INNOVATIVE APPROACHES IN PEDIATRIC SURGICAL ONCOLOGY

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PUBLISHED IN: Frontiers in Pediatrics and Frontiers in Surgery

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88976-969-8
DOI 10.3389/978-2-88976-969-8

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INNOVATIVE APPROACHES IN PEDIATRIC SURGICAL ONCOLOGY

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Citation: Pio, L., Sarnacki, S., eds. (2022). Innovative Approaches in Pediatric Surgical Oncology. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88976-969-8

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frontiers | Frontiers in Pediatrics

TYPE Editorial
PUBLISHED 04 August 2022
DOI 10.3389/fped.2022.989822

OPEN ACCESS

EDITED AND REVIEWED BY
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SPECIALTY SECTION
This article was submitted to Pediatric Surgery, a section of the journal Frontiers in Pediatrics

RECEIVED 08 July 2022
ACCEPTED 20 July 2022
PUBLISHED 04 August 2022

CITATION
Pio L and Sarnacki S (2022) Editorial: Innovative approaches in pediatric surgical oncology.
Front. Pediatr. 10:989822.
doi: 10.3389/fped.2022.989822

Editorial: Innovative approaches in pediatric surgical oncology

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KEYWORDS

pediatric tumors, pediatric oncology, pediatric surgical oncology, pediatric cancer, translational surgery

Editorial on the Research Topic
Innovative approaches in pediatric surgical oncology

Pediatric surgical oncology (PSO) represents one of the most challenging sub-specialties in the field of pediatric surgery, due to potentially life-threating procedures and the wide field of presentation and location of children's solid tumors.

In this Research Topic of Frontiers in Pediatrics we selected different studies covering both technical and strategical innovations which are necessary for a modern surgical management of pediatric solid tumors.

The first requirement for the optimal treatment of pediatric cancer is a well-trained surgeon in pediatric oncology; Losty in "*Training in Pediatric Surgical Oncology*" highlights the current required skills and related challenges in a worldwide setting of centers with a relative low case-load due to the rarity of pediatric malignancy.

To provide a structured surgical training according to the different tumors' location, vascular, thoracic and urology rotations are encouraged in the 1st years of residency, followed by a specific training in high volume centers with established international fellowship program in PSO (1).

Societies with special commitment in PSO (such as the International Society of Pediatric Surgical Oncology-IPSO) are promoting international fellowship programs and the current trend of improved centralization will help young surgeons to be trained in high-volume centers (2).

Pediatric tumors Show a heterogeneity in their clinical presentation, Persano et al. in "*Burned-Out Testicular Tumors in Adolescents: Clinical Aspects and Outcome*" describe one of these challenging situations. The authors define the clinical presentation of the burned-out testicular tumors as a well-distinct entity, with histology peculiarities in absence of local testicular symptoms, underlying their poor prognosis.

Other than clinical heterogeneity, surgeons should know the potential pitfalls of radiology presentation pf pediatric tumors at diagnosis. Carvalho et al. in "*Diagnostic Errors in Wilms' Tumors: Learning From Our Mistakes*" shares the experience and diagnosis complications in one of the largest Pediatric Oncology Institution of America Latina.

Authors emphasize the importance of a multidisciplinary team and the integration of biology in addition with radiologic findings to reach a high diagnostic accuracy for Wilms tumors.

Multidisciplinary strategy has a fundamental role in PSO as described by Theilen et al. in "*Multidisciplinary Treatment Strategies for Wilms Tumor: Recent Advances, Technical Innovations and Future Directions*," reviewing the role of imaging technology and genetics in clinical practice.

Once the diagnosis is confirmed and neo-adjuvant chemotherapy is initiated, several complications can be observed, some of them requiring surgical evaluation as described in "*Hemorrhage During Induction Chemotherapy in Neuroblastoma: Additional Risk Factors in High-Risk Patients*" by Voglino et al., reporting the effectiveness of conservative treatment for localized hemoperitoneum and thoracic drain placement for hemothorax.

Studies of large cohort of children with specific tumors included in national or international protocols can modify the surgical strategy as described by Machavoine et al. in "*Locoregional Control and Survival in Children, Adolescents, and Young Adults With Localized Head and Neck Alveolar Rhabdomyosarcoma—The French Experience*," enhancing the role of lymph node surgery and secondary resection of the primary tumor and their positive influence on event-free survival (EFS) for alveolar rhabdomyosarcoma.

Qi and Zhan in "*Roles of Surgery in the Treatment of Patients With High-Risk Neuroblastoma in the Children Oncology Group Study: A Systematic Review and Meta-Analysis*" discuss another important Research Topic about the intraoperative surgical strategy such as resection's completeness. The meta-analysis they provided confirms the recent studies regarding the positive influence of the extent of resection on EFS in High Risk Neuroblastoma (3, 4).

Surgical technique advances allow to change and extend indications as reported by Garnier et al. in "*Case Report: Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy Application in Intraperitoneally Disseminated Inflammatory Myofibroblastic Tumor and in the Youngest Patient in the World: New Indication and Modification of Technique*."

Authors describe an extension of the scope of indications of intraperitoneal chemotherapy (usually reserved for children>2 years old and desmoplastic tumors) to a myofibroblastic tumor with a peritoneal carcinomatosis, with excellent oncology outcome at low term follow up after 12 months. A modified hyperthermic intraoperative cytoreductive surgery is reported, with a reduced intraperitoneal normothermic infusion(30 min) and a modified dosage of doxorubicin.

Over the years, minimally invasive surgery (MIS) gained popularity in PSO (5); Ngo et al. in "*Case Report: Transoral Endoscopic Thyroidectomy via Vestibular Approach in Pediatric Thyroid Cancer*" provide an example of the increasing tendency to extend the application of MIS to several types of tumors locations, reporting for the first time an innovative approach in pediatric population providing didactic intraoperative views and trocar positioning description. Authors discuss their novel approach providing a comprehensive technical description and their surgical results.

Vatta et al. in "*Robotics-Assisted Pediatric Oncology Surgery—A Preliminary Single-Center Report and a Systematic Review of Published Studies*" shows the state of the art of a recent evolution of MIS by describing the application of robotics in PSO, with promising results in recently published cohorts of pediatric patients treated for different solid tumors (6).

Pediatric surgery recently benefits from several technology advances as described by Privitera et al. in "*Above and Beyond Robotic Surgery and 3D Modeling in Pediatric Cancer Surgery*." Authors present a comprehensive review of different techniques for loco-regional intraoperative cancer treatment such as photodynamic therapy and near-infrared photoimmunotherapy, describing their promising results in adult surgical oncology, with a potential application in children.

These innovative local treatments are currently available for adults, but not for children due to the much lower incidence of solid tumors in pediatric population and the subsequent difficulties to design clinical trials based on evidence medicine.

In addition, this review describes the theoretical basis of optical imaging improvement in surgery, including radio, spectroscopy and fluorescent-guided surgery (FGS).

FGS is one of the most promising technology advances in PSO and Abdelhafeez et al. with their original article "*Indocyanine Green–Guided Pediatric Tumor Resection: Approach, Utility, and Challenges*" describe the currently largest cohort of pediatric patients in literature who benefit from FGS. Authors describe the feasibility of FGS and the current limitations of this technique represented by tissue-penetration and background noise of adjacent organs with current fluorophore probes.

Surgeons must be aware of tumor's risk of local relapse after surgery, Pelizzo et al. in "*Proliferation Pattern of Pediatric Tumor-Derived Mesenchymal Stromal Cells and Role in Cancer Dormancy: A Perspective of Study for Surgical Strategy*" study investigate the role of mesenchymal stromal cells as a potential risk factor for local relapse, defining their role in cancer cells dormancy, in addition to their well-known role on drug sensitivity and the subsequent tumor progression and metastasis.

Sundquist et al. provide an example of multidisciplinary scientific collaboration in PSO with the study protocol presentation "*A Phase II Trial of a Personalized, Dose-Intense Administration Schedule of 177Lutetium-DOTATATE in Children With Primary Refractory or Relapsed High-Risk Neuroblastoma–LuDO-N*." This study protocol involves different subspecialities (nuclear radiologist, oncologist, surgeons, and pathologist) in a common project with the

aim of studying an alternative molecular radiotherapy for the most challenging Neuroblastoma presentations (refractory and relapsed high-risk neuroblastoma), in order to improve event free and overall survival rate in high-risk patient population (7).

