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Circulating microRNA profiles in Wilms tumour (WT): A systematic review and meta-analysis of diagnostic test accuracy

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ABSTRACT

Background: Wilms tumour (WT) is caused by aberrant embryonic kidney development and associated with dysregulated expression of short, non-protein-coding RNAs termed microRNAs (miRNAs). At present, there is no reliable circulating biomarker of WT, and this remains an urgent unmet clinical need. Such biomarkers may assist diagnosis, subtyping/prognostication, and disease-monitoring. Here, we established the list of dysregulated circulating miRNAs in WT from the existing published literature.

Methods: Regardless of publication date, PubMed, Scopus, Web-of-Science, and Wiley online library databases were searched for English/French studies on WT circulating miRNAs. The PRISMA-compliant search was registered in PROSPERO. The QUADAS tool measured retained article quality. The meta-analysis assessed the sensitivity and specificity of miRNAs for WT diagnosis.

Results: Qualitative analysis included 280 samples (172 WT patients; 108 healthy controls) from five of 450 published articles. The study uncovered 301 dysregulated miRNAs (144 up-regulated, 143 down-regulated, 14 conflicting). The pooled sensitivity, specificity, and AUC of the 49 significantly dysregulated microRNAs from two studies was 0.67 [0.62; 0.73], 0.95 [0.92; 0.96] and 0.77 [0.73; 0.81] respectively, indicating a stronger diagnostic potential for WT.

Conclusions: Circulating miRNAs show promise for WT diagnosis and prognosis. More research is needed to confirm these findings and determine associations with tumour stage/subtype.

Prospero registration number: CRD42022301597.

1. Introduction

1.1. Wilms tumour (WT)

Wilms tumour (WT), also known as nephroblastoma, is a malignant kidney tumour that accounts for 5% of all paediatric cancers and 95% of childhood malignant renal neoplasms [1], with an incidence of 1 in 10, 000 children [2]. It is caused by aberrant kidney development at the embryonic stage, during mesenchymal epithelial transition (MET) at the beginning of nephrogenesis [3]. It typically affects children aged 0–4 years of age [1], with a median diagnostic age between 3 and 4 years [2], and an equal distribution between males and females.

Whilst the majority of WT cases (90%–95%) are unilateral, 5–10% of cases affect both kidneys (synchronous bilateral or multifocal tumours) [4]. Approximately 98–99% of WT cases are sporadic, whereas 1–2% are familial [2,5]. Metastatic forms are observed in approximately 12% of cases [6]; they are 80% pulmonary, 10% hepatic [7], but lymph node or bone metastasis may be observed in rare cases [5]. Up to 20% of WTs are discovered inadvertently during routine check-up visits as tumours can become large without manifesting any specific symptoms [8,9].

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However, WT is generally characterised by the development of a palpable hard abdominal mass or swelling that may be associated with abdominal pain, intra-tumoral bleeding, microscopic haematuria (in 25% of cases), hypertension (25%), fever (20%) and vomiting [10–12]. Medical imaging, primarily ultrasound, helps establish the diagnosis, which may determine if the mass is intra- or extra-renal, solid or cystic. Three dimensional imaging (CT scan and/or MRI) are then performed, which are required for staging purposes and initial disease-monitoring [10,13].

WT is an undifferentiated mesodermal tumour comprising variable amounts of embryonic kidney elements (blastocyst, epithelium, and stroma). Based on prognosis, there are two distinct histopathological types - favourable (>90% of cases) and unfavourable (6–10%). Anaplastic and sarcomatous variants are unfavourable and associated with poor clinical outcomes [10]. WT is treated following either the North American NWTS protocol or the European SIOP protocol [14,15]. In Morocco and much of Europe, the SIOP protocol is implemented, with preoperative chemotherapy, then total nephrectomy with primary ligation of the vascular pedicle and lower resection of the ureter, followed by postoperative chemotherapy [16]; usually with combination chemotherapy [5]. Sometimes, chemotherapy is combined with post-operative radiation therapy, depending on the stage and histology of the tumour [17]. Nevertheless, whilst maximising survival, these therapies can have adverse side effects, including short-term toxicity, particularly gastrointestinal and cardiac, and long-term toxicity, which manifests in the slowing of bone growth with a risk of scoliosis as well as an increased risk of secondary malignancies [5,18].

1.2. Background to microRNAs (miRNAs)

MicroRNAs (miRNAs) are short, highly conserved, non-protein-coding, single-stranded RNA molecules of approximately 20–22 nucleotides in length [19]. They were first discovered and identified in 1993 to be post-transcriptional downregulators of protein-coding genes, by binding to the 3′ untranslated region (3′UTR) of messenger RNAs (mRNAs), inhibiting protein translation (translation silencing) via deadenylation (poly-A tail degradation) [20]. Circulating miRNAs are stable over time, even after repeated freeze-thaw cycles and are typically contained within membrane-bound particles termed exosomes/extracellular vesicles. A minority are bound to serum protein complexes (e.g., Argonaute, lipoproteins). Levels of miRNAs can change in body fluids when cellular damage or tissue injury occurs [21,22].

1.3. MiRNAs in cancer

As miRNAs cause mRNA degradation, in cancer, miRNA effects are therefore ‘opposite’ depending on whether the protein-coding target gene is an oncogene or a tumour suppressor gene [23]. Hence, up-regulation of oncomirs (miRNAs with an oncogenic effect) or down-regulation of suppressor-miRs (miRNAs with a tumour suppressor effect) may play a causal role in the generation or maintenance of tumours [19]. Thus, expression profiles of miRNAs are used to classify cancerous and normal tissues and for disease-monitoring and response to therapy [20]. The role of miRNAs is context-dependent. For example, a particular miRNA may act as an oncomir in one tissue/cancer and as a suppressor in another, based on the differing protein-coding transcriptomes, and thus mRNA targets, in different tissue types. Taking for example, miR-203 that acts as a tumour suppressor in pancreatic cancer by modulating DUSP5 expression [24], in renal cancer by targeting FDF2 [25], while it is an oncogene in breast cancer by targeting fibroblast growth factor 2 [26] as well as in melanoma cancer by targeting BMI1 gene [27]. Interestingly, miR-203 in ovarian cancer acts as a suppressor miR when targeting BIRC5 gene [28], but as an oncogene by regulating PDHB gene [29].

Bioinformatic data indicates that a single miRNA can bind to more than 200 target genes of different functions. Thus, miRNAs potentially control the expression of at least one-third of human mRNAs, highlighting their potential role in most cellular processes. Several studies have shown that miRNAs act as key regulators of cell proliferation, apoptosis, and cell differentiation. Therefore, deregulation or inhibition of miRNA expression has been involved in many diseases [23].

Interest is currently focused on studying alterations in miRNA expression levels in various tissues at different stages of diverse diseases [30]. For instance, miRNAs are implicated in numerous types of paediatric cancer, including childhood germ cell tumours [31]. Hence, profiling miRNA expression levels in cancer can become a tool for diagnosis and prognosis or for identification of therapeutic targets [21,32,33].

MiRNAs have emerged as promising theranostic agents in the last decade, owing to their small size and ability to regulate multiple cellular pathways by targeting specific genes [34]. This has spurred interest in developing miRNA-based therapeutics, which involves expressing or depleting specific miRNAs in diseased cells or organs using viral vectors, liposomes, antibodies or nanoparticles. However, delivering miRNAs to specific cells or tissues remains challenging [35,36]. Furthermore, some studies suggest that miRNAs could be used in combination therapy, due to the ability of some mimetic or antimimetic miRNAs to modulate the sensitivity of cancer cells to chemotherapeutic drugs [37–39].

