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miR-371a-3p Predicting Viable Tumor in Patients Undergoing Retroperitoneal Lymph Node Dissection for Metastatic Testicular Cancer: The SWENOTECA-MIR Study

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Study Need and Importance: Survival rates for metastatic testicular cancer are high, but treatments are associated with severe morbidity and excessive long-term mortality. There is a critical need for improved serum tumor markers to guide therapy and reduce overtreatment. The microRNA miR-371a-3p, a promising novel tumor marker and potential liquid biopsy for testicular cancer, has been the focus of research for the last decade. This prospective multicenter study represents the largest cohort to date on microRNA in predicting viable cancer preoperatively in testicular cancer patients undergoing retroperitoneal lymph node dissection (RPLND). We analyzed miR-371a-3p patterns in 114 Norwegian and Swedish patients before and after RPLND using reverse transcription–digital droplet polymerase chain reaction (PCR).

What We Found: In seminoma patients undergoing primary RPLND (n = 24), miR-371a-3p demonstrated high performance, with 74% sensitivity and 100% specificity, outperforming conventional serum tumor markers. The serum levels significantly decreased after surgery (violin plot in Figure). In prechemotherapy nonseminoma patients (n = 18) and in postchemotherapy patients (n = 72), miR-371a-3p showed low sensitivity and no significant differences before and after surgery, indicating limited utility. Teratomas where consistently negative.

Limitations: Despite being the largest cohort so far, the sample is still small, necessitating cautious interpretation of the results. Optimism-corrected performance estimates were used in an effort to reduce cohort size impact. The reverse transcription–digital droplet PCR is a novel technique compared to quantitative PCR, and the chosen threshold may introduce bias. To address this, we tested the method on 180 orchiectomy patients and 50 blood donors previously, with good performance.

Interpretation for Patient Care: The prognosis of testicular cancer is excellent, even if it has spread. Therefore, the main challenge is avoiding overtreatment. This prospective multicenter study indicates that miR-371a-3p is a valuable tumor marker for predicting viable tumors in prechemotherapy seminoma patients, but not for nonseminoma and postchemotherapy patients before lymph node surgery of the retroperitoneal space.

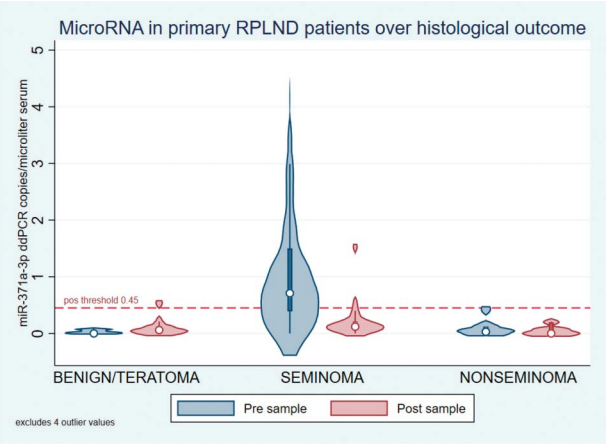

Figure. Violin plot illustrating the pre- and postoperative levels of miR-371a3p in patients treated with primary retroperitoneal lymph node dissection (RPLND), categorized by histological outcomes. A positive (pos) threshold is defined as miR-371a-3p exceeding 0.45 copies/μL serum. ddPCR indicates digital droplet polymerase chain reaction.

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miR-371a-3p Predicting Viable Tumor in Patients Undergoing Retroperitoneal Lymph Node Dissection for Metastatic Testicular Cancer: The SWENOTECA-MIR Study

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Purpose: The SWENOTECA-MIR prospective multicenter study aims to assess the clinical value of miR-371a-3p as a novel marker in metastatic germ cell tumor patients undergoing retroperitoneal lymph node dissection (RPLND), to predict the presence of viable residual tumor.

Materials and Methods: A total of 114 patients (86 nonseminomas, 28 seminomas) who underwent surgery for presumed metastatic disease pre

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Conflict of Interest Disclosures: Dr Kjellman reported receiving speaker honoraria from Janssen R&D and Astellas; participating in trials for Astellas, Janssen R&D, Bayer, and Immunicum; and being on the advisory board for Astellas. No other disclosures were reported.

Ethics Statement: This study received Institutional Review Board approval (REC Stockholm 2018/1730-31 and REC Central Norway 2015/1475).

Author Contributions:

Conception and design: Thor, Myklebust, Grenabo Bergdahl, Lundgren, Almås, Haugnes, Tandstad, Akre, Cohn-Cedermark, Dahl, Kjellman.
Data acquisition: Thor, Myklebust, Grenabo Bergdahl, Almås.
Data analysis and interpretation: Thor, Myklebust, Lundgren, Skokic, Dahl, Kjellman.
Drafting the manuscript: Thor.
Critical revision of the manuscript for scientific and factual content: Thor, Myklebust, Grenabo Bergdahl, Lundgren, Almås, Haugnes, Tandstad, Akre, Cohn-Cedermark, Dahl, Kjellman.
Statistical analysis: Thor, Lundgren, Skokic, Kjellman.
Supervision: Thor, Myklebust, Grenabo Bergdahl, Lundgren, Almås, Haugnes, Tandstad, Akre, Cohn-Cedermark, Dahl, Kjellman.
Anna Thor 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.

Data Availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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chemotherapy (primary RPLND) and post chemotherapy RPLND were included. The expression of miR-371a-3p was evaluated using reverse transcription–digital droplet polymerase chain reaction before and after RPLND. Pre- and postoperative miR-371a-3p levels were statistically compared, and optimism-corrected performance calculations compared with conventional serum tumor markers. Associations were evaluated by logistic regression. Patients who underwent primary RPLND were categorized into seminoma and nonseminoma groups.

Results: Among the seminoma patients (n = 24) undergoing primary RPLND, all had normal conventional markers. Six patients received adjuvant treatment before surgery. miR-371a-3p exhibited a sensitivity of 74%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 21% for viable tumor. The levels of miR-371a-3p significantly decreased after surgery. In the nonseminoma group (n = 18) treated with primary RPLND, 22% had elevated conventional markers and 3 had received prior adjuvant treatment. miR-371a-3p showed a sensitivity of 34%, specificity of 88%, positive predictive value of 67%, and negative predictive value of 62% for the primary nonseminoma patients. No association was observed between stage or prior adjuvant treatment and the outcome of the miR test. In the postchemotherapy group (n = 72), the miR-371a-3p sensitivity was 9%, reducing to 0 when excluding patients with seminoma (n = 4). Teratomas and benign histology were essentially negative.

Conclusions: Our study highlights miR-371a-3p as a fairly sensitive and highly specific marker for prechemotherapy seminomas, outperforming conventional markers. However, in prechemotherapy nonseminomas as well as in postchemotherapy patients, we observed low sensitivity and no significant differences in miR-371a-3p levels before and after surgery, suggesting limited utility for miR-371a-3p in this context.