Survivorship improvement of childhood cancers highlights the functional sequelae impact of chemotherapy and surgery (8).

The results of "*Special Considerations for Tympanoplasty Type I in the Oncological Pediatric Population: A Case-Control Study*" by Richard et al. raise the problem of chronic tympanic membrane perforation in children who survive after cancer treatment, providing surgical optimized strategies in terms of timing and technique.

Young You et al. in "*Considerations for Balance Between Fundamental Treatment and Improvement of Quality of Life of Pediatric Thyroid Cancer Patient: Comparative Analysis With Adult Using Propensity Score Matching*" further study the postoperative quality of life in children with thyroid malignancy, reporting promising outcomes and suggesting to limit total thyroidectomy when is safe and feasible for children, allowing a best functional outcomes.

This Research Topic and all these manuscripts illustrate the current advances on surgical management of pediatric malignancies, highlighting the role of a multidisciplinary approach and the emerging impact and effectiveness of translational surgery, a surgical discipline that serves as bridge across basic science, technology innovations, different medical specialty, and surgery.

Author contributions

Both authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Acknowledgments

LP is a student from the FIRE Ph.D. program funded by the Bettencourt Schueller Foundation and the EURIP Graduate Program (ANR-17-EURE-0012). The authors would like to thank Andrea Pevere for editing the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Fernandez-Pineda I, Sanders D, Rao BN, Shochat SJ, Davidoff AM. Outcomes of a pediatric surgical oncology fellowship in a pediatric cancer institution. *Pediatr Blood Cancer*. (2017) 64:pbc.26618.doi: 10.1002/pbc.26618

2. Wijnen M, Hulscher J. Centralization of pediatric surgical care in the Netherlands: lessons learned. *J Pediatr Surg*. (2021) 67:178–81. doi: 10.1016/j.jpedsurg.2021.10.023

3. von Allmen D, Davidoff AM, London WB, Van Ryn C, Haas-Kogan DA, Kreissman SG, et al. Impact of extent of resection on local control and survival in patients from the COG A3973 study with high-risk neuroblastoma. *J Clin Oncol*. 35:208–16. doi: 10.1200/JCO.2016.67.2642

4. Mullassery D, Farrelly P, Losty PD. Does aggressive surgical resection improve survival in advanced stage 3 and 4 neuroblastoma? A systematic review and meta-analysis. *Pediatr Hematol Oncol*. (2014) 31:703–16. doi: 10.3109/08880018.2014.947009

5. Gabra HO, Irtan S, Cross K, Lobos P, Froeba-Pohl A, Pio L, et al. Minimally invasive surgery for neuroblastic tumours: a SIOPEN multicentre study: proposal for guidelines. *Eur J Surg Oncol*. (2022) 48:283–91. doi: 10.1016/j.ejso.2021.08.013

6. Blanc T, Meignan P, Vinit N, Ballouhey Q, Pio L, Capito C, et al. Robotic surgery in pediatric oncology: lessons learned from the first 100 tumors-a nationwide experience. *Ann Surg Oncol*. (2022) 29:1315–26. doi: 10.1245/s10434-021-10777-6

7. Ladenstein R, Pötschger U, Pearson ADJ, Brock P, Luksch R, Castel V, et al. Busulfan and melphalan versus carboplatin, etoposide, and melphalan as high-dose chemotherapy for high-risk neuroblastoma (Hr-Nbl1/Siopen): an international, randomised, multi-arm, open-label, phase 3 trial. *Lancet Oncol*. (2017) 18:500–14. doi: 10.1016/S1470-2045(17)30070-0

8. Bhakta N, Liu Q, Ness KK, Baassiri M, Eissa H, Yeo F, et al. The cumulative burden of surviving childhood cancer: an initial report from the St Jude Lifetime Cohort Study (SJLIFE). *Lancet*. (2017) 390:2569–82. doi: 10.1016/S0140-6736(17)31610-0

frontiers in Pediatrics

ORIGINAL RESEARCH
published: 25 August 2021
doi: 10.3389/fped.2021.688021

Burned-Out Testicular Tumors in Adolescents: Clinical Aspects and Outcome

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OPEN ACCESS

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Specialty section:
This article was submitted to Pediatric Surgery, a section of the journal Frontiers in Pediatrics

Received: 30 March 2021
Accepted: 22 July 2021
Published: 25 August 2021

Citation:
Persano G, Crocoli A, De Pasquale MD, Cozza R, Alaggio R, Diomedi Camassei F, Beati F, Di Paolo P, Martucci C and Inserra A (2021) Burned-Out Testicular Tumors in Adolescents: Clinical Aspects and Outcome. Front. Pediatr. 9:688021. doi: 10.3389/fped.2021.688021

Purpose: Testicular germ cell tumors are the fourth most common neoplasm in adolescents, accounting for 8% of all tumors in the age group 15–19 years. On rare instances, the primary testicular lesion is not clinically or radiologically evident while nodal or visceral metastases represent the clinical manifestations of the disease. This phenomenon is described as "burned-out testicular tumor." In this paper, the authors report a single-institution experience with burned-out testicular tumors in adolescents and discuss their clinical implications.

Patients and Methods: All the patients diagnosed with metastatic testicular germ cell tumors at Bambino Gesù Children Hospital between January 1, 2010, and June 30, 2020, were included in the study. Patients were categorized into two groups: "primary testicular" and "burned out." All the patients were staged and treated according to the AIEOP–TCGM 2004 protocol.

Results: Eleven patients were classified as "primary testicular," and five patients were classified as "burned out." "Burned-out" tumors were associated with the presence of systemic symptoms compared to "primary testicular" tumors (80 vs. 0%; $p = 0.0027$) and higher aFP, hCG, and LDH levels ($p < 0.00001$). The "burned-out" population had a statistically significant higher incidence of relevant toxicity than the "primary testicular" population (80 vs. 18%; $p = 0.0357$) and a worse outcome in terms of both mean overall survival (15 vs. 43 months; $p = 0.0299$) and mean event-free survival (12 vs. 38 months; $p = 0.0164$).

Conclusion: "Burned-out" testicular tumors seem to be a well-distinct clinical entity with a high treatment-related toxicity and poor prognosis. Further studies are needed to clarify the "burned-out phenomenon" and to identify more effective therapeutic strategies for these patients.

Keywords: germ cell tumor, children, burned out germ cell tumor, adolescents, testis

INTRODUCTION

Testicular germ cell tumors are the fourth most common neoplasm in adolescents, accounting for 8% of all tumors in the age group 15–19 years (1) with an estimated prevalence in Europe of 24.5 cases per million inhabitants (2).

The prognosis of germ cell tumors is generally excellent, even though a subset of patients, i.e., patients older than 11 years, with elevated alfa-fetoprotein levels at diagnosis, extragonadal primary tumors, non-germinomatous tumors, and stage III and IV disease, have a worse outcome and therefore need an intensified treatment (3–6).

On rare instances, the primary testicular lesion is not clinically or radiologically evident while nodal or visceral metastases remain viable and represent the clinical manifestations of the disease (7). In these patients, the only histological evidence of a testicular origin of the tumor is a characteristic pattern of testicular scarring with hematoxylin staining bodies that contain calcium and DNA, often associated with peripheral atrophy and intratubular malignant germ cells (8). This phenomenon is described as "burned-out testicular tumor" or "spontaneously regressed testicular tumor" (8–11). Burned-out testicular tumors have been extensively described in the literature from a histological point of view; on the other hand, the clinical aspects of testicular burned-out tumors have not been fully characterized to date.

In the present paper, the authors report a single-institution experience with burned-out testicular tumors in adolescents and discuss their clinical implications.

MATERIALS AND METHODS

All the patients diagnosed with metastatic testicular germ cell tumors at Bambino Gesù Children Hospital between January 1, 2010, and June 30, 2020, were included in the study.