Clinical trials investigating the role of miRNAs in cancer treatment have shown promising results, such as the study of Xie et al. that found that miR-621 enhances chemosensitivity of breast cancer cells to paclitaxel plus carboplatin (PTX/CBP) by suppressing FBXO11-dependent inhibition of p53 [37]. Another study by Hsu et al. demonstrated that the delivery of miR-122 to a MYC-driven mouse model of hepatocellular carcinoma strongly inhibited tumorogenesis [40]. However, to date, there have been no clinical trials investigating the use of miRNA delivery for tumour suppression in Wilms tumour, and most studies on miRNAs and WT have been limited to in vitro experiments.

1.4. Circulating miRNA profiling in WT

WT is primarily managed using two distinct approaches. In Europe, neoadjuvant chemotherapy is often initiated without tissue biopsy; although 90–95% of patients benefit from not needing to undergo biopsy to start treatment, up to 5% of children retrospectively will be observed to have received inappropriate treatment [12,41]. In contrast, North American trials have identified pre-treatment prognostic biomarkers to stratify high-risk patients for intensified treatment, and upfront tumour resection or tissue biopsy is preferred. The identification of accurate blood-based biomarkers for WT, such as miRNAs, may help prevent misdiagnosis and mistreatment and may contribute to standardising treatment by providing valuable information on risk stratification. The main focus of research was to profile miRNAs in WT tissue. Among these miRNAs, miR-21, miR-140-5p, and miR-155-5p have been the most extensively studied. MiR-21 is consistently found to be overexpressed and is linked to poor prognosis of WT due to its correlation with down-regulation of PTEN expression [42–44]. On the other hand, miR-140-5p and miR-155-5p, which are typically down-regulated in WT, have demonstrated potential in suppressing cellular proliferation and metastasis. MiR-140-5p targets TGFBRI and IGF1R genes [45], while miR-155-5p targets CREB1 gene [46]. Despite these findings, no circulating miRNA has been widely studied as a diagnostic biomarker for WT to date. This systematic review and meta-analysis compiles the list of WT-associated circulating miRNAs identified to date, discusses their correlation with tumour histological types, where available, and assesses their diagnostic performance for WT.

2. Materials and methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) tool was used to ensure transparent reporting [47,48], and to perform the systematic review, which was recorded under

the registration number: CRD42022301597.

2.1. Search strategy and data sources

Two independent reviewers (SB and EE) performed a comprehensive literature search, using four different databases (PubMed, Scopus, Web of Science, and Wiley online Library) (Supplementary Table B1). The search ran from November 2021, to January 2022 inclusive. All articles retrieved from search queries and textual search, in English or in French, were saved, with no restriction on publication date. The search query, textual search and filtering strategy sought to collate all articles on circulating microRNA signatures in WT.

For preliminary selection, two authors (SB and EE) independently reviewed titles and abstracts from the initial search, blinded to author and institution. Then, full text papers were reviewed in more detail for eligibility determination. Doubts regarding the inclusion of any publication were resolved by discussion or involvement of a third reviewer (RA).

2.2. Rationale for study selection

We selected studies that identified circulating WT-associated miRNA profiles, as their use would be less invasive than direct miRNA expression profiling from WT tissue (which would require at least a biopsy) and due to the expectation that miRNAs would be differentially expressed in blood (serum, plasma, and/or whole blood) in the presence of WT.

2.3. Inclusion and exclusion criteria

We employed the PECO acronym (Participants, Exposition, Comparisons, and Outcomes) for predetermined criteria for inclusion (Supplementary Table B2). The systematic review included studies that met all of the following criteria: the publication (i) was a diagnostic study that recruited a control group; (ii) profiled miRNA expression in plasma, serum, or blood for patients at the time of diagnosis of WT; (iii) included patients under 15 years old, of all nationalities. Studies that met any of the following criteria were excluded: (i) research conducted on animals, *in vitro* or *in silico*; (ii) utilised non-molecular assays to detect miRNAs, (iii) studied miRNA expression in tumour tissue or biological matrix other than plasma, serum, or blood; (iv) profiled miRNAs in diseases other than WT; (v) patients diagnosed with WT and another disease at the same time; (vi) editorials, meeting abstracts, or review articles, including systematic reviews and/or meta-analyses; (vii) studies without a complete and reliable protocol or relevant outcomes; (viii) those without an endogenous control or control group.

2.4. Data extraction and assessment of clinical validity

From eligible articles with original data, we extracted the following information: first author, publication year, title, country, clinicopathological features of patients and controls, treatment received before sample collection, sample size and type, storage conditions, haemolysis levels/assessments, method for miRNA isolation, assay for miRNA detection, endogenous miRNA or other small RNA control, number of reported miRNAs, miRNA expression profile (up- or down-regulation, fold change, *p*-value), assay performance [area-under-the-curve (AUC), accuracy, sensitivity, specificity]. The extracted data are described in tabular form and Appendix A.

In some studies, the full published text did not report all the required information, such as patients' age, total number of profiled miRNAs, sensitivity and specificity of assay performance; in such cases, we retrieved this information from the appendix files, where available, and calculated the required data using Jamovi biostatistics tool [54]. As with the literature selection process, discrepancies were resolved by consensus among authors (SB, EE, and RA).

2.5. Nomenclature of miRNAs

During data extraction, the same miRNA was occasionally named differently from one study to another; certain studies adopted the old nomenclature system 'miR/miR*' to symbolise the miRNA localisation ('miR' for the miRNA in the guide strand and 'miR*' for the miRNA in the passenger strand), others adopted the updated nomenclature system '5p/3p' [55]. To facilitate data analysis, we adjusted the nomenclature of some miRNAs with identical numbers, but one mentioned with the miR* prefix and the other with miR-3p, referring to the most current version of miRBase, the online miRNA registry, (v22.1) (https://www.mirbase.org/) [55,56]. Subsequently, miR* was replaced by miR-3p (for example, hsa-miR-126* was replaced by hsa-miR-126-3p and hsa-miR-132* was replaced by hsa-miR-132-3p). In general, a standard nomenclature system is adopted to name miRNAs: the prefix 'mir-' is followed by a dash and a number, the latter often indicating order of naming. The capitalised 'miR-' refers to the mature form of miRNA. Origin of species is designated with a three-letter prefix, 'hsa-' refers to miRNA in human [57].

2.6. Selection of significantly dysregulated miRNAs

All studies used a two-tailed *t*-test to identify the significantly dysregulated circulating miRNAs between experimental groups, with p-value <0.05 considered significantly dysregulated in WT. This cut-off was retained for our analyses.

2.7. Risk of bias assessment

The quality of all included articles was evaluated by two authors (SB and RA), using the QUADAS-2 (quality assessment tool for diagnostic accuracy studies) scale [58,59]. This tool consisted of 18 questions to evaluate four domains: patient selection, index test, reference standard and flow and timing (the response is either yes, no, or some concerns). Each domain was evaluated for risk of bias and the first three were evaluated for applicability concerns (scored as low, high or some concerns). The QUADAS score of quality evaluation of the included studies was calculated by answering the 18 questions; each item in the scale was scored as 0 (no, high, or some concerns) or 1 (yes or low). A highest possible score of 18 indicated optimal study quality.