Key Words: biomarkers, microRNA, miR-371a-3p, RPLND, testicular cancer

Testicular germ cell tumor (TGCT) is the most common cancer in young males, with rising incidence.[1] Although treatment for metastatic TGCT is highly effective, the chemotherapy regimens used are associated with significant long-term side effects, including cardiovascular disease and secondary malignancies.[2] Survivors may also experience excess mortality due to prior therapy.[3]

Staging and monitoring of TGCTs involve repeated CT and MRI, and measuring serum protein biomarkers beta human chorionic gonadotropin (β-hCG), alpha fetoprotein (AFP), and lactate dehydrogenase. However, the markers lack sensitivity and specificity, with 40% of all TGCTs being marker negative, especially seminomas and teratomas.[4]

Chemotherapy typically includes bleomycin, etoposide, and cisplatin (BEP) courses varying by clinical stage (CS) and risk group. Adjuvant treatment by one course of carboplatin or BEP is offered to selected CS I patients, according to the SWENOTECA protocol. BEP was administered as adjuvant treatment to seminoma patients within the randomized ABC-study.[5] Surgery is indicated for nonseminoma patients with a visible residual tumor $\geq$ 10 mm in the retroperitoneum after first-line chemotherapy,[5] while for seminoma patients with small retroperitoneal metastases, surgery is being evaluated as a primary treatment in prospective clinical trials.[6-8] Retroperitoneal lymph node dissection (RPLND) is a complex surgical procedure, associated with perioperative morbidity and risk of long-term sequelae, including loss of antegrade ejaculation. In previous studies, 44% to 72% of RPLND patients harbored only necrosis or fibrosis in residual masses.[9-11] Reliable diagnostic tools are needed to distinguish patients requiring RPLND from those who can be spared.

In 2011, the microRNA clusters miR-302/367 and miR-371 to 373 were identified as potential novel markers for TGCTs.[12] Subsequent research revealed miR-371a-3p as the most promising micro-RNA marker, as it has been found to be present in nearly all cases of TGCTs other than teratoma.[13-16] Dieckmann et al demonstrated that miR-371a-3p expressed sensitivity and specificity over 90% in TGCTs at various CSs.[14] Further studies reported similar results for miR-371a-3p in post-chemotherapy patients with retroperitoneal residual masses or relapse.[17,18] Teratomas have consistently shown negative results in miR-371a-3p measurements across various studies.[19]

To determine the clinical value of the lead candidate miR-371a-3p as a tumor marker, large prospective clinical trials are required. The first part of the SWENOTECA-MIR study on miR-371a-3p in 180 orchiectomy patients showed better performance than conventional markers, with an overall sensitivity of 89%.[20] This study aims to assess miR-371a-3p expression before and after RPLND in TGCT patients, to evaluate its accuracy and potential as a predictor of viable disease, and to see whether cytoreductive surgery affects its values.

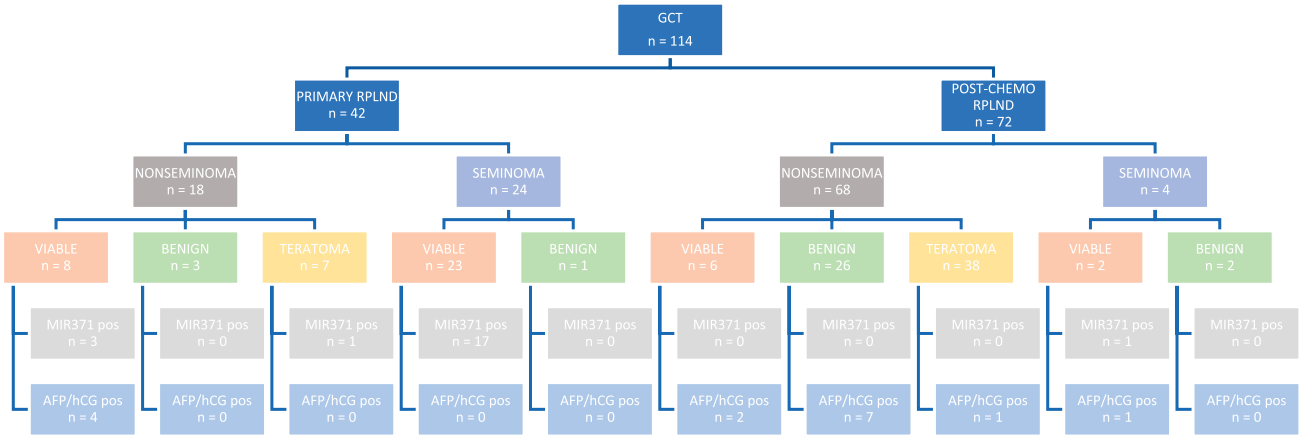

Figure 1. Comprehensive overview of the study cohort's characteristics and outcomes by histology and tumor markers. AFP indicates alpha fetoprotein; GCT, germ cell tumor; hCG, human chorionic gonadotropin; pos, positive; POST-CHEMO, postchemotherapy; RPLND, retroperitoneal lymph node dissection.

METHODS

Study Design and Participants

The SWENOTECA-MIR trial is a prospective binational multicenter study, with 3 cohorts based on different interventions: orchiectomy, chemotherapy, or RPLND. The primary outcomes are miR-371a-3p levels before and after each intervention. Inclusion criteria were males aged 18 to 70 without prior malignancy, diagnosed with TGCT, and planned for RPLND, either due to presumed metastatic disease pre chemotherapy (primary RPLND), or postchemotherapy RPLND. Exclusion criteria included previous malignancy or inability to understand the consent form due to a language barrier. This cohort comprises 114 patients who underwent RPLND from March 2017 to October 2022 in 3 tertiary hospitals. Unilateral or bilateral, open or robot-assisted RPLND was performed in templates according to SWENOTECA guidelines[5] by a small group of experienced surgeons affiliated with the study. All oncologists and urologists involved in patient treatment were kept blinded to the results. Clinical parameters including levels of AFP, β-hCG, and lactate dehydrogenase, CS according to Royal Marsden, age at RPLND, radiology examinations, orchiectomy and RPLND histology, and treatments were retrieved from medical records.

The study was approved by the Regional Ethics Committees (REC Stockholm 2018/1730-31 and REC Central Norway 2015/1475). All patients received oral and written information and gave written consent.