The clinical notes of all the patients with stage III and IV tumors were retrospectively reviewed and categorized in two broad groups: "primary testicular" patients, i.e., patients who presented with testicular pain or testicular enlargement at initial diagnosis and sonographic evidence of testicular mass, and "burned-out" patients, i.e., patients who had no testicular symptoms at initial presentation and non-specific findings at ultrasound, such as microcalcification (**Figure 1**) and parenchymal heterogeneity in one or both testes. At the time of admission, all the patients were staged and treated according to the AIEOP–TCGM 2004 protocol (5). Hepatic, renal, pulmonary, and cardiac function were evaluated before the initiation of treatment. All the patients received first-line treatment with PEB chemotherapy regimen (Cisplatin 25 mg/m^2/day iv for 4 days, Etoposide 100 mg/m^2/day iv for 4 days, Bleomycin 15 UI/m^2 on day 2), three courses for stage III disease, and four courses for stage IV disease; indications for resection of secondary lesions after chemotherapy were considered for individual cases.

Seven variables have been examined: age at diagnosis, clinical presentation, serum markers level, stage, primary histology, toxicity, and outcome. Follow-up time was calculated from the time of diagnosis until the time of last follow-up visit with a cutoff in October 2020. Statistical analyses were performed using Prism 9.0.0.121 (GraphPad Software, Inc., San Diego, CA). A value of $p < 0.05$ was considered statistically significant for each analysis.

Age at Diagnosis

Mean age at diagnosis has been calculated separately in the two groups and data have been compared using Student's *t*-test.

Clinical Presentation

The presence at initial diagnosis of "mass effect" symptoms, defined as extra-testicular palpable mass or symptoms related to compression due to metastatic mass, or "systemic" symptoms, defined as fever $> 38°C$, weight loss, and deep venous thrombosis, was searched for in the clinical notes of each patient in both groups. Data were compared using Fisher's test.

Serum Markers Level

Alpha-fetoprotein (aFP), human chorionic gonadotropin (hCG), and lactate dehydrogenase (LDH) levels were measured at initial presentation in all the patients. Mean and standard deviation for each serum marker were calculated separately in the two groups and data were compared using Student's *t*-test.

Stage

In each group, patients were divided into two subgroups according to the stage of the disease, i.e., stage III and IV. For stage IV subgroup, patients with extra-pulmonary visceral metastases were analyzed separately on the basis of the evidence of a distinct worse prognosis in adult population (12). Data were compared using Fisher's test.

Primary Histology

All the patients had their diagnosis confirmed by histological examination of the affected testis or a specimen from the metastatic mass. Patients were classified according to the AIEOP–TCGM 2004 protocol as mature teratoma, immature teratoma, yolk sac tumor, choriocarcinoma, embryonal carcinoma, seminoma, or mixed histology (5). Data were compared using Fisher's test.

Toxicity

Patients were defined as having experienced "relevant toxicity" during treatment if they had at least one episode of grade 3 or higher toxicity in at least one apparatus as defined in the "Common Terminology Criteria for Adverse Events (CTCAE) version 5.0" (13). Data were compared using Fisher's test.

Outcome

Patient's outcome has been registered at the time of last follow-up visit as "remission" if the patient had no clinical, radiological, or serological evidence of disease; "progression" if the patient had clinical, radiological, or serological signs of disease; or "death" if the patient had passed away. Event-free survival (EFS) was calculated from the date of initial treatment to the date of whichever came first among progression, death, and last follow-up visit. Overall survival (OS) was measured from the date of initial diagnosis to the date of death or last follow-up visit. Survival was analyzed with Kaplan–Meier plots and the log-rank test. A value of $p < 0.05$ was considered statistically significant.

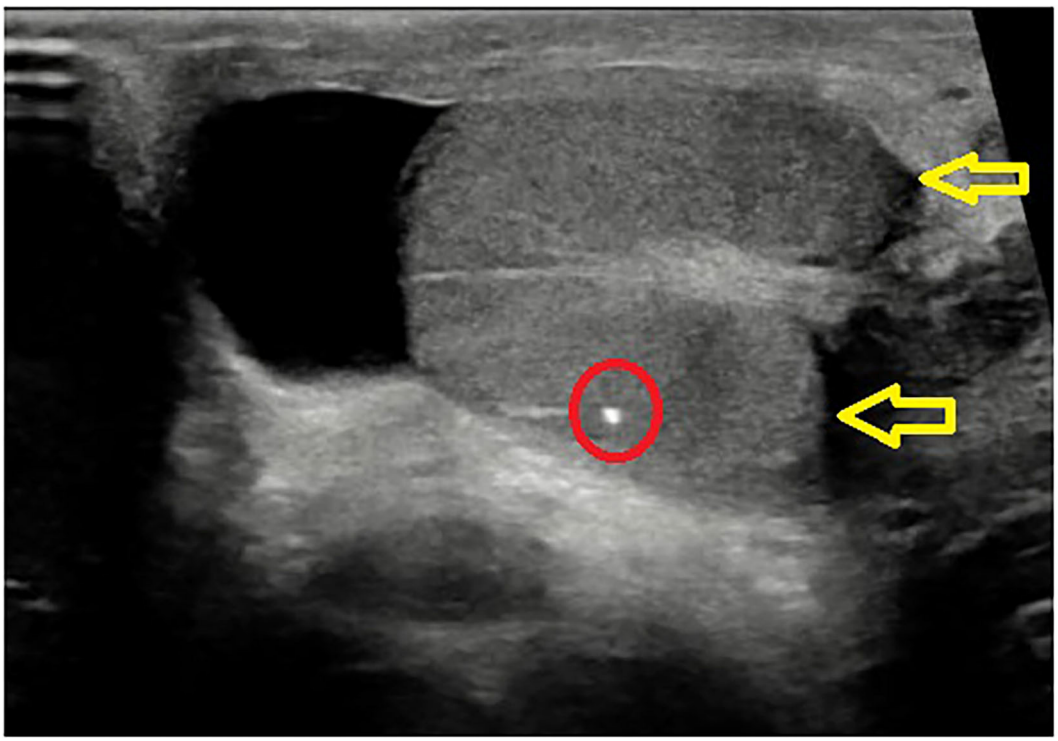

FIGURE 1 | Burned out echographic appearance: scrotal ultrasound showing testicle with microcalcifications and hypoechogenic nodule.

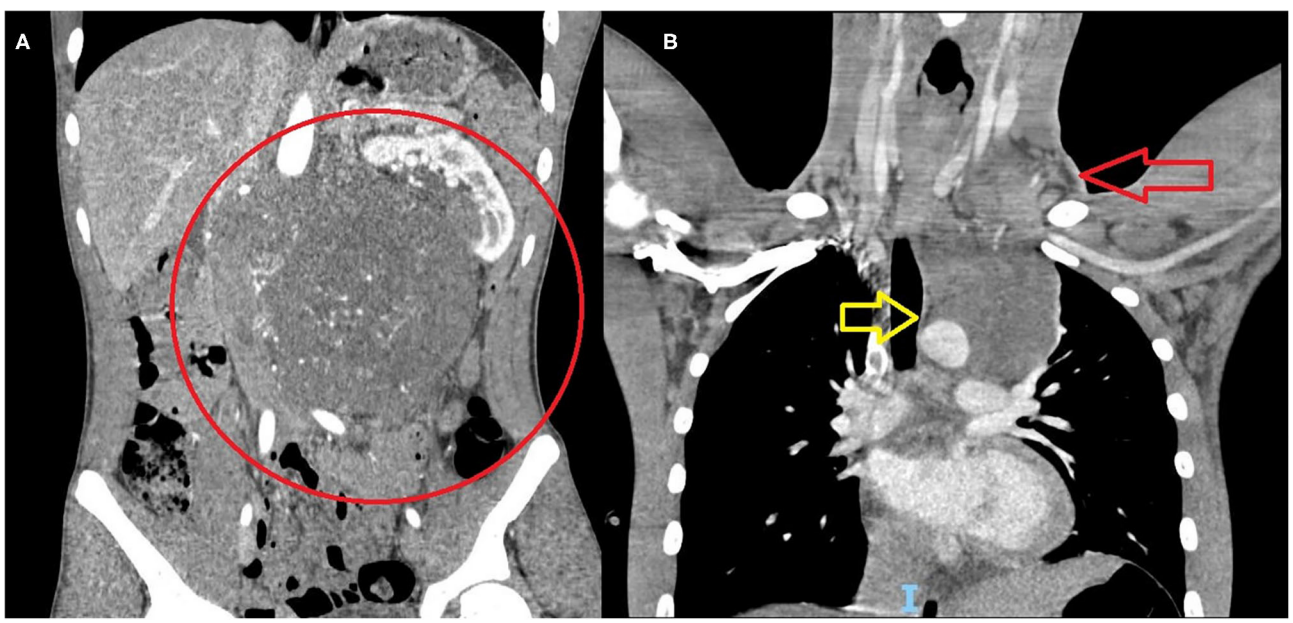

FIGURE 2 | Massive metastatic involvement of retroperitoneal **(A)** and mediastinal **(B)** lymph-nodes.