2.8. Selection and confusion bias

We considered that the risk of bias was low in patient selection if the study recruited WT patients with histologically confirmed diagnosis before preoperative chemotherapy, and healthy controls whose mean age was similar to that of the WT patients. In contrast, we considered there to be a high risk of bias if the study recruited patients affected with WT and other cancer or disease simultaneously. We considered risk of bias low in the conduct or interpretation of the index test, in this case RT-qPCR, if the study mentioned the methodology of miRNA profiling and identification of significantly dysregulated miRNAs and if it used an endogenous control for qPCR. We described the risk of bias as unclear in the conduct or interpretation of the reference standard, if the study didn't clearly state the WT histopathology. We considered the risk of bias low in flow and timing of study conduction if all patients and controls received the same reference standard at the same time of receiving the index test.

2.9. Data synthesis and statistical analysis

First, we performed a narrative description of dysregulated circulating miRNAs in WT. The qualitative synthesis covering the results of the included studies is summarised in tabular form with the presentation of the main judgment criteria. Then, we performed one–way ANOVA test to identify miRNAs that show a significantly different expression

between WT subtypes, at $p < 0.05$. Means differences of miRNA expression in the various histological types was carried out by unpaired *t*-tests. Using Stata software (StataCorp, United States of America), we computed AUC values, with the corresponding 95% confidence interval (CI95) and standard error, for differentiation of two groups with single miRNA. Using a bivariate model, we conducted a meta-analysis of the most significantly dysregulated miRNAs. We utilised Meta-DiSc 2.0 software [60] to compute the overall specificity and sensitivity of all miRNAs. Afterwards, the standardised and overall effects for miRNA specificity and sensitivity and their CI95 were displayed graphically using forest plots. Moreover, heterogeneity between studies was examined with I^2 statistics, which described the percentage of total variation across studies that was due to heterogeneity rather than chance. I^2 was expressed as a percentage (25%: low heterogeneity, 50% moderate heterogeneity and 75% for high heterogeneity). We also assessed the pooled diagnostic value of miRNAs through means of the SROC curve and AUC forest plot, using JASP software. Then, we performed subgroup analyses, in order to test the diagnostic potential of the different miRNA types (putative oncomirs or suppressor miRNAs) in the different studies and ethnicities.

3. Results

3.1. Studies identified by database searches

Initially, a total of 743 articles were obtained from all four databases (PubMed, Scopus, Web of Science and Wiley Online Library), using search queries and textual search, and 450 were recovered after removing duplicate and retracted articles. Subsequently, titles and abstracts were evaluated and 13 studies were subjected to full-text screening. Eventually, five studies met all eligibility criteria and were qualified for inclusion into the systematic review. Two studies [50,51] were included in the meta-analysis, since only two authors (MJM and NL) supplied us with the required data to calculate sensitivity and specificity (Fig. 1).

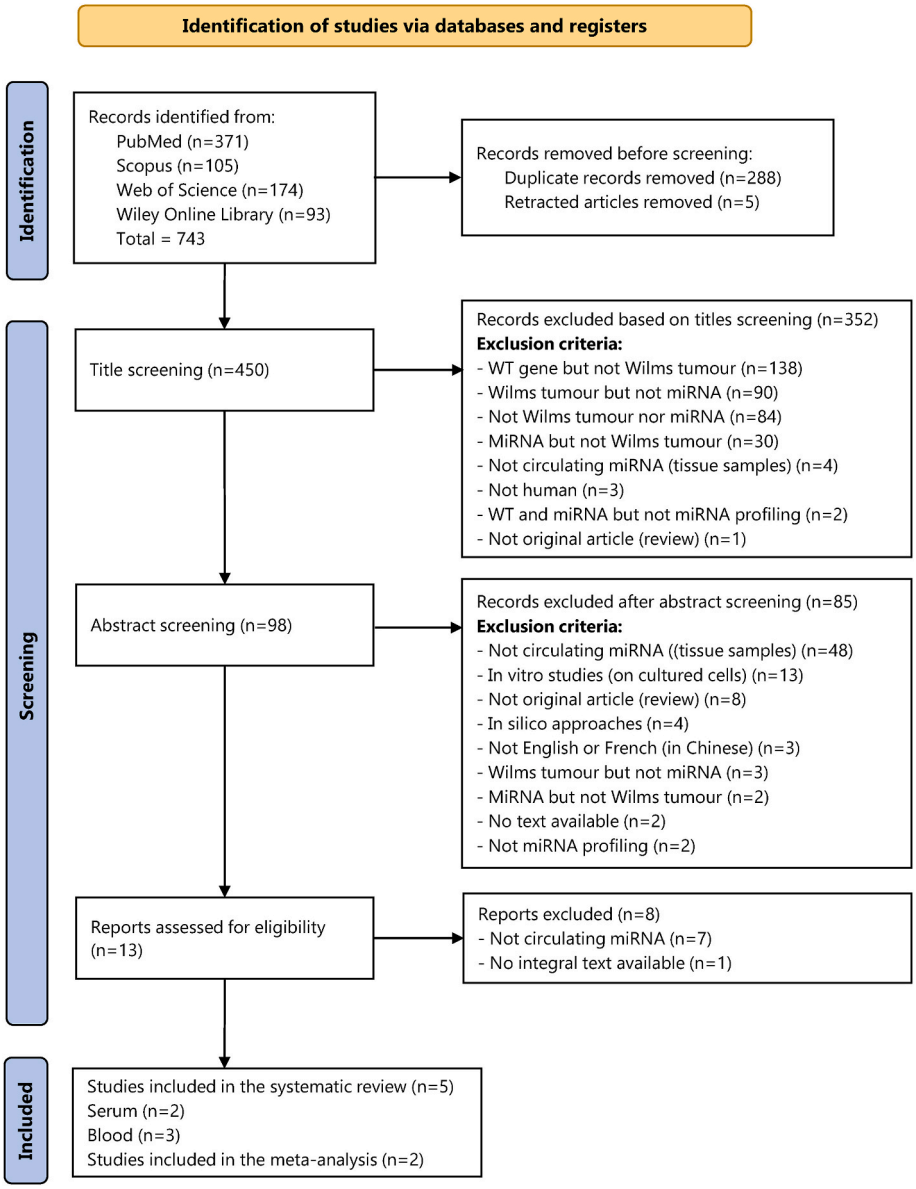

Fig. 1. PRISMA flow Diagram of database searching and study selection and identification [48].

Table 1
Characteristics of the five included studies profiling circulating miRNAs in patients with nephroblastoma (Wilms tumour; WT).