Laboratory Methods

Study samples were collected up to 1 week prior to and 18 to 24 hours after RPLND. Serum was stored at −80 °C until analysis. RNA was extracted from 200 µL serum using the miRNeasy Kit (Qiagen, P/N 217004). Reverse transcription (RT) was performed using the specific RT primers from the TaqMan assays miR-371a-3p (Thermo Fisher Scientific, ID 002124) and miR-30b-5p (Thermo Fisher Scientific, ID 000602). RT-digital droplet (dd) polymerase chain reaction (PCR) was performed using a QX200 AutoDG Droplet Digital PCR System for 96 well plates (Bio-Rad) and Supermix for Probes (Bio-Rad, P/N 186-024), as described previously.[20] The endogenously expressed miR-30b-5p was used as an internal control. Results for microRNA-371a-3p are given as copies per µL serum. Details regarding the analysis can be found in the Supplementary Protocol. The threshold for defining a sample as positive for miR-371a-3p was 0.45 copies per µL serum, as previously described.[20]

Statistical Analysis

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for the preoperative sample of miR-371a-3p and the conventional markers β-hCG/AFP. The gold standard in our calculations of sensitivity, specificity, PPV, and NPV is the postoperative pathology report of prevalence of viable tumor in the retroperitoneal lymph nodes. Teratomas were anticipated to yield true negative results in miR-371a-3p tests and were categorized as benign outcomes or disease-negative results in the performance calculations. To assess the performance of our predictive model and account for potential overfitting, bootstrap optimism correction was applied. To that end, the analysis was reformulated in terms of classification, treating preoperative positive miR-371a-3p and preoperative positive conventional tumor markers as predictors, and viable cancer in the pathohistological report as the outcome. A majority class classifier was used. In cases of equal estimated outcome class probabilities when training on a bootstrap sample, classes were randomly assigned with probability 0.5. In the cases of absence of a class in the predictor in a bootstrap sample, the overall class probabilities in the original outcome sample were used when applying the classifier to the original predictor values. A total of 10,000 bootstrap samples were used in the performance calculations.

Paired violin plots for histological outcomes were constructed according to miR-371a-3p levels before and after surgery, for the subgroups seminomas, nonseminomas, and benign/teratomas. Statistical significance between pre- and postoperative miR-371a-3p levels was determined using the Wilcoxon signed-rank test due to non-normal distribution of miR-371a-3p values. For the

Table 1. *Patients With Nonseminoma Treated With Retroperitoneal Lymph Node Dissection for Presumed Metastatic Germ Cell Tumor (Nonseminoma Primary Retroperitoneal Lymph Node Dissection Group, n = 18)*

Clinical stage, No. (%)		
CS I with somatically differentiated GCT	3	(17)
CS IIA	4	(22)
CS IIB	9	(50)
CS IIC	1	(5.5)
Unknown	1	(5.5)
Orchiectomy specimen histology, No. (%)		
Embryonal cancer	4	(22)
Teratoma	4	(22)
Mixed	8	(44)
With teratoma	5	
With seminoma	4	
With somatical differentiation	4	
Unspecified nonseminoma tumor	2	(11)
Chemotherapy, No. (%)		
Adjuvant (BEP × 1)	3	(17)
No chemotherapy	15	(83)
Serum tumor markers positive at RPLND, No. (%)	4	(22)
AFP elevated	1	
AFP and β-hCG elevated	1	
Unknown	2	
Marker negative	14	(78)
Age at RPLND, median (range), y	29	(18-61)
RPLND specimen histology, No. (%)		
Teratoma	7	(39)
Benign	3	(17)
Viable tumor	8	(44)
Embryonal carcinoma	3	
Seminoma	1	
Mixed tumor embryonal carcinoma + yolk sac	2	
Somatically differentiated GCT	1	
Yolk sac tumor	1	
Sensitivity serum tumor markers, optimism-corrected (%)	49	
Specificity serum tumor markers, optimism-corrected (%)	100	
PPV serum tumor markers, optimism-corrected (%)	98	
NPV serum tumor markers, optimism-corrected (%)	63	
True positive miR-371a-3p, No. (%)	3	(38)
False-positive miR-371a-3p, No. (%)	1	(13)
False-negative miR-371a-3p, No. (%)	4	(50)
Sensitivity miR-371a-3p optimism-corrected (%)	34	
Specificity miR-371a-3p, optimism-corrected (%)	88	
PPV miR-371a-3p, optimism-corrected (%)	67	
NPV miR-371a-3p, optimism-corrected (%)	62	

Abbreviations: AFP, alpha fetoprotein; β-hCG, beta human chorionic gonadotropin; BEP, bleomycin, etoposide, cisplatin; CS, clinical stage; GCT, germ cell tumor; NPV, negative predictive value; PPV, positive predictive value; RPLND, retroperitoneal lymph node dissection.

primary RPLND patients, logistic regression was used to assess associations between a high tumor stage or the absence of adjuvant chemotherapy, with a preoperative positive miR-371a-3p test. Bootstrapping of 1000 replications was employed to obtain robust estimates of the associations. Statistical significance was achieved at a level of $P < .05$ for 2-tailed analyses. Data analysis and graphics were created using STATA version 16.1 (StataCorp LCC, College Station, Texas) and R version 4.3.2 (The R Foundation, Vienna, Austria).

RESULTS

In total, the study included 114 patients, of whom 86 had nonseminomas and the remaining 28 had seminomas. The cohort was categorized into 2 groups based on the clinical indication for RPLND,

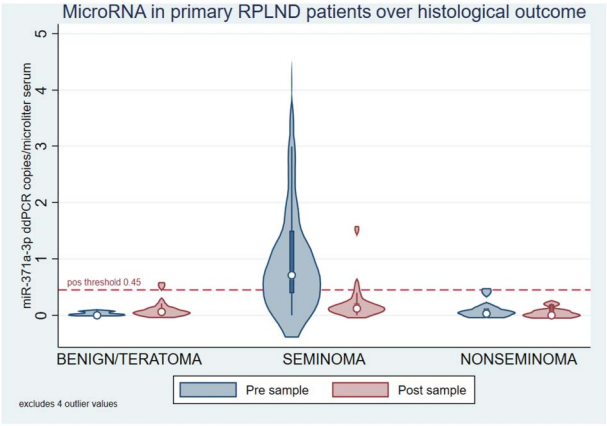

Figure 2. Violin plot illustrating the pre- and postoperative levels of miR-371a3p in patients treated with primary retroperitoneal lymph node dissection (RPLND), categorized by histological outcomes. A positive (pos) threshold is defined as miR-371a-3p exceeding 0.45 copies/μL serum. ddPCR indicates digital droplet polymerase chain reaction.

primary (n = 42) or post chemotherapy (n = 72). Among those who underwent primary RPLND for early-stage disease, further subgroups were created for seminomas (n = 24) and nonseminomas (n = 18; Figure 1).

