs would be a valuable decision tool in early-stage GCT derived from the evidence that miR levels correlate with treatment effects and seem to molecularly mirror the disease state [27,44,45] (Fig. 2). The miR half-life in CSI is <24 h [26,42]. In metastatic disease the velocity of decay is also rapid but dependent on disease burden [4,26,40,45,46]. miR-371a-3p levels can also distinguish patients with metastatic disease (CSII and CSIII) from those with localised disease (CSI), with an AUC of 0.759 (95% CI 0.715–0.803) [36].

miR-371a-3p levels significantly decrease after orchiectomy in 91.77% and 82.4% of patients with local and metastatic disease, respectively [36]. Of note, up to 13.3% of CSI patients retained elevated levels after orchiectomy, and approximately 10% of CSII patients experienced a decrease to normal levels postoperatively. These findings are unexplained so far, but may reflect the uncertainties of staging with STMs and imaging alone [35,36,40]. Accordingly, elevated miR371a-3p plasma levels predicted non-teratomatous metastases in patients with clinical CSI–IIA/B disease and negative STMs undergoing retroperitoneal lymph-node dissection (RPLND), with an AUC of 0.965, sensitivity of 100%, and specificity of 92% [47]. The accuracy of traditional GCT staging might increase from 65% to 94% for CSIA and from 50% to 92% for CSIB by implementing miR measurements, as recently suggested in a decision-tree model [48].

Serial measurements of circulating miR-371a-3p confirmed treatment efficacy in a series of 151 patients with CSI disease on active surveillance, with a decrease after orchiectomy, an increase at relapse, and another decrease following salvage therapy. Relapsing patients had elevated miR-371a-3p levels in 94.1% of cases, but only 38% had elevated STMs at the same time point. The risk of relapse was not associated with elevated post-orchiectomy miR-371a-3p levels or with the gradient of its decline. Strikingly, serial miR-371a-3p monitoring allowed earlier detection of relapse when compared to STM measurements [49].

No study to date has prospectively evaluated how circulating miRs can alter treatment decisions in CSI disease.

3.3.5. *Metastatic disease*

3.3.5.1. Chemotherapy treatment monitoring. The first evidence of miR levels mirroring response to treatment came in 2011 from a single case report showing decreasing miR-372 levels during chemotherapy that closely corresponded to AFP levels and clinical improvement [27]. That observation was confirmed 1 yr later by normalising miR levels in four patients with advanced disease undergoing chemotherapy [18].

Subsequent studies [26,33,39–41] provided further evidence corroborating the correlation with treatment response. First, miR-371a-3p levels have the ability to distinguish localised from metastasised disease with sensitivity of 83.4% and specificity of 60.1% [36,40]. Second, decreasing serum miR-371a-3p levels after chemotherapy mirror the efficacy of treatment, with a significant drop from the start of the first to the start of the second cycle of chemotherapy [36,40,41,50] (Fig. 2). Moreover, the rate of decline seems proportional to the burden of disease. In CSII, miR-371a-3p levels declined significantly between cycle one and two, with no significant decline thereafter, whereas in CSIII disease, significant declines occurred between cycles one and two, and again between cycles two and three. Thus, repeated measurements of miR-371a-3p