RESULTS

In the examined period, 16 patients were diagnosed with stage III and IV testicular germ cell tumors at our institution and all were included in the present study.

Eleven patients were classified as "primary testicular:" four patients (36%) had right testicular tumor and seven (64%) had tumor in the left testis. Five patients were classified as "burned out:" two patients (40%) had right-side tumor, two had left-side tumor (40%), and in one patient (20%), the location of the primitive tumor could not be determined.

None of the patients had pre-existing comorbidities.

Age at Diagnosis

"Burned-out" patients were significantly older (mean age 17 years 7 months, range 15 years 10 months to 19 years 11 months) than "primary testicular" patients (mean age 15 years, range 0 years 6 months to 24 years 0 months, $p < 0.00001$).

Clinical Presentation

None of the patients classified as "primary testicular" presented with "mass related" symptoms or "systemic" symptoms.

All the "burned-out" patients presented with symptoms related to mass effect due to metastases. Four "burned-out" patients (80%) presented with systemic symptoms (i.e., weight loss in three patients, fever $> 38^{\circ}$C and deep venous thrombosis in two patients). The association between "burned-out" tumors and systemic symptoms was statistically significant ($p = 0.0027$).

Serum Markers Level

aFP, hCG, and LDH levels were significantly higher in the "burned-out" population than in "primary testicular" patients: mean aFP 2,215 ng/ml (range 2–10,997 ng/ml) vs. 979 ng/ml (range 2.05–4,604.3 ng/ml), $p < 0.00001$; mean hCG 14,831 mIU/ml (range 2–73,564.5 mIU/ml) vs. 530 mIU/ml (range 2–1,863.5 mIU/ml), $p < 0.00001$; mean LDH 1,558 IU/L (range 372–4,052 IU/L) vs. 424 IU/L (range 238–620 IU/L), $p < 0.00001$.

Stage

In the "primary testicular" population, six patients (55%) were stage III and five patients (45%) were stage IV; one patient (9%) had extrapulmonary visceral metastases in the central nervous system.

In the "burned-out" population, one patient (20%) was stage III and four patients (80%) were stage IV; three patients (60%) had extrapulmonary visceral metastases, including liver (three patients), bones (two patients), and central nervous system (one patient) (**Figure 2**).

There was no statistically significant difference in the stage distribution between the two groups ($p = 0.3077$). A trend toward a higher incidence of extra-pulmonary visceral metastases was noted in the "burned-out" population (three patients vs. one patient in the "primary testicular" group), although such difference failed to prove statistically significant ($p = 0.0632$).

Data are summarized in **Table 1**.

Primary Histology

All the patients in both groups had non-seminomatous tumors.

In the "primary testicular" group, all the patients underwent primary orchiectomy: eight patients had mixed histology (73%), two patients had yolk sac tumor (18%), and one patient had immature teratoma (9%).

In the "burned-out" group, diagnoses were made on biopsies from metastatic sites. Two patients had choriocarcinoma (40%), two patients had embryonal carcinoma (40%), and one patient had mixed histology (20%). Four patients underwent orchiectomy after the diagnosis of metastatic germ cell tumor had been established: in all four cases, histology revealed *in situ* neoplasia associated with areas of fibrosis and interstitial lymphoplasmacytic infiltrates. One patient affected by choriocarcinoma with multiple pulmonary metastases progressively developed respiratory distress and was considered unfit to undergo surgery.

TABLE 2 | Toxicity and outcome results.

		Primary testicular	Burned-out	*p*-value
Toxicity grade 3+		2/11 (18%)	4/5 (80%)	0.0357
Outcome mean (range)	EFS	38 m (5–119 m)	12 m (2–28 m)	0.0164
	OS	43 m (13–119 m)	15 m (6–28 m)	0.0299

TABLE 1 | Patients' characteristics.

		Primary testicular	Burned-out	*p*-value
Age (mean)		17 years 7 months	15 years	< 0.00001
Systemic symptoms		0/11	4/5	0.0027
Serum markers mean (range)	aFP (ng/ml)	979 (2.05–4,604.3)	2,215 (2–10,997)	<0.00001
	hCG (mIU/ml)	530 (2–1,863.5)	14,831 (2–73,564.5)	<0.00001
	LDH (IU/L)	424 (238–620)	1,558 (372–4,052)	<0.00001
Stage				
	III	6/11 (55%)	1/5 (20%)	0.3077
	IV	5/11 (45%)	4/5 (80%)	
	Extra-pulmonary metastases	1/11 (9%)	3/5 (60%)	0.0632

A trend toward a higher incidence of choriocarcinoma and embryonal carcinoma in the "burned-out" group was noted, although it was not statistically significant ($p = 0.0833$).

Toxicity

In the "primary testicular" population, two patients (18%) experienced "relevant toxicity" during treatment; both had grade 3 anemia and thrombocytopenia (18%), one had infection (9%), and one had renal and hepatic grade 3 toxicity associated with grade 3 arterial hypertension (9%).

In the "burned-out" population, four patients (80%) had "relevant toxicity;" all of them had grade 3 anemia and thrombocytopenia (80%), three had infectious complications (60%), two had simultaneous renal and pancreatic grade 3 toxicity (40%), two had neurological complications (20%), one had a severe hypertensive crisis (20%), one had chronic pulmonary toxicity (20%), one patient with massive pulmonary metastases had acute respiratory distress and pleural hemorrhage secondary to "choriocarcinoma syndrome" (14, 15) that required oxygen supplementation and chest tube placement (20%), and one had an allergic reaction (20%).

There were no toxicity-related deaths in both groups.

The "burned-out" population had a statistically significant higher incidence of relevant toxicity than "primary testicular" population ($p = 0.0357$) (**Table 2**).

Outcome

The 11 patients in the "primary testicular" group were followed up for a mean time of 43 months (range 13 to 119 months). Five patients underwent surgery on residual secondary lesions after chemotherapy. At the last follow-up visit, 10 patients (91%) were alive and in complete clinical, radiological, and serological remission while one patient (9%) had radiological evidence of progression of disease; this patient decided to refer to a different institution for further treatment and was subsequently lost at follow-up. A second patient experienced relapse 29 months after the initial diagnosis and was successfully treated by salvage therapy with surgical resection of residual masses followed by high-dose chemotherapy and autologous stem cell transplantation. No deaths were recorded. Mean OS time for the "primary testicular" group was 43 months (range 13–119 months) and mean EFS was 38 months (range 5 to 119 months).

The five patients in the "burned-out" group were followed up for a mean time of 15 months (range 6 to 28 months). At the last follow-up, two patients (40%) affected by pure embryonal carcinoma were alive and in complete remission at 28 and 27 months, respectively; one of them underwent surgery on residual metastases after chemotherapy. One patient (20%) initially diagnosed with mixed tumor experienced clinical and radiological signs of progression of disease with negative serum markers at 3 months with histological evidence of mature teratoma, a condition described as "growing teratoma syndrome" (16); this patient underwent multiple debulking procedures of the residual lesions for symptomatic relief and was alive 7 months after initial diagnosis with progressive growing teratoma syndrome. Two patients (40%) affected by pure choriocarcinoma experienced progression of disease during treatment 2 months after the initial diagnosis; they both underwent surgery on secondary lesions for symptomatic relief and died due to progressive disease at 6 months. Mean OS time for the "burned-out" group was 15 months (range 6 to 28 months) and mean EFS was 12 months (range 2–28 months).

There was a statistically significant difference between "primary testicular" and "burned out" both in terms of OS ($p = 0.0299$) and EFS ($p = 0.0164$) as shown by the Kaplan–Meier plots (see **Figure 3**).