In the 18 patients with CS I or II nonseminoma treated with RPLND, postoperative histopathology revealed viable cancers other than teratoma in 8 (44%; Table 1). Among these, 4 (22%) expressed positive conventional markers at surgery, while only 3 patients tested positive for miR-371a-3p. Two

Table 2. *Patients With Seminoma Treated With Retroperitoneal Lymph Node Dissection for Presumed Metastatic Germ Cell Tumor (Seminoma Primary Retroperitoneal Lymph Node Dissection Group, n = 24)*

Clinical stage, No. (%)		
CS I with progression	10	(42)
CS IIA	12	(50)
CS IIB	2	(8)
Chemotherapy, No. (%)		
Adjuvant	6	(25)
Carboplatin × 1	5	
BEP × 1	1	
No chemotherapy	18	(75)
Serum tumor markers positive at RPLND, No. (%)	0	(0)
Median age at RPLND, median (range), y	42	(29-53)
RPLND specimen histology, No. (%)		
Benign	1	(4)
Seminoma	23	(96)
True positive miR-371a-3p, No. (%)	17	(74)
False-negative miR-371a-3p, No. (%)	6	(26)
Sensitivity miR-371a-3p, optimism-corrected (%)	74	
Specificity miR-371a-3p, optimism-corrected (%)	100	
PPV miR-371a-3p, optimism-corrected (%)	100	
NPV miR-371a-3p, optimism-corrected (%)	21	

Abbreviations: BEP, bleomycin, etoposide, cisplatin; CS, clinical stage; GCT, germ cell tumor; NPV, negative predictive value; PPV, positive predictive value; RPLND, retroperitoneal lymph node dissection.

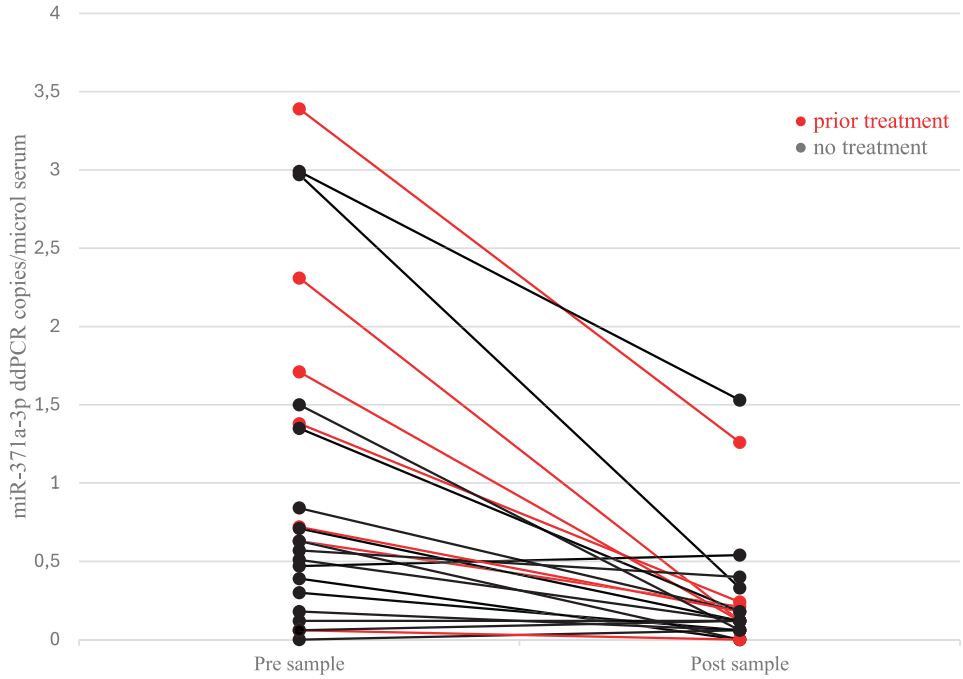

Figure 3. miR-371-3p levels pre and post retroperitoneal lymph node dissection (RPLND) for patients with outcomes of viable seminoma. The decrease in miR-371a-3p is significant ($P = .001$). Red indicates patients having received prior chemotherapy. ddPCR indicates digital droplet polymerase chain reaction.

patients with viable nonseminoma were positive for both conventional markers and miR-371a-3p at surgery. However, one patient with teratoma showed a postoperative miR-371a-3p positive result after testing negative preoperatively, indicating a false-positive result. No significant difference was observed in levels of miR-371a-3p pre- or postoperatively within this group ($P = .6$; Figure 2). Logistic regression was deemed inappropriate because of the low count of positive tests. The miR-371a-3p test had an optimism-corrected sensitivity of 34%, specificity of 88%, PPV of 67%, and NPV of 62%. The conventional markers had a sensitivity of 49%, specificity of 100%, PPV 98%, and NPV 63%. Among the 3 patients with a viable cancer and a positive miR-371-3p test result, one patient had received 1 course of BEP before surgery, while the other 2 were chemotherapy naïve.

The primary seminoma group included 24 patients (Table 2). Among them, 42% (10 patients) were relapses in CS I, with 6 receiving adjuvant carboplatin or BEP prior to primary RPLND. All were negative in conventional markers at surgery. Postoperative histology identified viable cancers in 23 and 1 benign case. Among the viable seminomas 17 tested positive for miR-371a-3p. The optimism-corrected sensitivity was 74%, specificity 100%, PPV 100%, and NPV 21%. Logistic regression analysis indicated no association between a higher CS and a positive miR-371a-3p test (odds ratio [OR] 0.7 [95% CI: 0.1-9], $P = .8$) or prior adjuvant treatment and a positive miR-371a-3p test (OR 2.1 [95% CI: 0.2-23], $P = .6$). After adding bootstrapping to the logistic regression, the results remained virtually unchanged, both for CS (OR 0.7 [95% CI: 0.1-4], $P = .7$) and prior adjuvant treatment (OR 2.1 [95% CI: 0.4-12], $P = .4$). The preoperative sample measurements of miR-371a-3p in patients with viable seminomas were significantly higher than the postoperative, showing a rapid decline in serum levels after surgery ($P = .001$; Figures 2 and 3). Details of patients with postoperative viable disease, excluding teratomas, are displayed in Tables 3 and 4.

In the postchemotherapy group of 72 patients (68 nonseminomas, 4 seminomas) 65% were CS II at diagnosis (Table 5). Indications for postchemotherapy RPLND in seminoma patients were progression of residual tumor, inconclusive radiology, or elevated AFP levels suggesting nonseminomatous elements. Overall, 15% had elevated conventional markers at surgery. The postoperative histology revealed 8 (11%) viable cancers, 38 (53%) teratomas, and 26 (36%) benign findings. Among the 8 viable cancers, 2 expressed conventional markers. One patient had a positive result in miR-371a-3p. The histology revealed a large seminoma tumor of 5 cm, and the patient was negative in conventional markers.