Reference	Article title	Country	Sample Type	main goal	Assay	main results	QUADAS Score
Schmitt et al., 2012 [49]	Treatment-independent miRNA signature in blood of Wilms tumour patients	Germany	blood	- Comparing miRNA signature in WT patients prior and after preoperative chemotherapy. - Comparing miRNA profiles in 23 WT patients prior to chemotherapy with 19 HC.	Microarray and RT-qPCR	- No significant difference between miRNA signature of both groups (prior and after preoperative chemotherapy) - Comparing to HC, 176 miRNA were found significantly dysregulated in WT blood patients, both, prior and after chemotherapy, including 79 down-regulated and 97 up-regulated.	15/18
Ludwig et al., 2015 [50]	Circulating Serum miRNAs as Potential Biomarkers for Nephroblastoma	Germany	Serum	Test whether miRNAs overexpressed in WT tissue can be detected in WT serum and if they may be exploited as circulating biomarkers for WT diagnosis and subtypes discrimination	RT-qPCR	Fourteen miRNAs revealed considerably higher abundance in serum of patients with WT than in controls.	15/18
Murray et al., 2015 [51]	Solid Tumours of Childhood Display Specific Serum microRNA Profiles	United Kingdom	Serum	Quantitative-RT-PCR miRNA profiling from 53 serum samples with 6 WT patients	RT-qPCR	- 33 miRNAs were overexpressed in WT group. - miR-143-3p was increased in WT compared with the 'other tumour' group ($p = 0.003$) - In differential diagnosis plots, miR-143-3p distinguished WT from MYCN NB/NB ($p = 0.0005$), with miR-129-5p being over-expressed in six of eight MYCN-NB/NB cases versus WT	14/18
An et al., 2018 [52]	Up-regulation of miR-190b promoted growth, invasion, migration and inhibited apoptosis of Wilms tumour cells by repressing the PTEN expression	China	Whole blood	Profiling miR-190b in WT patients' blood sample compared to healthy controls using RT-qPCR.	RT-qPCR	- MiR-190b was over-expressed in WT blood samples compared to healthy controls.	13/18
Luo et al., 2020 [53]	MiR-155-5p exerts tumour-suppressing functions in Wilms tumour by targeting IGF2 via the PI3K signalling pathway	China	Whole blood	- Identifying differentially expressed miRNAs (DE-miRNAs) in WT blood and tissues, using miRNA microarray. - Explore miR-155-5p expression In WT tissue using RT-qPCR	Microarrays	- A total of 105 miRNAs were differentially expressed in WT group compared to the uronephrosis group (24 miRNAs were up-regulated, 81 miRNAs were down-regulated) - MiR-155-5p was down-regulated in both blood and tissue from WT patients who did not receive preoperative chemotherapy but was up-regulated in tissues from WT patients who had received preoperative chemotherapy.	13/18

Abbreviations: RT-qPCR: Real-time quantitative polymerase chain reaction; WT: Wilms tumour, HC: Healthy controls; QUADAS: Quality Assessment Tool for Diagnostic Accuracy Studies, MYCN-NB: MYCN-amplified high-risk neuroblastoma, NB: non-MYCN-amplified low-risk neuroblastoma.

Table 2
Patients' characteristics and methods for miRNA profiling.

Reference	Groups	Mean age	Patients' gender	Clinicopathological features	Sample type	Storage conditions	level of haemolysis	Isolation method	Detection method	Spike-in/ Endogenous control	No. of reported miRNAs	NO. of significantly dysregulated miRNA
Schmitt et al., 2012 [49]	23 untreated WT (HCD/ biopsy) before PC	4.9 yr ± 3.9	11 F 12 M	41 unilateral 2 bilateral	Whole blood	2.5 mL Paxgene Blood RNA tubes −20 °C	NA	miRNeasy Kit (Qiagen)	**microarrays** using the Geniom RT Analyser **RT-qPCR:** miScript SYBR Green PCR System (Qiagen, Valencia, CA, USA)	snRNA RNU6B	214	**Significantly dysregulated**: 176 Significantly up-regulated: 97 Significantly down-regulated: 79 The expression profile of six miRNAs was confirmed using RT-qPCR
	20 WT after PC (HCD)	3.3 yr ± 2.2	10 F 10 M	NA								
	19 HC	37.8 yr ± 14.2	12 F 7 M	Healthy								
Ludwig et al., 2015 [50]	32 untreated WT Before PC (HCD/ Biopsy)	<18 yr.	6F, 3 M 6F, 5 M 6F, 6 M	9 blastemal 11 regressive 12 triphasic 7 metastatic	Serum	−80 °C	all samples with high risk of haemolysis (ΔCtmiR-23a - miR-451 > 7) were excluded	miRNeasy Serum/Plasma kit (Qiagen)	**RT-qPCR** miScript II RT kit (Qiagen) miScript SYBR1-Green PCR Kit (Qiagen)	cel-miR-39	19	**Significantly dysregulated:** 15 Significantly up-regulated: 14 Significantly down-regulated: 1
	12 HC		5F,7 M	Healthy								
	Validation set: 43 WT (HCD) 9 other RC		21F, 22 M 4F, 5 M 2F, 11 M	13 healthy 9 RC 16 regressive WT 12 triphasic WT 8 stroma WT 4 diffuse anaplasia WT 2 epithelial WT 1 necrotic WT								
	13 HC											
Murray et al., 2015 [51]	6 WT (HCD/ biopsy)	38.1 mo ±29.8	5 F 2 M	6 unilateral 1 bilateral	Serum	Serum separator tubes −80 °C	No macroscopic evidence of haemolysis on visual inspection	miRNeasy Mini-Kit (Qiagen)	**RT-qPCR** - miRCURY-LNA Universal-RT microRNA PCR, polyadenylation and cDNA synthesis Kit (Exiqon) - microRNA Ready-to-Use PCR Human Panels I&II (Exiqon)	UniSp2 UniSp6 RNA	564	**Significantly dysregulated:** 34 miRNAs Significantly up-regulated: 33 Significantly down-regulated: 1 32 miRs differentiated WT from HC 2 miRs differentiated WT from NB
	20 HC	74 mo ±52.8	9 F 11 M	Healthy								
	8 NB	31.1 mo ± 34.2	2 F 6 M	4 MYCN-NB 4 NB								
An et al., 2018 [52]	44 WT (HCD)	≥24 mo: 16 WT <24 mo : 28 WT	20 F 24 M	23 left WT 18 right WT 3 bilateral 14 Lymph node metastasis 33 I-II NWTS-5 stage 11 III-IV NWTS-5 stage 36 FH 8 UH	Whole blood	liquid nitrogen	NA	TRIzol reagent (Invitrogen)	**RT-qPCR** - Genepharma Reverse transcription kit - Taqman kit (Genepharma)	snRNA RNAU6	1	Up-regulated: miR-190b
	23 HC	NA	NA	Healthy								

(*continued on next page*)

Table 2 (*continued*)

Reference	Groups	Mean age	Patients' gender	Clinicopathological features	Sample type	Storage conditions	level of haemolysis	Isolation method	Detection method	Spike-in/ Endogenous control	No. of reported miRNAs	NO. of significantly dysregulated miRNA
Luo et al., 2020 [53]	4 WT before PC (HCD) 4 UNP 172 WT patients 108 Controls: 87 HT 21 Other diseases	NA NA	NA NA	32 left WT 28 right WT 42 I + II TNM 18 III + IV TNM 45 FH 15 UH 28 perinephric metastasis 10 lymphatic metastasis NA	Whole blood –	−80 °C –	NA –	mirVana miRNA Isolation kit (Invitrogen)	**Microarray** Genisphere FlashTag kit was used to label the total RNA, and Affymetrix GeneChip miRNA Arrays were used for hybridization –	NA –	105 **903**	**Significantly dysregulated:** 105 miRNAs Up-regulated: 24 Down-regulated: 81 **Significantly dysregulated:** 331 with duplicate, 301 without duplicate Up-regulated: 144 Down-regulated: 143 Conflicting: 14
Total												

Abbreviations: WT: Wilms Tumour; HCD: Histopathologically confirmed diagnosis; HC: Healthy controls; PC: Preoperative chemotherapy; MYCN-NB: MYCN-amplified high-risk neuroblastoma; NB: non-MYCN-amplified low-risk neuroblastoma; RC: Renal carcinoma; UNP: uronephrosis; yr.: years; mo.: month; F: Female; M: Male; TNM: Tumour-node-metastasis; FH: Favourable histology; UH: Unfavourable histology; NWTS-5: National Wilms Tumour Study grade classification; RT-qPCR: reverse transcription quantitative real-time polymerase chain reaction; NA: Not applicable/Not available.