DISCUSSION

A burned-out testicular tumor is defined as spontaneous regression of a testicular germ cell tumor, which after metastatic spread manifests at its primary location as a scarring lesion with characteristic histological alterations (17). Such phenomenon was first described in 1927 by Prym in a patient with extragonadal choriocarcinoma (18) and has been reported both in adolescents and in adults (19). The histological features of burned-out testicular tumors were described by Azzopardi et al. in 1961; in this report, the authors described 17 adults who died due to metastatic choriocarcinoma and embryonal carcinoma and found a specific pattern of fibrous scarring associated with amorphous hematoxylin-staining deposits in dilated seminiferous tubules, mainly consisting in phospholipid, protein debris, and DNA, in some cases accompanied by small teratomatous structures and microscopic foci of seminoma (8). Subsequent studies have confirmed the characteristic microscopic appearance but have challenged the association of the "burn out" phenomenon with choriocarcinoma and embryonal carcinoma, describing "burned-out" tumors of all the histologic subtypes with a prevalence of seminoma, both pure and in association with other histotypes (10, 20).

The mechanism behind primary tumor regression has not been determined yet. One of the two main hypotheses postulate an immunological response mediated by cytotoxic T lymphocytes; the presence of a lymphoplasmacytic infiltrate and hemosiderin-containing macrophages in most histologic specimens from "burned-out" patients might support such hypothesis (10, 20). The second hypothesis postulates an ischemic response in the primary neoplasia, secondary to the blood supply deficit due to high metabolic rates and/or intermittent testicular torsion (21); such hypothesis is supported by the presence of testicular atrophy associated with scarring, reduced spermatogenesis, areas of necrosis, and coarse, large intratubular calcifications (11, 20).

Burned-out tumors clinically manifest with mass symptoms secondary to retroperitoneal, mediastinal, or supraclavicular lymph nodes or visceral metastases from germ cell tumors, in the absence of clinically apparent testicular masses (7, 22). To date, one case series has described the occurrence of weight loss and deep venous thrombosis associated with burned-out tumors (21), while two case reports have described the association with paraneoplastic neurological symptoms (i.e., ataxia and limbic encephalitis) (23, 24). Such clinical presentation and the presence of elevated LDH serum levels may contribute to initial misdiagnosis of lymphoproliferative disease in some patients (21) and provoke further delay in the correct diagnosis. Germ cell-specific serum markers, i.e., aFP and hCG, are

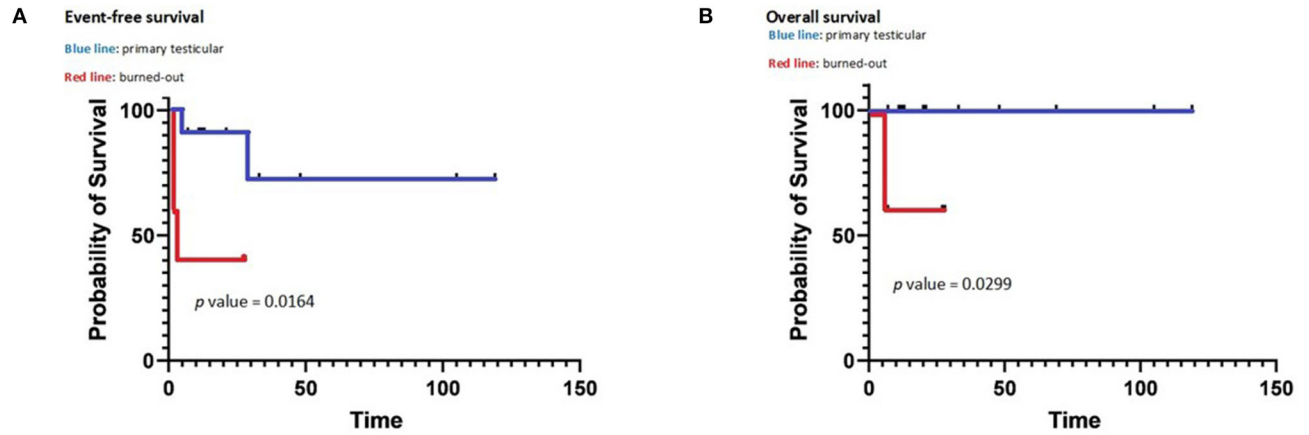

FIGURE 3 | Event-free survival **(A)** and overall survival **(B)** of primary and burned out testicular tumors.

often evaluated only after the correct diagnosis has been made on biopsy from metastatic sites and are elevated in case of non-seminomatous histology, especially when yolk sac or choriocarcinoma components are present, respectively (17).

Ultrasound scans in burned-out tumors typically show hypoechoic areas with irregular margins and heterogeneous adjacent parenchyma, diffuse microlithiasis and poor or absent vascular signals (25–27). Such findings, however, are non-specific, because also non-neoplastic lesions, such as hematomas or infarctions, may present with a similar sonographic pattern (28). One study by El Sanharawi et al. recently analyzed the vascularization of burned-out testicular tumor by dynamic contrast-enhanced magnetic resonance, demonstrating poor or absent enhancement in burned-out tumors (29), which is consistent with the typical histological appearance of fibrous scar and peripheral atrophy (8, 10).

To date, most papers about burned-out tumors consist in case reports or small case series; therefore, there are limited data about the outcome for burned-out patients. In the largest published series, tumor-related mortality ranges from 13% (22) to ∼25% (17); however, these articles describe only adult patients and include both seminomatous and non-seminomatous tumors, which have a different prognosis (12). Moreover, there are no published data about treatment-related toxicity.

In the present work, all the patients in both groups are affected by stage III and stage IV non-seminomatous tumors. Patients in the "burned-out" group tended to be older than patients in the "testicular primary" group (mean age 17 years 10 months vs. 15 years 0 months); despite this difference, both populations are comprised in the range 13 to 19 years, which is reported to be the age range at highest risk of adverse events in testicular germ cell tumors (30). Histology and stage distribution did not differ significantly between the two groups and no patient had pre-treatment comorbidity. All the patients in both groups were treated according to the same protocol.

We may therefore assume that the two groups are comparable and that the differences in terms of clinical presentation, treatment-related toxicity, and outcome are attributable to the "burned-out" vs. "primary testicular" status.

All the patients in the "burned-out" population presented with a palpable metastatic mass, and four out of five patients also presented with systemic symptoms, i.e., weight loss, fever, and deep venous thrombosis, while none of the "primary testicular" patients in our series presented mass-related symptoms or systemic symptoms ($p = 0.0027$).

Serum markers at diagnosis were significantly higher in the "burned-out" population than in the "primary testicular" group: mean aFP 2,215 ng/ml (range 2–10,997 ng/ml) vs. 979 ng/ml (range 2.05–4,604.3 ng/ml), $p < 0.00001$; mean hCG 14,831 mIU/ml (range 2–73,564.5 mIU/ml) vs. 530 mIU/ml (range 2–1,863.5 mIU/ml), $p < 0.00001$; mean LDH 1,558 IU/L (range 372–4,052 IU/L) vs. 424 IU/L (range 238–620 IU/L), $p < 0.00001$.

The differences in clinical presentation and serum markers at diagnosis may be interpreted as a sign of higher tumor burden in the "burned-out" group; a different explanation could be a substantially different biological behavior of "burned-out" tumors compared to "primary testicular" tumors even in the face of similar disease stage. At the state of the art, there is poor evidence about the biological mechanism of the "burned-out" phenomenon and its clinical implications; further studies are needed to clarify this issue.

In the present case series, "burned-out" patients had a significantly higher incidence of relevant toxicity than "primary testicular" patients ($p = 0.0357$); such event is unexpected since both groups received the same therapeutic regimen and no patient had pre-existing comorbidities. One patient in the "burned-out" group experienced "choriocarcinoma syndrome," a treatment-related complication that occurs in patients affected by choriocarcinoma with multiple pulmonary metastases at the beginning of chemotherapy, characterized by acute respiratory distress and pulmonary hemorrhage (14, 15). Apart from "choriocarcinoma syndrome," the higher incidence of relevant toxicity observed in the "burned-out" population is unanticipated; it might be simply related to the small sample size,

or it could be the result of a higher tumor burden in "burned-out" patients. Metastatic germ cell tumors and tumors with high LDH levels are at risk for tumor lysis syndrome during induction chemotherapy (31–34); "burned-out" patients present with both features and therefore might be at higher risk of renal toxicity. However, such mechanism needs further supporting evidence and would only explain renal toxicity. The treatment-related toxicity experienced by "burned-out" patients is currently an unexplained phenomenon; since the present paper is the first study that systematically addresses this issue, further studies are necessary in this field.