The optimism-corrected diagnostic performance calculations of miR-371a-3p for the postchemotherapy

Table 3. *Characteristics of Nonseminoma Patients With Viable Histology Results Other Than Teratoma After RPLND (n = 14)*

Pt No.	Group	Clinical stage at diagnosis	Orchiectomy histology	Testicular tumor size (mm)	Prior chemotherapy	Age at RPLND (y)	Clinical stage at RPLND	RPLND histology	No. positive lymph nodes	Largest tumor diameter (mm)	Positive markers at surgery	Pre miR-371a-3p copies/μL serum (threshold 0.45)	Post miR-371a-3p copies/μL serum (threshold 0.45)	Positive miR-371a-3p test
1	NS	IIB	EC	-	0	48	IIB	YST	1	10.0	Yes	0.00	0.00	No
2	NS	IVC	-	-	PEI × 4	48	IVC	YST, T	2	05.5	No	0.00	0.18	No
3	NS	IIC	YST	15.0	PEI × 4	25	IIB	YST, T	2	05.0	No	0.00	0.00	No
4	NS	IIC	S (AFP+)	15.0	BEP × 3	30	IIC	YST, T	3	11.0	No	0.00	0.00	No
5	NS	IIB	EC, YST, T, S	47.0	0	41	IIB	S	1	27.0	Yes	0.12	0.20	No
6	NS	IIA	EC, YST, S	-	0	24	IIA	EC	1	03.2	No	0.06	0.00	No
7	NS	IVC	-	-	PEI × 2, TIP × 2, HD × 2	24	IVC	CC	-	-	Yes	0.25	0.29	No
8	NS	I	-	16.0	BEP × 1	51	IIA	EC, YST, undefined malignant GCT	-	-	Yes	103.7	19.9	Yes
9	NS	IIA	-	40.0	0	51	IIA	EC, YST	-	-	Yes	38.0	5.70	Yes
10	NS	IIC	YST, T	45.0	BEP × 4	20	IIC	YST, T	-	-	Yes	0.21	0.07	No
11	NS	IIA	EC	11.0	0	21	IIA	EC	-	-	No	0.43	0.21	No
12	NS	IIA	T	08.0	0	18	IIB	EC	-	-	No	9.51	0.21	Yes
13	NS	IIB	EC, YST, T	40.0	BEP × 3	30	IIB	YST	-	-	No	0.00	0.15	No
14	NS	I	T, SOMATIC	29.0	0	49	I	SOMATIC	-	-	No	0.12	0.19	No

Abbreviations: AFP+, alpha fetoprotein serum marker positive; BEP, bleomycin, etoposid, cisplatin; EC, embryonal carcinoma; GCT germ cell tumor; HD, high-dose chemotherapy; NS, nonseminoma; PEI, cisplatin, etoposid, ifosfamide; Pt, patient; RPLND, retroperitoneal lymph node dissection; S, seminoma; SOMATIC, somatically diffentiated germ cell tumor; T, teratoma; TIP, paclitaxel, ifosfamid, cisplatin; YST, yolk sac tumor.

group yielded sensitivity 9%, specificity 100%, PPV 100%, and NPV 64%. The sensitivity was reduced to 0 when the seminoma patients were excluded. The conventional markers exhibited sensitivity of 36%, specificity of 87%, PPV of 0%, and NPV of 67% within the entire group of postchemotherapy patients.

Table 6 presents the performance metrics calculated using both standard methods and bootstrap resampling.

DISCUSSION

This study represents the largest evaluation to date on the utility of miR-371a-3p predicting histological outcomes post RPLND. Given centralized treatment for metastatic TGCTs in our countries, our aim was to provide population-based data of miR-371a-3p expression in patients undergoing RPLND. As a result, the data pertaining to Swedish patients are population based (83 of 114 patients). The

Table 4. *Characteristics of Seminoma Patients With Viable Histology Results After Primary RPLND (n = 23)*

Pt No.	Group	Clinical stage at diagnosis	Orchiectomy histology	Testicular tumor size (mm)	Prior chemotherapy	Age at RPLND (y)	Clinical stage at RPLND	RPLND histology	No. positive lymphnodes	Largest tumor diameter (mm)	Positive markers at surgery	Pre-op miR-371a-3p copies/μL serum (threshold 0.45)	Post-op miR-371a-3p copies/μL serum (threshold 0.45)	Positive miR-371a-3p test
1	S	I	S	24.0	Carbo × 1	41	IIA	S	1	14.0	No	0.63	0.21	Yes
2	S	I	S	19.0	0	46	IIA	S	4	16.0	No	0.51	0.12	Yes
3	S	IIA	S	58.0	0	42	IIA	S	1	11.5	No	0.63	0.00	Yes
4	S	I	S	12.0	0	41	IIA	S	1	15.0	No	0.00	0.06	No
5	S	IIA	Burned out	-	0	29	IIA	S	3	08.0	No	0.39	0.00	No
6	S	I	S	22.0	Carbo × 1	46	IIA	S	1	-	No	1.71	0.12	Yes
7	S	IIA	S	82.0	0	30	IIA	S	2	20.0	No	2.97	0.33	Yes
8	S	I	S	52.0	0	48	IIB	S	2	24.0	No	4.14	14.9	Yes
9	S	I	S	90.0	Carbo × 1	51	IIB	S	1	13.0	No	2.31	0.12	Yes
10	S	IIA	S	60.0	BEP × 1	47	IIA	S	1	19.0	No	1.38	0.24	Yes
11	S	IIA	S	80.0	0	36	IIA	S	1	02.5	No	0.12	0.12	No
12	S	IIA	S	25.0	0	52	IIA	S	1	19.0	No	0.84	0.18	Yes
13	S	IIA	S	30.0	0	39	IIA	S	1	19.0	No	0.18	0.06	No
14	S	I	S	35.0	0	32	IIB	S	1	10.7	No	2.99	1.53	Yes
15	S	I	S	-	0	34	IIA	S	1	20.0	No	0.57	0.40	Yes
16	S	I	S	-	Carbo × 1	32	IIC	S	1	25.0	No	0.06	0.00	No
17	S	I	S	70.0	0	38	IIA	S	-	-	No	0.47	0.54	Yes
18	S	I	S	52.0	Carbo × 1	53	IIA	S	-	-	No	0.72	0.18	Yes
19	S	I	S	45.0	0	35	IIA	S	-	-	No	1.50	0.06	Yes
20	S	I	S	48.0	0	47	IIA	S	-	-	No	1.35	0.18	Yes
21	S	IIA	S	38.0	0	43	IIA	S	-	-	No	0.06	0.12	No
22	S	I	S	32.0	0	51	IIA	S	-	-	No	0.78	-	Yes
23	S	IIA	S	80.0	0	45	IIA	S	-	-	No	0.71	0.12	Yes

Abbreviations: BEP, bleomycin, etoposide, cisplatin; Carbo, carboplatin; Pt, patient; RPLND, retroperitoneal lymph node dissection; S, seminoma.