3.2. Study characteristics and quality evaluation

The characteristics of the included studies are summarised in Tables 1 and 2. Overall, the five studies recruited a total of 172 patients with Wilms tumour (WT) and 108 controls (87 healthy controls and 21 patients with diseases other than WT), and reported the expression profile of 903 miRNAs. The countries of origin of the included studies were China (n = 2), Germany (n = 2) and United Kingdom (n = 1). Two studies quantified circulating miRNA expression in serum and three studies in whole blood using miRNA microarrays and/or quantitative Real-Time PCR. Three studies adopted miRNeasy Kit (Qiagen) for miRNA isolation from serum or blood, one used TRIzol reagent (Invitrogen) and one used mirVana miRNA Isolation kit (Invitrogen) for miRNA extraction from blood. Two studies performed RT-qPCR using SYBR Green PCR system from Qiagen, one used microRNA Ready-to-Use PCR Human Panels I&II from Exiqon, and one used Taqman kit RT-qPCR system from Genepharma. Three studies used U6 snRNA as endogenous control. The risk of bias assessment of the included studies, using the QUADAS-2 scale is represented using scores and shown in Fig. 2. The scores were between 13 and 15 for the included studies, indicating high study quality.

3.3. Circulating miRNA profiles

Of the 903 detected circulating miRNAs in WT patients at diagnosis, 331 were significantly dysregulated and could differentiate WT patients from controls (threshold values of $\geq$2- and $\leq$-2-fold change and p-value $<$0.05). After duplicate removal, we were left with 301 miRNAs, with 144 up-regulated miRNAs, 143 down-regulated miRNAs and an overlap of 14 miRNAs that were expressed differently between studies (Supplementary Figure B1). The fourteen overlapped miRNAs are denoted in Supplementary Figure B5, with the corresponding fold changes.

The significantly dysregulated circulating miRNAs in WT with the corresponding relative expression profiles, p-values, fold change values and assay performance values (AUC, accuracy, sensitivity, and specificity) are listed in Appendix A. A histogram in Supplementary Figure B2 shows the reported number of significantly up- and down-regulated circulating miRNAs in each of the included studies. Half of the circulating miRNAs were up-regulated, with miR-342-3p, −190b, −99b, −658, -615-3p, -126-3p, −130b-3p, -143-3p, -17-5p, -24-3p and *let-7f-5p* being the most frequently reported up-regulated miRNAs in the majority of the included studies (Supplementary Figure B3).

In more details, ***Schmitt et al*** detected 97 significantly up-regulated and 79 down-regulated circulating miRNAs, with miR-766, -1246, −197, and −224 showing the most significantly up-regulation, with an increased median expression of 3.0–9.6 fold compared with the control group. The most significantly down-regulated miRNAs were miR-20a, −20b, -144-3p and −144, which displayed median expression changes of 2.9–6.9 fold in WT patients compared with controls [49]. In the other hand, ***Ludwig et al*** reported 14 significantly up-regulated and one down-regulated circulating miRNAs, with miR-130b-3p, -100-5p, -143-3p, -17-5p and -223-3p have the highest diagnostic potential for WT. These miRNAs exhibited no correlation with haemolysis, suggesting that their expression levels are unaffected by differences in serum collection [50]. Just as in the previous study, ***Murray et al*** identified miR-130b-3p and miR-143-3p as from the top-ranking significantly up-regulated circulating miRNAs with potential for differential diagnosis, along with miR-10a-5p, −30b-3p, 34a-3p, −106b-3p, -141-5p, −577, -877-5p and 24 more up-regulated miRNAs. MiR-143-3p was more prominent in the WT group than in the 'other tumours' group (p = 0.003) and distinguished WT from MYCN-amplified high-risk neuroblastoma (MYCN-NB) and non-MYCN-amplified low-risk neuroblastoma (NB) (p = 0.0005). Moreover, miR-129-5p was over-expressed in six of eight MYCN-NB/NB cases versus WT [51]. ***An et al*** found that miR-190b was over-expressed in 44 blood samples from WT patients compared with 23 healthy controls (HC) ($p < 0.001$). Moreover, higher circulating

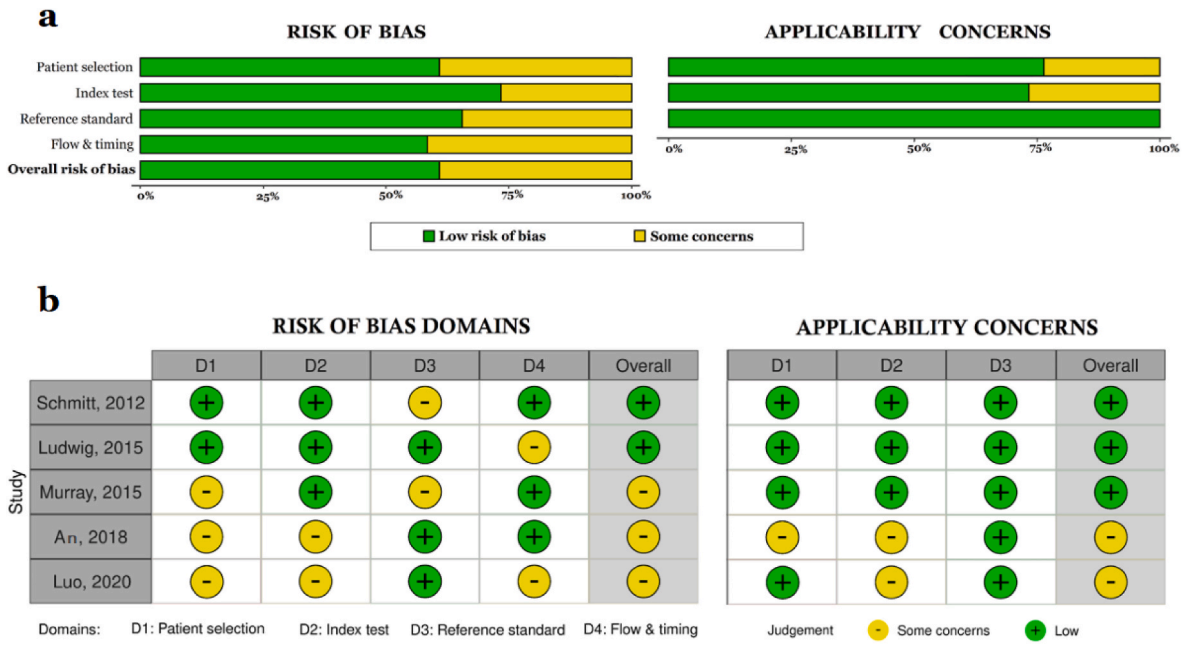

Fig. 2. Overall quality of the included studies as assessed using the QUADAS-2 tool. **a**. Weighted bar plots of the distribution of risk-of-bias judgments within each bias domain. **b**. 'Traffic light' plots of the domain-level judgments for each individual result.

miR-190b levels correlated with unfavourable histology, lymph node metastasis and III-IV NWTS stage [52]. ***Luo et al*** identified 24 up-regulated and 81 down-regulated miRNAs in the WT group compared with the uronephrosis group. MiR-126-3p was the most up-regulated and miR-338-5p and −3195 were the most down-regulated [53].

3.4. Differential abundance and biomarker potential of circulating miRNAs in WT subtypes

To evaluate the potential of circulating miRNAs for differentiating between WT subtypes, we analysed the data provided by ***Ludwig et al, 2015***, as it provided delta CT (ΔCT) of miRNA profiles for each patient and control. We evaluated *p*-values using one–way ANOVA test for nine up-regulated miRNAs, since they were among the most frequently reported in the included studies. In particular, we found that miR-143-3p, -223-3p were more abundant in serum of patients with regressive WT compared with patients with triphasic WT. Only miR-143-3p was differentially expressed between triphasic and blastemal. Furthermore, miR-320-a discriminated regressive from blastemal WT (Fig. 3).