The present data show that "burned-out" patients have a worse outcome compared to "primary testicular" patients in terms of both OS ($p = 0.0299$) and EFS ($p = 0.0164$). To our knowledge, this is the first study to compare outcome between "burned-out" and "primary testicular" patients. Several factors may contribute to such difference. In the present series, "burned-out" patients had significantly higher levels of serum markers (i.e., aFP, hCG, and LDH) than "primary testicular" patients: elevated aFP, hCG, and LDH are known adverse prognostic factors in adults (12), although in pediatric patients, such association has been demonstrated for aFP only (4, 35). "Burned-out" patients also showed a trend toward a higher incidence of extra-pulmonary visceral metastases, although not statistically significant ($p = 0.0632$); the presence of such secondary lesions is a documented poor prognostic factor in adults (12) but not in the pediatric population at the state of the art.

The different clinical behavior could be explained by a biological difference between "burned-out" and "primary testicular" tumors. Further studies are needed to clarify this issue.

The present study has an obvious limitation in its retrospective nature. A second limitation is the small number of patients included in the study.

On the other hand, the two groups compared in this study showed a similar distribution in terms of age, stage, and histology; moreover, all the patients were treated according to the same protocol (5). It is reasonable to conclude that all the differences observed between the two groups may be secondary to the "burned-out" vs. "primary testicular" status.

In conclusion, "burned-out" testicular tumors seem to be a well-distinct clinical entity with a higher treatment-related toxicity and poorer prognosis compared to metastatic "primary testicular" tumors. Further studies are needed to clarify the "burned-out phenomenon" from a biological point of view and to identify more effective therapeutic strategies for these patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

GP, AC, MD, RC, and AI contributed to conception and design of the study. FB and CM organized the database and revised the data. RA and FD revised the pathology specimens. PD revised radiology images. All authors contributed to manuscript revision and read and approved the submitted version.

REFERENCES

1. Ward E, DeSantis C, Robbins A, Kohler B, Jemal A. Childhood and adolescent cancer statistics, 2014. *CA Cancer J Clin.* (2014) 64:83–103. doi: 10.3322/caac.21219
2. Steliarova-Foucher E, Stiller C, Kaatsch P, Berrino F, Coebergh JW, Lacour B, et al. Geographical patterns and time trends of cancer incidence and survival among children and adolescents in Europe since the 1970s (the ACCISproject): an epidemiological study. *Lancet.* (2004) 364:2097–105. doi: 10.1016/S0140-6736(04)17550-8
3. Rescorla FJ. Pediatric germ cell tumors. *Semin Surg Oncol.* (1999) 16:144–583. doi: 10.1002/(SICI)1098-2388(199903)16:2<;144::AID-SSU6>;3.0.CO;2-M
4. Mann JR, Raafat F, Robinson K, Imeson J, Gornall P, Sokal M, et al. The United Kingdom children's cancer study group's second germ cell tumor study: carboplatin, etoposide, and bleomycin are effective treatment for children with malignant extracranial germ cell tumors, with acceptable toxicity. *J Clin Oncol.* (2000) 18:3809–18. doi: 10.1200/JCO.2000.18.22.3809
5. Terenziani M, De Pasquale MD, Bisogno G, Biasoni D, Boldrini R, Collini P, et al. Malignant testicular germ cell tumors in children and adolescents: The AIEOP (Associazione Italiana Ematologia Oncologia Pediatrica) protocol. *Urol Oncol.* (2018) 36:502.e7–502.e13. doi: 10.1016/j.urolonc.2018.07.001
6. Frazier A L, Hale J P, Rodriguez-Galindo C, Dang H, Olson T, Murray M J, et al. Revised risk classification for pediatric extracranial germ cell tumors based on 25 years of clinical trial data from the United Kingdom and United States. *J Clin Oncol.* (2015) 33:195–201. doi: 10.1200/JCO.2014.58.3369
7. Fabre E, Jira H, Izard V, Ferlicot S, Hammoudi Y, Theodore C, et al. "Burned-out" primary testicular cancer. *BJU Int.* (2004) 94:74–8. doi: 10.1111/j.1464-410X.2004.04904.x
8. Azzopardi J G, Mostofi F K, Theiss E A. Lesions of testes observed in certain patients with widespread choriocarcinoma and related tumors. The significance and genesis of hematoxylin-staining bodies in the human testis. *Am J Pathol.* (1961) 38:207–25.
9. Sesterhenn I A, Davis C J Jr. Pathology of germ cell tumors of the testis. *Cancer Control.* (2004) 11:374–87. doi: 10.1177/107327480401100605
10. Balzer BL, Ulbright TM. Spontaneous regression of testicular germ cell tumors: an analysis of 42 cases. *Am J Surg Pathol.* (2006) 30:858–65. doi: 10.1097/01.pas.0000209831.24230.56
11. Williamson SR, Delahunt B, Magi-Galluzzi C, Algaba F, Egevad L, Ulbright TM, et al. The world health organization 2016 classification of testicular germ cell tumours: a review and update from the international society of urological pathology testis consultation panel. *Histopathology.* (2017) 70:335–46. doi: 10.1111/his.13102
12. International Germ Cell Cancer Collaborative Group. International Germ Cell Consensus Classification: a prognostic factor-based staging system for metastatic germ cell cancers. *J Clin Oncol.* (1997) 15:594–603. doi: 10.1200/JCO.1997.15.2.594
13. U.S. Department Of Health And Human Services, National Institutes of Health, National Cancer Institute. *Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0* (2017).
14. Kirch C, Blot F, Fizazi K, Raynard B, Theodore C, Nitenberg G. Acute respiratory distress syndrome after chemotherapy for lung metastases from non-seminomatous germ-cell tumors. *Support Care Cancer.* (2003) 11:575–80. doi: 10.1007/s00520-003-0481-5
15. Rejlekova K, Cursano M C, De Giorgi U, Mego M. Severe complications in testicular germ cell tumors: the choriocarcinoma syndrome. *Front Endocrinol.* (2019) 10:218. doi: 10.3389/fendo.2019.00218