Table 5. *Patients Treated With Postchemotherapy Retroperitoneal Lymph Node Dissection (Postchemotherapy Group, n = 72)*

Orchiectomy specimen histology, No. (%)		
Nonseminoma	68	(94)
Seminoma	4	(6)
Clinical stage, No. (%)		
CS II A-C	47	(65)
CS III A-C	7	(10)
CS IV A-C	18	(25)
Chemotherapy, No. (%)		
Standard (BEP, PEI, EP; × 3-4)	61	(85)
Intensified (TIP, GOP, EMA/CO)	10	(14)
Unknown	1	(1)
Serum tumor markers positive at RPLND, No. (%)	11	(15)
AFP elevated	4	
β-hCG elevated	2	
AFP and β-hCG elevated	1	
Elevated serum tumor markers, unknown type	4	
Unknown	1	(1)
Marker negative	60	(83)
Age at RPLND, median (min-max), y	29	(18-58)
RPLND specimen histology, No. (%)		
Teratoma	38	(53)
Benign	26	(36)
Viable tumor	8	(11)
Yolk sac tumor	1	
Choriocarcinoma	1	
Seminoma	2	
Mixed tumor teratoma + yolk sac tumor	4	
Sensitivity serum tumor markers, optimism-corrected (%)	36	
Specificity serum tumor markers, optimism-corrected (%)	87	
PPV serum tumor markers, optimism-corrected (%)	0	
NPV serum tumor markers, optimism-corrected (%)	67	
True positive miR-371a-3p, No. (%)	1	(13)
False-negative miR-371a-3p, No. (%)	7	(88)
Sensitivity miR-371a-3p, optimism-corrected (%)	9	
Specificity miR-371a-3p, optimism-corrected (%)	100	
PPV miR-371a-3p, optimism-corrected (%)	100	
NPV miR-371a-3p, optimism-corrected (%)	64	

Abbreviations: AFP, alpha fetoprotein; β-hCG, beta human chorionic gonadotropin; CS, clinical stage; EMA/CO, etoposide, methotrexate, actinomycin D, cyclophosphamide, vincristine; EP, cisplatin, etoposide; GOP, gemcitabine, oxaliplatin, paclitaxel; max, maximum; min, minimum; NPV, negative predictive value; PEI, cisplatin, etoposide, ifosfamide; PPV, positive predictive value; RPLND, retroperitoneal lymph node dissection; TIP, paclitaxel, ifosfamide, cisplatin.

Table 6. *Optimism-Corrected Estimates for Performance Calculations Using Bootstrap Resampling*

	Sensitivity	Specificity	PPV	NPV
Primary nonseminoma group (n = 18)				
miR371a-3p				
Original estimates	0.38	0.9	0.75	0.64
Optimisms	0.04	0.02	0.08	0.03
Optimism-corrected estimates	0.34	0.88	0.67	0.62
Conventional tumor markers				
Original estimates	0.5	1	1	0.69
Optimisms	0.01	0.00	0.02	0.07
Optimism-corrected estimates	0.49	1	0.98	0.63
Primary seminoma group (n = 24)				
miR371a-3p				
Original estimates	0.74	1	1	0.14
Optimisms	0.00	0.00	0.00	−0.07
Optimism-corrected estimates	0.74	1	1	0.21
Conventional tumor markers				
Original estimates	0	1	-	0.04
Optimisms	0	0	−6.7	-
Optimism-corrected estimates	0	1	-	-
Postchemotherapy group (n = 72)				
miR371a-3p				
Original estimates	0.13	1	1	0.9
Optimisms	0.04	0	0	0.26
Optimism-corrected estimates	0.09	1	1	0.64
Conventional tumor markers				
Original estimates	0.38	0.87	0.27	0.92
Optimisms	0.01	0.00	0.28	0.25
Optimism-corrected estimates	0.36	0.87	−0.01	0.67

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

SWENOTECA-MIR study's initial phase compared ddPCR to quantitative PCR in 180 orchiectomy patients and 50 healthy donors, demonstrating ddPCR's high performance with an overall sensitivity of 89% in all TGCTs except teratomas.[20] Evaluating our choice of method in a large patient cohort and demonstrating its robust characteristics is a strength of the present study. We also observed a clear relationship between testicular tumor size and miR-371a-3p levels in our prior study. In this study, miR-371a-3p appeared to be fairly good in predicting viable cancer in seminomas treated with surgery, with a sensitivity of 74% and specificity of 100%. This demonstrates an advantage over conventional markers, which, as anticipated, were negative for all primary seminoma patients. However, in primary nonseminoma patients, the sensitivity and specificity were lower, 34% and 90%, respectively. This small sample of 18 patients treated with primary RPLND included one false-positive patient with a teratoma. miR-371a-3p did not perform better than conventional markers in these patients.

The weakness for potential clinical use of miR-371a-3p analyzed with ddPCR for seminomas lies in its relatively low NPV of 21% in our dataset, indicating a significant potential for missing a detectable cancer. However, it is important to recognize the influence a high prevalence has on the NPV, which is the case in this group. Published research on miR-371a-3p in primary seminoma patients undergoing RPLND is sparse, but our findings align with Konneh et al,[21] who studied 15 seminoma patients. Lafin et al studied 24 chemotherapy-naïve seminomas and nonseminomas in CS I and II treated with RPLND, demonstrating impressive miR-371a-3p sensitivity of 100% and specificity of 92%.[22] Notably, the study successfully identified viable cancer less than 5 mm in size, even though histological subtypes are unclassified. Additionally, Seelemeyer et al reported 24 GCT patients (15 seminomas, 9 nonseminomas) undergoing primary RPLND, demonstrating miR-371a-3p sensitivity of 91%.[23] To investigate our false-negative results, we collected available data on positive lymph nodes and retroperitoneal tumor size, although size measurements were not available for all cases, in some due to the absence of size specifications in the pathology reports. We observed a weak positive correlation between tumor size and miR-371a-3p levels in seminoma patients but not in nonseminomas, although the sample size was insufficient to draw any

conclusions. In our material, a notable portion of viable primary nonseminoma specimens were yolk sac tumors, a subtype recognized for its low expression of miR-371a-3p. Typically, yolk sac tumors are associated with elevated AFP levels. Thus, cases exhibiting negativity for both miR-371a-3p and AFP may suggest a tumor biology where neither marker is released into the bloodstream, potentially indicating a genuine absence rather than a technical false-negative result or an incorrectly selected threshold level.

Approximately 10% of the whole cohort expressed conventional markers at surgery, underscoring the clinical need of a more reliable marker to avoid unnecessary extensive surgery in patients without viable disease other than teratoma. In this regard, miR-371a-3p may prove to be a useful, noninvasive, nonradiation, feasible, and cost-effective analysis in prechemotherapy seminoma patients and could be a valuable tool for confirming the presence of a suspected seminoma relapse on radiological examination. Furthermore, it may even have the potential to detect relapses before evident on radiology, but further studies are required to validate this possibility.

In the postchemotherapy patients, predominantly nonseminomas, miR-371a-3p showed limited utility without advantages over conventional markers. Leão et al[17] described 82 nonseminoma patients in the RPLND setting using a different method of PCR, and reported sensitivity for miR-371a-3p at 100%, but specificity at 54%, including false-positive tests for both benign histology and teratomas, making the clinical utility of the marker uncertain. The threshold for miR-371a-3p in our study was established prior to the present study based on technical performance of the detection method used and expression levels in serum from healthy men,[20] but the chosen threshold may contribute to the discrepancy compared to other studies. A previous publication also proposed caution when interpreting results within an indeterminate range for very lowly expressed markers such as miR-371a-3p.[24]

Despite being the largest study thus far to evaluate the diagnostic performance of miR-371a-3p among patients undergoing RPLND, the limited sample size hampers conclusive inference. In an effort to mitigate these effects, optimism-corrected values for performance metrics are reported throughout this paper. As expected, the bootstrap procedure generally resulted in slightly decreased performance estimates. However, as described, a degree of randomness was introduced into the classifier used in the bootstrap procedure, leading in some cases to negative, but small, optimism estimates. Consequently, in some cases, this resulted in optimism-corrected estimates outside the theoretical boundaries. In these cases, the estimates were truncated at the theoretical boundaries.