3.5. Overall specificity and sensitivity (meta-analysis)

Analytical study could be performed for two studies included in our systematic review [50,51], comprising a total of 70 patients (38 with WT, 32 Healthy controls). The analysis of forest plots (Fig. 4) showed very high overall sensitivity and specificity of circulating miRNAs as biomarkers for discriminating between WT and controls. The pooled sensitivity was 0.67 (95%CI: 0.62; 0.72, $I^2\% = 0\%$) and the pooled specificity was 0.95 (95%CI: 0.92; 0.97, $I^2\% = 0\%$), indicating a very low heterogeneity among these studies. MiR-130b-3p had the highest overall sensitivity (88% [71%–96%]) and specificity (100% [74%–100%]) in differentiating 32 WT from 12 healthy controls, while miR-129-5p had the highest sensitivity (90% [68%–99%]) and specificity (100% [54%–100%]) in distinguishing 6 WT from 20 healthy controls. Point estimates of diagnostic accuracy of the remaining miRNAs ranged from 17% to 100% for sensitivity and from 65% to 100% for specificity across the two studies. The forest plot of AUC values is shown in Supplementary Figure B9, and summary receiver operating characteristic curves (SROCs) of the pooled diagnostic accuracy value of miRNAs is represented in Fig. 5. These results, when combined, reported that the summary AUC value of serum miRNAs for discriminating between WT patients and controls was 77% (CI95: [73%–81%], 95%PI (Prediction Interval) [51.6%, 91.2%]).

In order to test the diagnostic potential of the different miRNA types (oncomirs or suppressor miRs) in both studies, we carried out subgroup analyses and we summarised the statistics in Supplementary Table B3.

3.6. Functional role of the most reported dysregulated miRNAs in WT

Most of the circulating miRNAs found here to be differentially expressed in WT have previously been reported to be related to cancer, and their mechanism of regulation and their target genes has being studied. Supplementary Table B4 summarises dysregulated miRNAs in WT with known function.

4. Discussion

Identifying circulating biomarkers of Wilms tumour (WT) remains a major unmet clinical need. Our meta-analysis and further detailed analyses of two existing studies with available data has revealed that circulating miRNA expression profiles may segregate WT patients from healthy controls [50,51]. Furthermore, such expression profiles are correlated with metastasis, WT histological subtype and stage [50,52]. Whilst most researchers have focused on examining miRNA signatures in tissue, there is growing evidence that circulating miRNAs isolated from body fluids (such as serum, plasma, or blood) can be used as informative markers for non-invasive WT diagnosis and prognosis.

In the present study, which is the first systematic review and meta-analysis on circulating miRNAs in WT, we collated all the original published studies and provided a detailed list of all significantly dysregulated circulating miRNAs in WT. Furthermore, we assessed the relationship between miRNA profiles and WT progression and histological subtype, and evaluated the diagnostic potential of the most significantly dysregulated miRNAs.

In summary, the expression levels of several circulating miRNAs were repeatedly found to be associated with WT. Of these, half the miRNAs were up-regulated in 172 WT patients and could distinguish

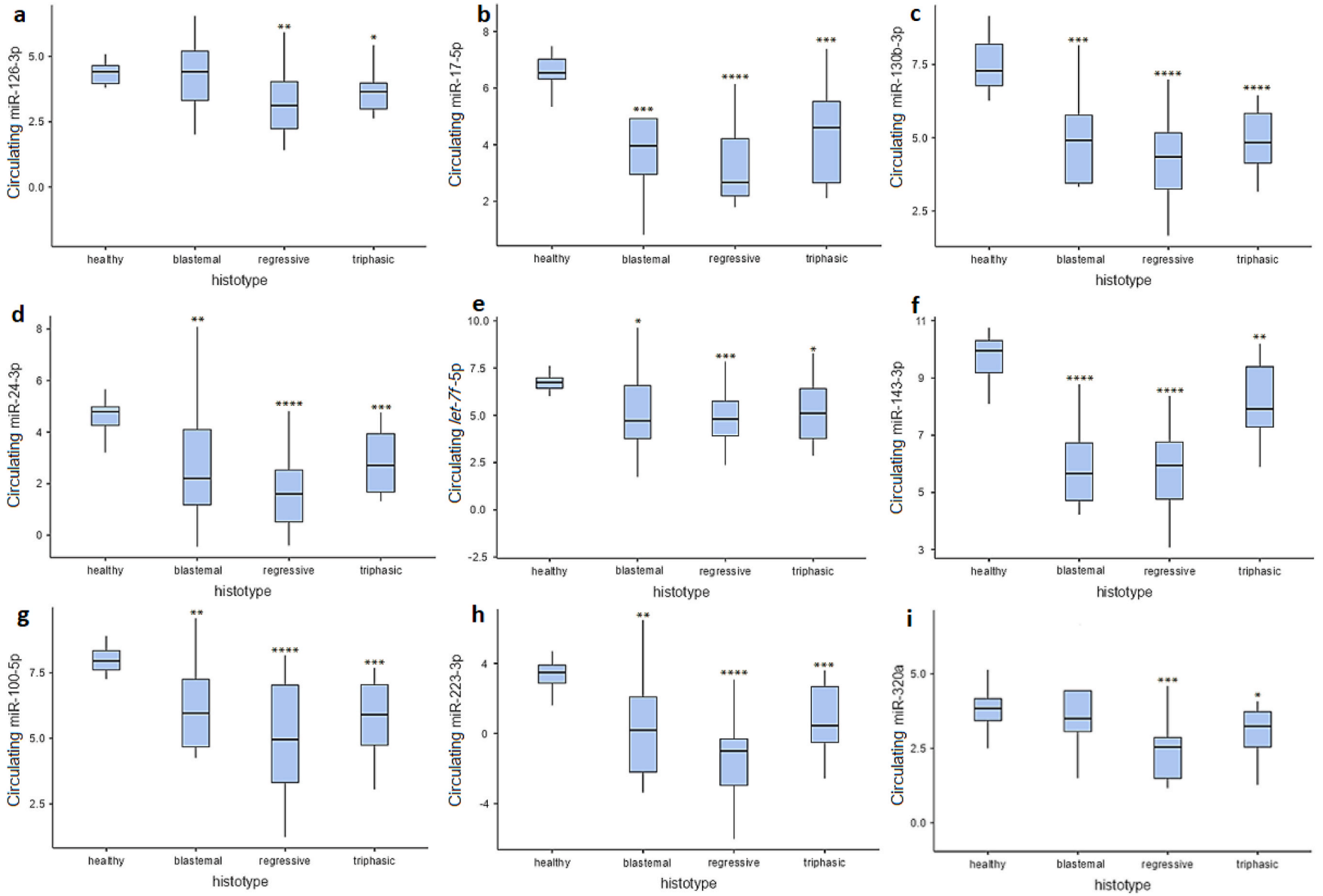

Fig. 3. Circulating miRNA expression in different WT subtypes. Box-whisker plots for expression of **a**. miR-126-3p, **b**. miR-17-5p, **c**. miR-130b-3p, **d**. miR-24-3p, **e**. let-7f-5p, **f**. miR-143-3p, **g**. miR-100-5p, **h**. miR-223-3p and **i**. miR-320a in serum of patients with triphasic, regressive and blastemal WT, and healthy controls. For each group, the boxes indicate the second and third quartile of miRNA expression, the whiskers show minimum and maximum value. The horizontal black lines indicate the median expression of the miRNA in the group. Lower ΔCt values indicate higher expression. Significant expression differences in each WT subtype compared to controls are marked with asterisks (levels for *p*-value in linear regression vs. healthy): *<0.5, **≤0.05, ***≤0.01, ****≤0.001).