16. Billmire D F. Malignant germ cell tumors in childhood. *Semin Pediatr Surg.* (2006) 15:30–6. doi: 10.1053/j.sempedsurg.2005.11.006
17. Angulo J C, González J, Rodríguez N, Hernández E, Núñez C, Rodríguez-Barbero J M, et al. Clinicopathological study of regressed testicular tumors (apparent extragonadal germ cell neoplasms). *J Urol.* (2009) 182:2303–10. doi: 10.1016/j.juro.2009.07.045
18. Prym P. Spontanheilung eines bosartigen, wahrscheinlich chorionepitheliometoses gewachses im hoden. *Virchows Arch [A] Pathol Anat Histol.* (1927) 265:239. doi: 10.1007/BF01894164
19. Fosså S D, Aass N, Heilo A, Daugaard G, Skakkebaek NE, Stenwig AE, et al. Testicular carcinoma *in situ* in patients with extragonadal germ-cell tumours: the clinical role of pretreatment biopsy. *Ann Oncol.* (2003) 14:1412–8. doi: 10.1093/annonc/mdg373
20. Ulbright TM. Germ cell tumors of the gonads: a selective review emphasizing problems in differential diagnosis, newly appreciated, and controversial issues. *Mod Pathol.* (2005) 18(Suppl. 2):S61–79. doi: 10.1038/modpathol.3800310
21. Astigueta JC, Abad-Licham MA, Agreda FM, Leiva BA, De la Cruz JL. Spontaneous testicular tumor regression: case report and historical review. *Ecancermedicalscience.* (2018) 12:888. doi: 10.3332/ecancer.2018.888
22. Scholz M, Zehender M, Thalmann GN, Borner M, Thöni H, Studer UE. Extragonadal retroperitoneal germ cell tumor: evidence of origin in the testis. *Ann Oncol.* (2002) 13:121–4. doi: 10.1093/annonc/mdf003
23. Ishikawa H, Kawada N, Taniguchi A, Odachi K, Mizutani A, Asahi M, et al. Paraneoplastic neurological syndrome due to burned-out testicular tumor showing hot cross-bun sign. *Acta Neurol Scand.* (2016) 133:398–402. doi: 10.1111/ane.12469
24. Freifeld Y, Kapur P, Chitkara R, Lee F, Khemani P, Bagrodia A. Metastatic "burned out" seminoma causing neurological paraneoplastic syndrome-not quite "burned out". *Front Neurol.* (2018) 9:20. doi: 10.3389/fneur.2018.00020
25. Rocher L, Ramchandani P, Belfield J, Bertolotto M, Derchi LE, Correas JM, et al. Incidentally detected non-palpable testicular tumours in adults at scrotal ultrasound: impact of radiological findings on management radiologic review and recommendations of the ESUR scrotal imaging subcommittee. *Eur Radiol.* (2016) 26:2268–78. doi: 10.1007/s00330-015-4059-7
26. Rocher L, Glas L, Bellin M F, Ferlicot S, Izard V, Benoit G, et al. Burned-out testis tumors in asymptomatic infertile men: multiparametric sonography and MRI findings. *J Ultrasound Med.* (2017) 36:821–31. doi: 10.7863/ultra.15.08037
27. Rocher L, Criton A, Gennisson JL, Creze M, Albiges L, Ferlicot S, et al. Characterization of testicular masses in adults: performance of combined quantitative shear wave elastography and conventional ultrasound. *Ultrasound Med Biol.* (2019) 45:720–31. doi: 10.1016/j.ultrasmedbio.2018.10.027
28. Luzurier A, Maxwell F, Correas J M, Benoit G, Izard V, Ferlicot S, et al. Qualitative and quantitative contrast-enhanced ultrasonography for the characterisation of non-palpable testicular tumours. *Clin Radiol.* (2018) 73:322.e1–9. doi: 10.1016/j.crad.2017.10.007
29. El Sanharawi I, Correas J M, Glas L, Ferlicot S, Izard V, Ducot B, et al. Non-palpable incidentally found testicular tumors: differentiation between benign, malignant, and burned-out tumors using dynamic contrast-enhanced MRI. *Eur J Radiol.* (2016) 85:2072–82. doi: 10.1016/j.ejrad.2016.09.021
30. Cost NG, Lubahn JD, Adibi M, Romman A, Wickiser JE, Raj GV, et al. A comparison of pediatric, adolescent, and adult testicular germ cell malignancy. *Pediatr Blood Cancer.* (2014) 61:446–51. doi: 10.1002/pbc.24773
31. Coiffier B, Altman A, Pui CH, Younes A, Cairo MS. Guidelines for the management of pediatric and adult tumor lysis syndrome: an evidence-based review. *J Clin Oncol.* (2008) 26:2767–78. doi: 10.1200/JCO.2007.15.0177
32. Feres GA, Figueira Salluh JI, Gil Ferreira C, Soares M. Severe acute tumor lysis syndrome in patients with germ-cell tumors. *Indian J Urol.* (2008) 24:555–7. doi: 10.4103/0970-1591.44267
33. Cairo MS, Coiffier B, Reiter A, Younes A, TLS Expert Panel. Recommendations for the evaluation of risk and prophylaxis of tumour lysis syndrome (TLS) in adults and children with malignant diseases: an expert TLS panel consensus. *Br J Haematol.* (2010) 149:578–86. doi: 10.1111/j.1365-2141.2010.08143.x
34. Kobatake K, Kato M, Mita K. Advanced testicular cancer associated with life-threatening tumour lysis syndrome and choriocarcinoma syndrome. *Can Urol Assoc J.* (2015) 9:62–4. doi: 10.5489/cuaj.2499
35. Lo Curto M, Lumia F, Alaggio R, Cecchetto G, Almasio P, Indolfi P, et al. Malignant germ cell tumors in childhood: results of the first Italian cooperative study "TCG 91." *Med Pediatr Oncol.* (2003) 41:417–25. doi: 10.1002/mpo.10324

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frontiers in Pediatrics

ORIGINAL RESEARCH
published: 20 September 2021
doi: 10.3389/fped.2021.689612

Indocyanine Green–Guided Pediatric Tumor Resection: Approach, Utility, and Challenges

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Incomplete tumor resection increases the risk of local recurrence. However, the standard of care approach to distinguishing tumor tissue is less than optimal, as it depends on a conglomeration of preoperative imaging and visual and tactile indicators in real time. This approach is associated with a significant risk of inadequate resection; therefore, a novel approach that delineates the accurate intraoperative definition of pediatric tumors is urgently needed. To date, there is no reliable method for the intraoperative assessment of tumor extent and real-time differentiation between tumor- involved tissues and tumor-free tissues. Use of intraoperative frozen sections is challenging, time consuming, and covers a small surface area. Increased vascular permeability and impaired lymphatic drainage in the tumor microenvironment leads to an enhanced permeability and retention effect of small molecules. ICG is a fluorescent dye that when administered intravenously accumulates passively in the tumor because of EPR, thereby providing some tumor contrast for intraoperative real-time tumor recognition. Preclinical and clinical studies suggest that the tumor-to-background fluorescence ratio is optimized when imaging is obtained 24 h after dye injection, and many studies suggest using a high dose of ICG to optimize dye retention in the tumor tissue. However, in childhood cancers, little is known about the ideal dosing, applications, and challenges of ICG-guided tumor resection. This retrospective study examines the feasibility of ICG-guided tumor resection in common childhood solid tumors such as neuroblastoma, sarcomas, hepatic tumors, pulmonary metastases, and other rare tumors. Pediatric dosing and challenges related to the optimization of tumor-to-background ratio are also examined.

Keywords: indocyanine green, fluorescence, pediatric solid tumor, near infrared spectroscopy, intraoperative imaging

OPEN ACCESS

Edited by:
Sabine Sarnacki, Assistance Publique Hopitaux De Paris, France

Reviewed by:
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Specialty section:
This article was submitted to Pediatric Surgery, a section of the journal Frontiers in Pediatrics

Received: *01 April 2021*
Accepted: *25 August 2021*
Published: *20 September 2021*

Citation:
Abdelhafeez A, Talbot L, Murphy AJ and Davidoff AM (2021) Indocyanine Green–Guided Pediatric Tumor Resection: Approach, Utility, and Challenges. Front. Pediatr. 9:689612. doi: 10.3389/fped.2021.689612

INTRODUCTION

Complete resection of solid tumors is critical for curing pediatric patients. The standard of care intraoperative approach to distinguishing tumor tissue depends on surgeons building a mental map of the tumor by using preoperative imaging and intraoperative visual and tactile indicators. However, this approach is associated with the risk of inadequate resection due to the lack of intraoperative real-time delineation of 3D tumor anatomy. The rate of local tumor recurrence for patients may be decreased and the survival increased by ensuring the completeness of resection by using fluorescence-guided surgery. Fluorescence-guided surgery uses a real-time tool for enhancing the visualization of the 3D tumor anatomy and facilitates differentiation between tumor and normal tissue.

Indocyanine Green (ICG) is a Food and Drug Administration (FDA)–approved water-soluble tricarbocyanine fluorophore that was initially used in clinical settings for measuring cardiac output, liver function, retinal angiography, and more recently for blood, biliary, and lymphatic flow imaging. ICG has been safely used in clinical studies for over 50 years at an FDA-approved pediatric maximum dose of 2 mg/kg IV. ICG is the most studied fluorophore, has a good safety profile, and is rarely associated with adverse reactions.

Many animal models have examined the utility of ICG-guided tumor localization, and more recently adult clinical trials have demonstrated the high sensitivity of this technique in identifying tumor cells (1–23). The difference in retention of ICG between tumor and normal tissue is the result of increased vascular permeability and impaired lymphatic drainage in the tumor microenvironment, which creates an enhanced permeability and retention (EPR) effect in small molecules (6).