Adopting a wider perspective for novel tumor markers, the alternative marker miR-375-3p, in combination with miR-371a-3p, has been studied for its utility in detecting teratoma and viable cancer in blood and tissue, yielding various results.[25-28] A previous study by our group[29] showed no advantage of either miR-375-3p or miR-371a-3p as circulating markers of teratoma.

The potential solution for predicting the histological outcome following RPLND may lie in the integration of artificial intelligence and advanced radiomics, as this captivating field continues to evolve. Baessler et al conducted a retrospective study involving 80 postchemotherapy nonseminomas, where a trained machine learning classifier achieved 88% sensitivity, 72% specificity, and NPV of 88% in categorizing postoperative histology.[30] The next phase for this exciting methodology involves validation through large prospective clinical trials to compare its efficacy against conventional and contemporary tumor markers.

CONCLUSIONS

In summary, miR-371a-3p emerges as a promising clinical tool for predicting metastatic disease in seminoma patients undergoing primary RPLND. However, in postchemotherapy patients, we were unable to demonstrate a significant difference of miR-371a-3p values before and after surgery, indicating limitations in its diagnostic or predictive utility in this subgroup.

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REFERENCES

1. Gurney JK, Florio AA, Znaor A, et al. International trends in the incidence of testicular cancer: lessons from 35 years and 41 countries. *Eur Urol.* 2019;76(5):615-623. doi:10.1016/j.eururo.2019.07.002
2. Haugnes HS, Bosl GJ, Boer H, et al. Long-term and late effects of germ cell testicular cancer treatment and implications for follow-up. *J Clin Oncol.* 2012;30(30):3752-3763. doi:10.1200/JCO.2012.43.4431
3. Kvammen Ø, Myklebust T, Solberg A, et al. Long-term relative survival after diagnosis of testicular germ cell tumor. *Cancer Epidemiol Biomarkers Prev.* 2016;25(5):773-779. doi:10.1158/1055-9965.EPI-15-1153
4. Murray MJ, Huddart RA, Coleman N. The present and future of serum diagnostic tests for testicular germ cell tumours. *Nat Rev Urol.* 2016;13(12):715-725. doi:10.1038/nrurol.2016.170
5. SWENOTECA. SWENOTECA X management program of testicular cancer. 2020. https://www.swenoteca.org.
6. Daneshmand S, Cary C, Masterson T, et al. Surgery in early metastatic seminoma: a phase II trial of retroperitoneal lymph node dissection for testicular seminoma with limited retroperitoneal lymphadenopathy. *J Clin Oncol.* 2023;41(16):3009-3018. doi:10.1200/JCO.22.00624
7. Heidenreich A, Paffenholz P, Nestler T, Pfister DA. Nerve sparing retroperitoneal lymph node dissection in clinical stage IIA/B seminoma: the COTRIMS trial. *J Clin Oncol.* 2022;40(6_suppl):418. doi:10.1200/jco.2022.40.6_suppl.418
8. Hiester A, Che Y, Lusch A, et al. Phase 2 single-arm trial of primary retroperitoneal lymph node dissection in patients with seminomatous testicular germ cell tumors with clinical stage IIA/B (PRIMETEST). *Eur Urol.* 2023;84(1):25-31. doi:10.1016/j.eururo.2022.10.021
9. Stephenson AJ, Bosl GJ, Motzer RJ, Bajorin DF, Stasi JP, Sheinfeld J. Nonrandomized comparison of primary chemotherapy and retroperitoneal lymph node dissection for clinical stage IIA and IIB nonseminomatous germ cell testicular cancer. *J Clin Oncol.* 2007;25(35):5597-5602. doi:10.1200/JCO.2007.12.0808
10. Heidenreich A, Albers P, Hartmann M, et al; German Testicular Cancer Study Group. Complications of primary nerve sparing retroperitoneal lymph node dissection for clinical stage I nonseminomatous germ cell tumors of the testis: experience of the German Testicular Cancer Study Group. *J Urol.* 2003;169(5):1710-1714. doi:10.1097/01.ju.0000060960.18092.54
11. Gerdtsson A, Thor A, Grenabo Bergdahl A, et al. Unilateral or bilateral retroperitoneal lymph node dissection in nonseminoma patients with postchemotherapy residual tumour? Results from RETROP, a population-based mapping study by the Swedish Norwegian Testicular Cancer Group. *Eur Urol Oncol.* 2022;5(2):235-243. doi:10.1016/j.euo.2021.02.002
12. Murray MJ, Halsall DJ, Hook CE, Williams DM, Nicholson JC, Coleman N. Identification of microRNAs from the miR-371∼373 and miR-302 clusters as potential serum biomarkers of malignant germ cell tumors. *Am J Clin Pathol.* 2011;135(1):119-125. doi:10.1309/AJCPOE11KEYZCJHT
13. Belge G, Grobelny F, Radtke A, et al. Serum levels of microRNA-371a-3p are not elevated in testicular tumours of non-germ cell origin. *J Cancer Res Clin Oncol.* 2021;147(2):435-443. doi:10.1007/s00432-020-03429-x
14. Dieckmann KP, Radtke A, Geczi L, et al. Serum levels of microRNA-371a-3p (M371 test) as a new biomarker of testicular germ cell tumors: results of a prospective multicentric study. *J Clin Oncol.* 2019;37(16):1412-1423. doi:10.1200/JCO.18.01480
15. Syring I, Bartels J, Holdenrieder S, Kristiansen G, Muller SC, Ellinger J. Circulating serum miRNA (miR-367-3p, miR-371a-3p, miR-372-3p and miR-373-3p) as biomarkers in patients with testicular germ cell cancer. *J Urol.* 2015;193(1):331-337. doi:10.1016/j.juro.2014.07.010
16. Dieckmann KP, Radtke A, Spiekermann M, et al. Serum levels of microRNA miR-371a-3p: a sensitive and specific new biomarker for germ cell tumours. *Eur Urol.* 2017;71(2):213-220. doi:10.1016/j.eururo.2016.07.029
17. Leão R, van Agthoven T, Figueiredo A, et al. Serum miRNA predicts viable disease after chemotherapy in patients with testicular nonseminoma germ cell tumor. *J Urol.* 2018;200(1):126-135. doi:10.1016/j.juro.2018.02.068
18. Nappi L, Thi M, Lum A, et al. Developing a highly specific biomarker for germ cell malignancies: plasma miR371 expression across the germ cell malignancy spectrum. *J Clin Oncol.* 2019;37(33):3090-3098. doi:10.1200/JCO.18.02057
19. Nappi L, Kollmannsberger C, Nichols C. The role of micro-RNAs in management of germ cell tumors: future directions. *Curr Opin Urol.* 2020;30(2):258-263. doi:10.1097/MOU.0000000000000726
20. Myklebust MP, Thor A, Rosenlund B, et al. Serum miR371 in testicular germ cell cancer before and after orchiectomy, assessed by digital-droplet PCR in a prospective study. *Sci Rep.* 2021;11(1):15582. doi:10.1038/s41598-021-94812-2
21. Konneh B, Lafin JT, Howard J, et al. Evaluation of miR-371a-3p to predict viable germ cell tumor in patients with pure seminoma receiving retroperitoneal lymph node dissection. *Andrology.* 2023;11(4):634-640. doi:10.1111/andr.13317
22. Lafin JT, Singla N, Woldu SL, et al. Serum microRNA-371a-3p levels predict viable germ cell tumor in chemotherapy-naïve patients undergoing retroperitoneal lymph node dissection. *Eur Urol.* 2020;77(2):290-292. doi:10.1016/j.eururo.2019.10.005
23. Seelemeyer F, Pfister D, Pappesch R, Merkelbach-Bruse S, Paffenholz P, Heidenreich A. Evaluation of a miRNA-371a-3p assay for predicting final histopathology in patients undergoing primary nerve-sparing retroperitoneal lymphadenectomy for stage IIA/B seminoma or nonseminoma. *Eur Urol Oncol.* 2024;7(3):319-322. doi:10.1016/j.euo.2023.10.021
24. Lafin JT, Scarpini CG, Amini A, et al. Refining the serum miR-371a-3p test for viable germ cell tumor detection. *Sci Rep.* 2023;13(1):10558. doi:10.1038/s41598-023-37271-1
25. Belge G, Grobelny F, Matthies C, Radtke A, Dieckmann KP. Serum level of microRNA-375-3p is not a reliable biomarker of teratoma. *In Vivo.* 2020;34(1):163-168. doi:10.21873/invivo.11757
26. Nappi L, Thi M, Adra N, et al. Integrated expression of circulating miR375 and miR371 to identify teratoma and active germ cell malignancy components in malignant germ cell tumors. *Eur Urol.* 2021;79(1):16-19. doi:10.1016/j.eururo.2020.10.024
27. Kremer L, von Brandenstein M, Wittersheim M, et al. The combination of microRNA-371a-3p and 375-5p can distinguish viable germ cell tumor and teratoma from necrosis in postchemotherapy retroperitoneal lymph node dissection specimens. *Transl Androl Urol.* 2021;10(4):1647-1655. doi:10.21037/tau-20-1349
28. Lafin JT, Kenigsberg AP, Meng X, et al. Serum small RNA sequencing and miR-375 assay do not identify the presence of pure teratoma at post-chemotherapy retroperitoneal lymph node dissection. *Eur Urol Open Sci.* 2021;26:83-87. doi:10.1016/j.euros.2021.02.003
29. Myklebust MP, Søviknes AM, Halvorsen OJ, Thor A, Dahl O, Ræder H. MicroRNAs in differentiation of embryoid bodies and the teratoma subtype of testicular cancer. *Cancer Genomics Proteomics.* 2022;19(2):178-193. doi:10.21873/cgp.20313
30. Baessler B, Nestler T, Pinto Dos Santos D, et al. Radiomics allows for detection of benign and malignant histopathology in patients with metastatic testicular germ cell tumors prior to post-chemotherapy retroperitoneal lymph node dissection. *Eur Radiol.* 2020;30(4):2334-2345. doi:10.1007/s00330-019-06495-z