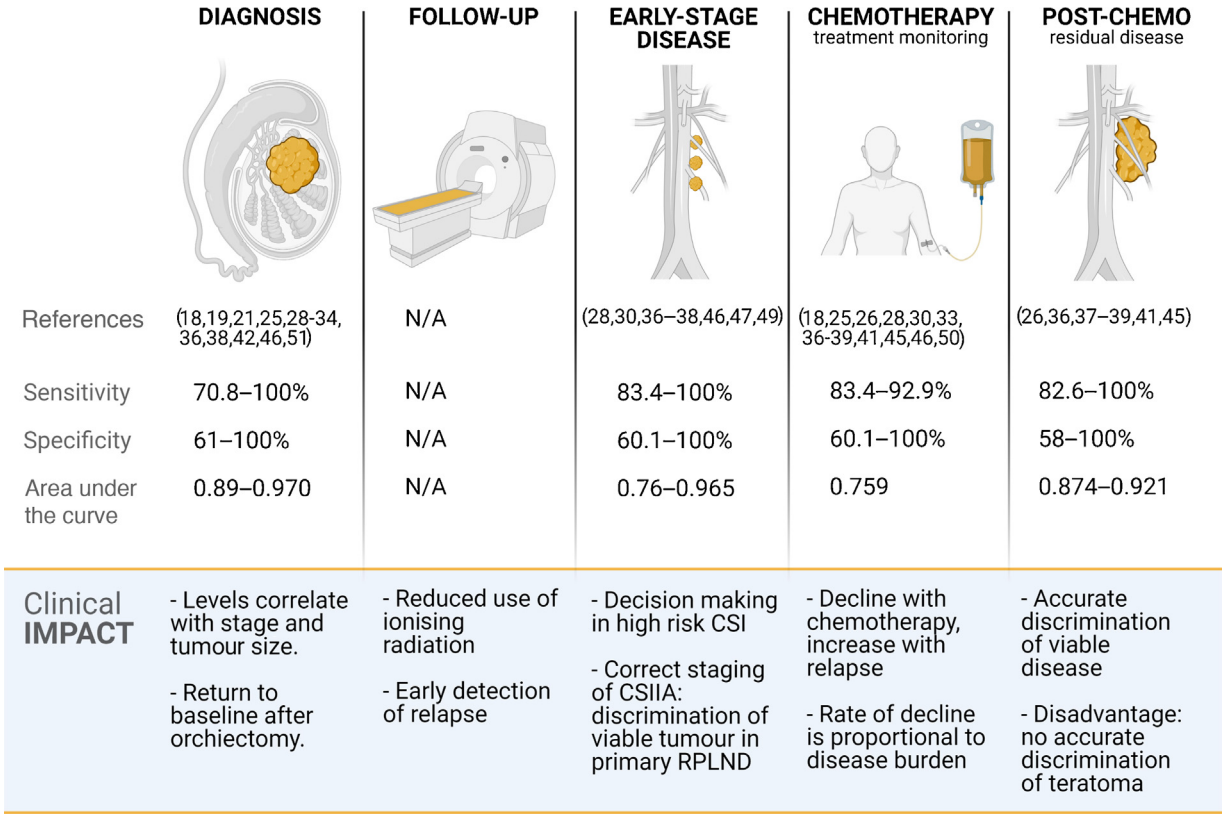

Fig. 3 – Serum miR-371a-3p in clinically relevant scenarios. Summary of the literature on the use of circulating miR-371a-3p in diagnosis, follow-up, early-stage disease, treatment monitoring, and postchemotherapy detection of residual disease, and the potential clinical impact of its use in each of these scenarios. Created in Biorender (www.biorender.com). RT-qPCR = real-time qualitative polymerase chain reaction; βhCG = β human chorionic gonadotropin; AFP = α-fetoprotein.

during chemotherapy might provide valuable information regarding treatment response even when STMs are normal [36].

Circulating miR-371a-3p levels provide prognostic information, as inferior progression-free survival and overall survival was reported for patients with elevated miR-371a-3p levels before chemotherapy [40]. However, the association between miR levels and outcome lost significance after adjusting for IGCCCG risk groups, suggesting that miR levels may only serve as a surrogate for disease burden. In other studies, patients who achieved a durable response to chemotherapy had significantly lower miR levels before chemotherapy (p = 0.022) than those achieving incomplete responses [41,50]. Among patients with chemorefractory disease despite normal STMs, 86.6% had at least one elevated miR during follow-up [33,36,39,41]. The sensitivity of this marker for detection of relapse is 82.6%, with an AUC of 0.921 [36].

3.3.5.2. Postchemotherapy residual disease. Among 82 patients with nonseminomatous tumours who underwent post-chemotherapy RPLND, levels of circulating miR-371a-3p predicted viable cancer with an AUC of 0.885 and, importantly, showed sensitivity of 100% and an NPV of 100% for retroperitoneal lesions >3 cm in diameter [39]. Two subsequent studies confirmed the ability of miR-371a-3p serum levels to predict residual active GCT when compared with teratoma (p = 0.0228) and necrosis/fibrosis (p = 0.0348) [26,41] (Fig. 2).

The inability of miR-371a-3p to detect teratoma lesions and GCT components with somatic malignancy is a considerable limitation from a clinical perspective. In a series of 21 patients with nonseminoma with confirmed relapses, only 11 (57%) had elevated miR-371 levels and among those with negative levels, two had histologically confirmed teratoma [26,41]. In an attempt to overcome this diagnostic gap for circulating miR371a-3p, serum/plasma levels of miR-375-3p were evaluated for their ability to detect teratoma [43]. It had been reported that miR-375-3p is highly expressed in teratoma tissue, but initial studies did not reveal any diagnostic value [26,47,51]. According to a recent report, measurement of miR-375 in plasma and combined measurement of miRs 375 and 371a-3p appeared to have superior accuracy for teratoma detection, with an AUC of 0.77 (95% CI 0.62–0.93) [52]. In light of the conflicting data, further studies are needed.

miR-885-5p and miR-448, two other candidates for teratoma detection, failed to distinguish teratoma from necrosis and viable cancer lesions in the postchemotherapy setting [26].