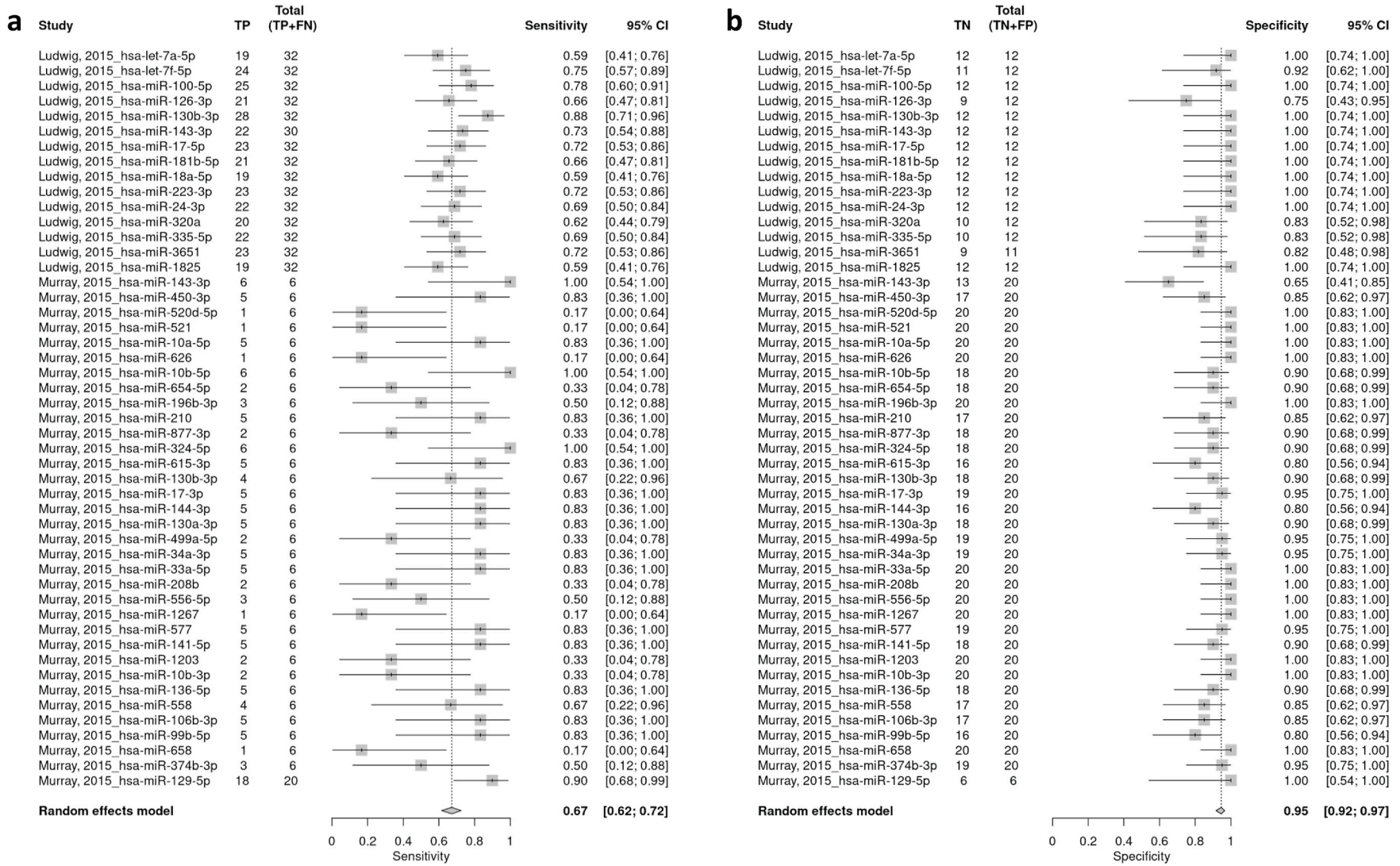

Fig. 4. Forest plots of diagnostic value index of altered miRNA expression in Wilms tumour (WT). **a.** Sensitivity of altered miRNA expression in diagnosis of WT. **b.** Specificity of altered miRNA expression in diagnosis of WT.

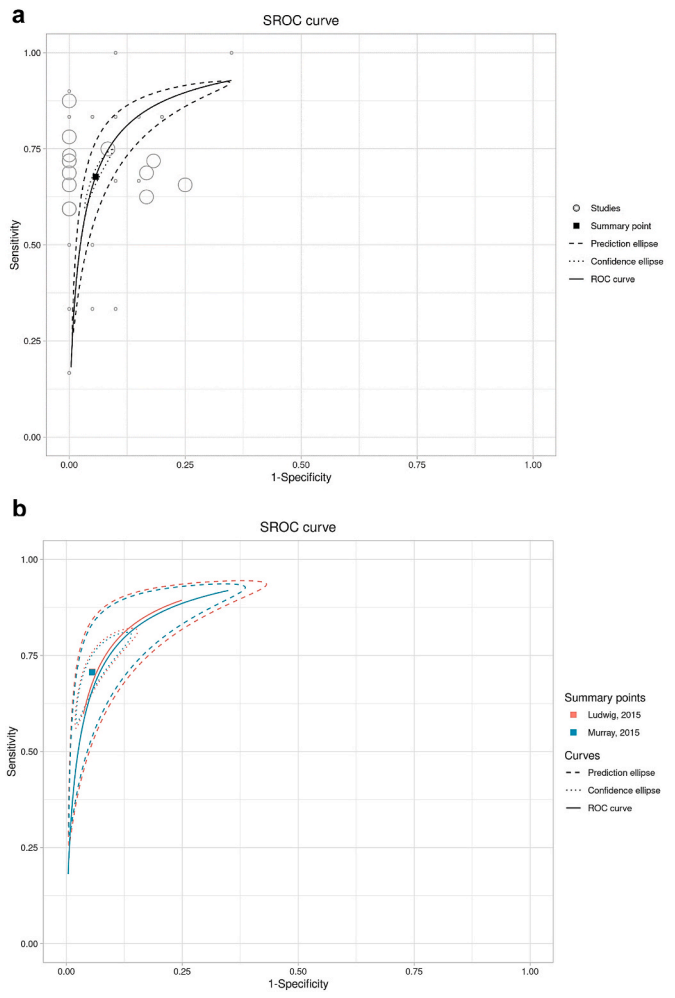

Fig. 5. Summary receiver operator characteristic (SROC) of altered miRNA expression in WT. **a.** SROC of altered miRNA expression in WT **b.** SROC of altered miRNA expression in WT classified by study.

them from 108 controls (87 healthy controls and 21 patients with other diseases) [49–53].

Whilst a signature of 301 circulating miRNAs could distinguish WT patients from controls, it did not appear to accurately reflect treatment effects; there was no difference in miRNA signature prior to, and after, preoperative chemotherapy [49]. In another study however, miR-155-5p was down-regulated in both blood and tissue from WT patients who did not receive preoperative chemotherapy but was up-regulated in tissues from WT patients who had received it [53]. MiR-155-5p is mainly defined as an oncomir in several cancers, such as non-small cell lung cancer. However, it is suggested that miR-155-5p functions as a tumour suppressor in WT through inactivating the PI3K/AKT/mTOR signalling pathway by directly targeting IGF2 [53]. Either way, miRNAs that are downregulated in the blood at diagnosis are not typically ideal candidates for biomarkers; optimal biomarkers are those that are elevated at diagnosis, with levels subsequently falling as expected with delivery of successful definitive treatment.