EDITORIAL COMMENT

The study by Thor et al offers valuable insights by evaluating miR-371a-3p for predicting viable residual tumor in testicular cancer patients undergoing retroperitoneal lymph node dissection (RPLND) in both the chemotherapy-naïve and postchemotherapy settings.[1] Their results with respect to performance of miR-371a-3p are sobering given the enthusiasm for miR-371a-3p to optimize patient selection for RPLND.

If we group the patients into 3 categories, (1) primary RPLND for seminoma, (2) primary RPLND for nonseminoma, and (3) postchemotherapy RPLND, clinically useful high sensitivity/specificity was only noted for seminomas in the primary setting. The performance characteristics of miR-371a-3p for patients receiving primary RPLND for nonseminoma or postchemotherapy RPLND was disappointing with sensitivities of 34% and 9%, respectively.

These results are somewhat incongruent with existing reports addressing the performance of microRNAs in the pre-RPLND setting. Lafin et al report higher sensitivity and specificity in the chemotherapy-naïve settings,[2] while Leão et al published encouraging results in the postchemotherapy setting.[3]

Several considerations arise from this study. Nearly certainly there are lab-specific considerations, including thresholding, that may impact the results.[4] There may be an opportunity to examine clinical state-specific thresholding as well. The authors employed digital droplet polymerase chain reaction, a relatively novel approach for evaluation of miR-371a-3p, noting its superior performance over quantitative polymerase chain reaction in the pre-orchiectomy setting, where tumor burden is high. An important addition to the literature would be a detailed comparison between the 2 assay techniques in the RPLND setting where assay sensitivity is critical due to lower tumor burden. Furthermore, associations between biomarker levels and lymph node sizes need to be carried out with larger sample sizes to draw valid conclusions. The potential value of miR-371a-3p to clinically available details (mass size, clinical stage 1 with relapse vs clinical stage 2 at presentation) will also be interesting to dissect.

The authors are to be congratulated for their analysis of miR-371a-3p in the pre-RPLND setting. Such studies are critically important, raising significant questions on the safe and responsible incorporation of miR-371a-3p into the clinical management of patients being considered for RPLND.

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REFERENCES

1. Thor A, Myklebust MP, Grenabo Bergdahl A, et al; SWENOTECA Working Group. miR-371a-3p predicting viable tumor in patients undergoing retroperitoneal lymph node dissection for metastatic testicular cancer: the SWENOTECA-MIR study. *J Urol.* 2024;212(5):720-730. doi:10.1097/JU.0000000000004164

2. Lafin JT, Singla N, Woldu SL, et al. Serum microRNA-371a-3p levels predict viable germ cell tumor in chemotherapy-naïve patients undergoing retroperitoneal lymph node dissection. *Eur Urol.* 2020;77(2):290-292. doi:10.1016/j.eururo.2019.10.005

3. Leão R, van Agthoven T, Figueiredo A, et al. Serum miRNA predicts viable disease after chemotherapy in patients with testicular nonseminoma germ cell tumor. *J Urol.* 2018;200(1):126-135. doi:10.1016/j.juro.2018.02.068

4. Lafin JT, Scarpini CG, Amini A, et al. Refining the serum miR-371a-3p test for viable germ cell tumor detection. *Sci Rep.* 2023;13(1):10558. doi:10.1038/s41598-023-37271-1