3.4. Costs

Implementation of new clinical markers always involves expenses that must be considered by health systems. Two model calculations suggested that replacing computed tomography scans with miR measurements could save considerable costs. Moreover, use of miR-371a-3p measurements in follow-up for CSI NSGCT might facilitate early detection of relapse and allow for more individually tailored and possibly cost-effective treatment [48,53]. Although economic benefits are foreseeable, the true costs of universal implementation need to be addressed.

3.5. Discussion

The data reviewed here suggest that serum or plasma miR-371a-3p is on the cusp of becoming the next-generation serum biomarker for GCTs (Fig. 3). The question is whether miR-371a-3p is indeed ready for prime time. In 1987, Lange and Winfield postulated seven independent features of an ideal biomarker that are still valid today: (1) the candidate substance is produced only by the malignancy itself; (2) it is secreted into body fluids; (3) it can be measured in a reproducible fashion; (4) levels in body fluids correlate with the amount of tumour present; (5) the substance can be detected even in early disease; (6) measured levels correlate with response to treatment; and (7) the half-life of the substance is short [54]. Weighing the miR data against the Lange-Winfield criteria confirms that all seven conditions are met by miR-371a-3p.

It is worth reviewing each feature critically. Regarding whether miR-371a-3p is produced only by TGCTs, there is ample evidence that GCTs are the only source of circulating miRs from the miR-371-3 cluster. The miRs have been documented in TGCT tissue and to a much lesser degree in normal testicular tissue, but not in extratesticular tissues [17,23–25]. Furthermore, elevated serum levels have been found only in TGCT patients and not in patients with testicular tumours of other origin [25,32] or in patients with other malignancies [31].

The second Lange-Winfield condition—secretion of the substance into body fluids—is clearly fulfilled, as numerous reports have documented the presence of miRs in serum and plasma and other fluids such as testicular vein blood, hydrocele fluid, seminal plasma, cerebrospinal fluid, and pleura effusion fluid [31,40,44].

The third criterion relates to the reproducibility of measurements. Quantitative real-time PCR is the technique used to measure miR levels in body fluids or tissue. The PCR techniques used in the studies reviewed here vary with regard to minor steps in the multistep measurement technique. However, international exchange of serum samples has revealed that the results are principally well reproducible [26,34]. Although each of the PCR techniques currently in use is reliable and valid, an international standard for measurement of serum miR levels is highly desirable.

The fourth attribute of an ideal biomarker is correlation between serum levels and the amount of tumour present. Many studies have demonstrated that serum miR-371a-3p is associated with tumour stage [32,37,41], primary tumour size [36,38], and overall GCT burden.

The fifth feature of a valuable tumour marker is its detectability in early-stage disease. There is no doubt that the diagnostic accuracy of miR-371a-3p is highest in high-volume metastatic disease (AUC 0.998) and decreases as the disease burden lessens. However, even in CSI disease the biomarker still has exceptional sensitivity (AUC 0.958), so it maintains its utility in this setting. It is likely that there is a threshold of detection below which miR-371a-3p will not be useful. This is suggested from studies of seminomas of $<$1 cm, for which miR-371a-3p sensitivity fell to 58% [36], from GCNIS cases, for which the sensitivity fell to 50% [21], and from post-orchiectomy miR levels in CSI cases, for which miR-371a-3p measurements failed to detect micrometastases among patients who eventually experienced relapse [49]. That said, there is more than enough utility demonstrated to satisfy the biomarker criterion of detectability in early-stage disease.

The sixth criterion is correlation between marker levels and treatment response. The observations that miR-371a-3p levels decline in 90% of CSI cases after orchiectomy [26,30,33] and that levels decrease precipitously in response to chemotherapy [39–41] and RPLND [26,39] confirm the fulfilment of this criterion.

The final characteristic of a useful biomarker is a short half-life. AFP, one of the classic STMs, has a long half-life of 5–7 d, and this slow decay usually implies several weeks of observation in CSI cases to confirm the diagnosis [55]. By contrast, circulating miR-371a-3p has a half-life of $<$24 h in CSI cases; although it is somewhat longer in metastatic disease, decay is still rapid in comparison to conventional STMs [26,42].