Importantly, circulating WT-associated miRNA signatures appear to be associated with histological subtype and tumour stage [61]. In this regard, *Luo* et al. demonstrated that miR-155-5p levels were correlated with tumour-node-metastasis (TNM) stage and lymphatic metastasis. These data suggest that miR-155-5p might be involved in WT development and progression [53]. Furthermore, *An* et al. found that higher circulating miR-190b levels were correlated with unfavourable histology, III-IV NWTS-5 stage and lymphatic metastasis in WT [52]. Moreover, *Ludwig* et al. demonstrated that specific miRNAs were differentially expressed in different WT histological subtypes. For example, circulating miR-143-3p, -223-3p, and −3651 were the most overexpressed miRNAs in regressive compared with triphasic, WTs. In blastemal WT, circulating miR-143-3p was significantly over-expressed compared with triphasic WT and miR-320a was significantly down-regulated compared with regressive WT [50]. In addition to circulating miRNAs that are diagnostic for WT, and following further confirmatory studies, such apparently WT subtype-specific miRNAs may play a future role in facilitating selection of optimal post-nephrectomy treatment, including chemotherapy schedules.

Another important aspect to consider for biomarker discovery work is potential differential diagnoses. For WT, this includes the clinical distinction from neuroblastoma (NB), another childhood cancer most often presenting with an abdominal mass. In fact, due to their anatomical proximity, it may be clinically and radiologically challenging to differentiate between them; indeed, some NB may be intra-renal [22,23]. Hence, it is not only crucial to discriminate WT from healthy controls, but also from patients with NB, to confirm the specificity of circulating WT-associated miRNA candidates, in order to arrive at an accurate diagnosis in a timely and optimal fashion. Indeed, *Murray* et al. studied miRNA signatures in both groups and demonstrated that circulating miR-143-3p (up-regulated in WT) and miR-129-5p (up-regulated in NB) discriminated patients with WT from those with NB [51].

MiRNAs were also found to be predictors of chemoresponsiveness in WT. Watson et al. identified 29 differentially expressed miRNAs between post-treatment intermediate risk and high risk groups. Some of these miRNAs, such as miR-17, miR-106b, miR-20a, and miR-223, which were up-regulated in post-treatment high-risk cases were also mentioned in this systematic review [62]. Moreover, in vitro studies, have investigated the inhibitory effects of miRNAs on WT proliferation and metastasis [39]. For example, Liu et al. found that MiR-144-3p inhibits the proliferation and metastasis of WT by regulating Girdin [63], while Shen et al. demonstrated that silencing of lncRNA MEG8 represses the viability, migration, and invasion of WT cells through mediating miR-23a-3p/CRK Axis [64].

This retrospective meta-analysis and review has a number of limitations, mostly beyond our control. These include the limited number of studies on the topic, and unreported/non-available data from these studies. Such data include lack of specificity and sensitivity of individual miRNAs for a WT diagnosis, non-availability of miRNA expression profile data for each individual patient, required to calculate sensitivity and specificity, and lack of information regarding histological subtype of WT included, preventing a more detailed subgroup analysis in the meta-analysis. Furthermore, healthy adults were included as a control group in one study [49], potentially affecting reliability of results and interpretation, and use of different systems for miRNA nomenclature over time. To overcome this latter limitation, we have updated all the miRNAs listed in this review to the most recent version of miRBase, the formal online miRNA registry. The other limitations described here are beyond our control for a retrospective review, but in future we advocate adoption of the standard systems for reporting biomarker studies, for example the REMARK guidelines for tumour marker prognostic studies [65].

5. Conclusions

Through our review, we have identified a key set of circulating miRNAs associated with WT development and progression, and demonstrated that circulating miRNAs can currently be detected in 67% of WT cases and have the potential to differentiate between WT and healthy controls in 95% of cases.

According to the meta-analysis, we recommend prioritizing research on specific circulating miRNAs in WT, based on the criteria of repeated detection, potential in differential diagnosis, number of patients studied, as well as specificity and sensitivity. These miRNAs include miR-130b-

3p, miR-450-3p, miR-100-5p, Let-7f-5p, and miR-17-5p, which have been frequently detected in the larger patient group, and consistently have 100% specificity and 88%, 83% 78%, 75%, and 72% sensitivity respectively. As well as miR-129-5p and miR-143-3p, which have been consistently detected and have potential in distinguishing between WT and NB with a sensitivity of 90%, and 73% respectively and a specificity of 100%.

These data are promising, but ultimately, more studies are necessary to study the expression of the most dysregulated miRNAs and evaluate their correlation with stage and progression in a larger cohort of WT patients. Moreover, such biomarker work needs to ensure that such circulating miRNA candidates can reliably differentiate WT form other lesions, including other malignant abdominal masses such as NB, but also other intra-renal lesions such as clear cell sarcoma of the kidney, rhabdoid tumour or mesoblastic nephroma. With such studies, patients will likely benefit from less invasive diagnosis and accurate disease-monitoring, which in turn will ultimately improve patient outcomes.

Data accessibility

All data collected, generated, or analysed during this study are included in this published article, or available as supplementary data.

Ethics approval and consent to participate

Ethics approval was granted for each individual study included in this review.

Consent for publication

Not applicable.

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CRediT authorship contribution statement

Sara Benlhachemi: Conceptualization, Formal analysis, Data curation, Methodology, Writing – original draft. **Redouane Abouqal:** Validation, Formal analysis, Methodology, Supervision. **Nicholas Coleman:** Writing – review & editing. **Matthew Jonathan Murray:** Validation, Writing – review & editing. **Elmostafa El fahime:** Validation, Supervision, Writing – review & editing. **Mohammed Khattab:** Validation.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ncrna.2023.05.007.

Abbreviations list

AUC	area-under-the-curve
95CI	95% Confidence interval
CT scan	computed tomography scan
Down	Down-regulated
F	Female
FH	Favourable histology
FPR	False positive rate
HC	Healthy controls
HCD	Histopathologically confirmed diagnosis
Hsa-miR	human microRNA
LCI95	95% Lower confidence interval
LR-	Negative likelihood ratio
LR+	Positive likelihood ratio
M	Male
MET	Mesenchymal epithelial transition
mo.	month
MRI	magnetic resonance imaging
mRNA	messenger RNA
MYCN-NB =	MYCN-amplified high-risk neuroblastoma
NA	Not applicable/Not available
NB =	non-MYCN-amplified low-risk Neuroblastoma
NWTS	National Wilms tumour study – The national Committee for Research of nephroblastoma
NWTS-5	National Wilms Tumour Study grade classification
PC	Preoperative Chemotherapy
PRISMA	The Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROSPERO	International prospective register of systematic reviews
QUADAS	Quality Assessment Tool for Diagnostic Accuracy Studies
RC	Renal Carcinoma
RNA	Ribonucleic acid
RT-qPCR	reverse transcription quantitative real-time polymerase chain reaction
Se	sensitivity
SIOP	International Society of Paediatric Oncology
Sp:	specificity
SROC:	Summary receiver operator characteristic
TNM	Tumour-node-metastasis
UCI95	95% Upper Confidence Interval
UH	Unfavourable histology
UNP	uronephrosis
Up:	Up-regulated
UTR	Untranslated region
WT	Wilms tumour
WT1	tumour suppressor gene 1
yr.	years

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