Overall, the novel GCT biomarker miR371a-3p satisfies many features of an ideal biomarker. Before implementing the use of a novel marker, the question to be answered is which features of the new marker are superior to those of the classic ones. There are two attributes of miR-371a-3p in particular that outperform the classic STMs: the exceptionally high sensitivity and specificity, and the short half-life. The ability to detect both nonseminoma and seminoma with approximately 90% sensitivity renders miR-371a-3p a universal biomarker for GCT, in stark contrast to the classic STMs with sensitivity of 50–60% overall and even lower (30%) for seminoma [4,38]. Such ubiquitous sensitivity would allow miR-371a-3p to serve a role even in primary diagnosis of TGCT, for which conventional STMs are of limited value.

Despite the overwhelmingly positive results published, the evidence supporting miR-371a-3p is not without limitations. A substantial weakness is the retrospective nature of the majority of the studies, together with low patient numbers recruited in some of them. Thus, particular information is often determined by a single outlying study, which is the reason why a formal meta-analysis is not feasible. Another important limitation of miR-371a-3p is the inability to detect teratoma. Clinically, this diagnostic gap is a major drawback, as teratoma always requires

particular therapeutic attention because of its insensitivity to chemotherapy and its independent association with cancer-specific mortality [56]. While miR-375 shows promise, the data reported to date are conflicting. More studies rooted in answering clinical management questions are needed.

Once miR-371a-3p is introduced into clinical practice, the question will arise as to whether the classic STMs will be completely replaced by miR-based assays. While LDH could easily be omitted given its poor sensitivity and specificity, it is likely that βhCG and AFP will continue to serve a role; they are cheap, serum protein-based tests that form the backbone of current staging and prognostic systems. Classic STMs still play an important part in adding information to histological work-up, with implications for disease management. It is conceivable that other nucleic acid–based circulating compounds, such as circulating tumour DNA containing the isochromosome 12p alteration, might also have a role as GCT-specific biomarkers [57,58].

miR-371a-3p will probably be used in the scenarios for which the inferior accuracy of STMs has so far hampered their application. Thus, miR-371a-3p is expected to play a prominent role in GCT follow-up. It is conceivable that many imaging procedures could be avoided during follow-up, thus saving costs and reducing the cumulative radiation dosage for individual patients. Overtreatment or otherwise inappropriate treatment could be avoided by correctly assessing early stages with no STM expression. In addition, serial miR-371a-3p measurements might serve as an important marker during chemotherapy to monitor its efficacy and eventually provide a means for tailoring more individualised treatment. Other applications of miR-371a-3p include primary diagnosis of small testicular masses, diagnostic assessment of postchemotherapy residual masses, and clinically unresolved scenarios such as marker-negative lymphadenopathy and investigation of cases with cancer of unknown primary origin [38,39,46,49]. There are high expectations for ongoing clinical trials, including SWOG1823, AGCT1531 (NCT03067181), NCT04435756, P3BEP (NCT02582697), and DRKS00019223, addressing the role of miRs over the natural history of TGCT and their clinical applications. The results collected are promising; however, for clinical implementation it is crucial to define a specific and validated pipeline of miRs for analysis, a standardised protocol (isolation techniques, amount of sample used, preamplification protocol, normalisation), standard quantification, and a reporting methodology.

4. Conclusions

Circulating miRs, and in particular miR-371a-3p, are a promising novel tool with potential to address areas of unmet clinical need in TGCT. Validation of the currently available evidence is expected to come from ongoing prospective trials that may ultimately open the door for clinical implementation of miRs.

Author contributions: Klaus-Peter Dieckmann had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Dieckmann, Leão, Albersen, Hamilton, Looijenga.
Acquisition of data: Leão, Dieckmann.
Analysis and interpretation of data: Tandstad, Culine, Belge, Coleman, Murray, Kollmannsberger, Leão.
Drafting of the manuscript: Leão, Hamilton, Dieckmann.
Critical revision of the manuscript for important intellectual content: Albersen, Looijenga, Tandstad, Culine, Belge, Coleman.
Statistical analysis: Leão.
Obtaining funding: None.
Administrative, technical, or material support: Albersen.
Supervision: Dieckmann.
Other: None.

Financial disclosures: Klaus-Peter Dieckmann certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: Klaus-Peter Dieckmann and Gazanfer Belge own shares in miRdetect GmbH, a start-up company aiming to produce a commercially available laboratory kit for measurement of miRNAs in serum. L.H.J. Looijenga has filed a patent application covering the use of miR-885-5p and miR-448 as molecular markers for teratoma. The remaining authors have nothing to disclose.

Funding/Support and role of the sponsor: None.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.eururo.2021.06.006.

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