Preclinical and clinical studies suggest that the tumor-to-background fluorescence ratio is optimized when imaging is obtained 24 h after injecting the intravenous dye (2, 21, 22), However, for liver primary tumors, a 72-h window has been suggested by others to allow greater washout of ICG from the adjacent normal liver tissue and improve the ability to identify tumors (24, 25). Adult trials have used 3–5 mg/kg ICG to optimize the tumor-to-background ratio, which is more than double the maximum FDA-approved pediatric dose. However, the necessity and safety of higher doses in children has not yet been studied. Moreover, the tumor biology of childhood tumors is different from that of adult tumors and ICG plasma clearance is significantly greater compared to adults (26). The aim of this study is to retrospectively examine ICG-guided tumor resection applications, dosing, and outcomes in pediatric oncology patients.

METHODS

This study was approved by the St. Jude institutional review board and waiver of informed consent was approved. We retrospectively reviewed the charts of all patients who underwent fluorescence-guided tumor resection at our institution from 2019 to 2020. Data on preoperative diagnosis and imaging findings, ICG dose, timing of ICG administration, operative notes, Iridium system (Visionsense Corp, Philadelphia, PA) video recording, intraoperative fluorescence, background noise, histopathology report, and complications were collected. All patients received a 1.5 mg/kg of ICG intravenous infusion over 15 min the day before surgery, except for two patients with hepatoblastoma who received 1.5 mg/kg of ICG 72 h before surgery. Intraoperative visualization was conducted with the Iridium system optimized to detect ICG. The Iridium system provides excitation light at 805 nm, causing ICG to emit bands between 825 and 850 nm that are captured by a near infrared (NIR) camera. This system generates a real-time fused image of surgical anatomy by allowing the capture of the normal white light image simultaneously with the ICG fluorescence image. The fused images enable the surgeon to proceed with tumor resection guided by fluorescing tumor dimensions without the need to switch off the normal white light view necessary for field visualization and to safely conduct tumor resection.

Measurements of the primary tumor as well as the surrounding tissue were completed to determine if the tumor was consistently fluorescent compared to the background normal tissue adjacent to the tumor. Background noise was defined as persistent fluorescence of nearby normal organs.

Histology is the gold standard for determining the presence of tumor. Thus, the diagnostic test ICG was compared to the final pathology report to calculate true positives, true negatives, sensitivity, and specificity.

RESULTS

Fifty-five patients (28 males and 27 females; median age 10 years [range < 1–21 years]) underwent fluorescence-guided tumor resection. Of them, eight underwent two procedures and one patient underwent three procedures. The total number of procedures done was 65, including 37 thoracic, 19 abdominal (other than nephron-sparing resections) and nine trunk and extremity operations (**Table 1**). Cancer was confirmed by histology in 52 procedures (80%), and no malignant tumors were found in 13 procedures (20%). The 13 procedures in which lesions were found other than tumors included 10 pulmonary wedge resections that were histologically confirmed to be the following: four lymph nodes, two granulomas, one nodular pulmonary ossification, one dystrophic calcification, one histoplasma, and one post-therapy Wilms tumor with no morphologic evidence of viable tumor. The three abdominal biopsies that yielded diagnoses other than tumors were confirmed by histology to be the following: one lymph node with sinus histiocytosis, one granuloma, and one reactive fibroblastic proliferation.

Of the 52 procedures that confirmed tumors, 46 (88%) were identified with NIR guidance, while of the 13 nonmalignant lesions only three (23%) lesions were fluorescent. The 46 fluorescent tumors included nine hepatoblastomas (HBs) and two hepatocellular carcinomas (HCCs), nine osteosarcomas (OSs), six neuroblastomas (NBs), six non-rhabdomyosarcoma soft tissue sarcomas (NRSTSs), five rhabdomyosarcomas (RMSs), three Ewing sarcomas (ESs), two germ cells tumors (GCTs), one chondroblastoma (CB), one solid pseudopapillary neoplasm of the pancreas (SPNP), one lymphoma, and one myoepithelial carcinoma of the chest wall (**Table 1**).

The sensitivity of NIR to identify tumor was 88% and specificity was 77% (**Table 2**). NIR imaging could not identify two primary adrenocortical tumors (ACTs). NIR imaging also could not detect one retroperitoneal metastatic lymph node and three pulmonary metastases, including two osteosarcoma metastases and one Wilms tumor metastasis. Two of these pulmonary metastases were small (<0.5 cm) and subpleural.

Background noise from adjacent organs was observed during a total of 37 procedures (57%), including all trunk and extremity resections, 68% of abdominal procedures (13), and 40% of thoracic procedures (15). Interestingly, background noise from

TABLE 1 | Tumor localization with ICG: utility, dosing, dosing interval, and challenges.

Procedures *n* = 65	Fluorescent tumors (true positive) *n* = 46	Fluorescent nodules but not tumors (false positive) *n* = 3	Nonfluorescent nodules but not tumors (true negative) *n* = 10	Nonfluorescent tumors (false negative) *n* = 6	Background noise	Source of background noise (for open vs. MIS)	Fluorescence-guided identification of tumors not detected by standard of care
Thorax *n* = 37 (pulmonary lesions = 36, Mediastinal NB = 1)	24 (OS = 9, HB = 5, HCC = 1, ES = 3, NB = 2, NRSTS = 2, RMS = 1, CB = 1)	2 (Histoplasma = 1, reactive lymph node = 1)	8 (3 lymph nodes = 3, granulomas = 2, nodular pulmonary ossification = 1, dystrophic calcification = 1, post-therapy Wilms tumor with no morphologic evidence of viable tumor = 1)	3 (OS = 2, WT = 1)	Total: 15 (40%) Open (11): 11(100%) MIS (26): 4 (15%)	Open: Skin, diaphragm, and chest wall MIS: Diaphragm, chest wall	3 lesions seen only by NIR (HB = 2, HCC = 1)
Abdomen *n* = 19	13 (HB = 3; HCC = 1, NB = 4, GCT = 2, NRSTS = 1, lymphoma = 1, SPNP = 1	1 (granuloma)	2 (Reactive fibroblastic proliferation = 1, lymph nodes with sinus histiocytosis = 1)	3 (ACC = 2, NB = 1)	Total: 13 (68%) Open (8): 8 (100%) MIS (11): 5 (45%)	Open: Skin, bowel, kidney, gall bladder MIS: Kidney, bowel	None
Trunk and extremities *n* = 9	9 (NRSTS = 4 RMS = 4 Myoepithelial carcinoma = 1)	0	0	0	9 (100%)	Skin	1 (Tumor extension seen only by NIR)

ICG, Indocyanine Green; MIS, minimally invasive; OS, osteosarcoma; HB, hepatoblastoma; ES, Ewing sarcoma; RMS, rhabdomyosarcoma; NRSTS, non-rhabdomyosarcoma soft tissue sarcoma; ATC, anaplastic thyroid cancer; CB, chondroblastoma; NB, neuroblastoma; HCC, hepatocellular carcinoma; WT, Wilms tumor; GCT, germ cell tumor, SPNP, solid pseudopapillary neoplasm of pancreas; ACT, adrenocortical tumor.

TABLE 2 | Sensitivity and specificity of ICG-guided tumor localization.

Sensitivity	88%
Specificity	77%
Positive predictive value	94%
Negative predictive value	63%
Accuracy	86%

ICG, Indocyanine Green.

adjacent organs was observed in all open abdominal and open thoracic procedures; however, this was seen in only 45% of minimally invasive (MIS) abdominal procedures (11) and only 15% of thoracoscopies (4) (**Table 1**).

NIR fluorescence provided localization guidance for three thoracoscopic resections of lung metastases of liver primary tumors (two HBs and one HCC) that were otherwise not seen by standard of care white light (**Figure 1**). NIR also provided surgical guidance for complete resection of a chest wall myoepithelial carcinoma with a medial tumor extension not differentiated from normal muscles with standard of care white light and tactile feedback (**Figure 2**). No adverse reactions were noted after administration of 1.5 mg/kg ICG in this study. Surgical complication rate was low in this cohort (5%), two patients developed postoperative seroma resolved after drainage and one patient developed air leak after pulmonary wedge resection responded to drainage.

DISCUSSION

We show that an ICG dose of 1.5 mg/kg is safe in pediatric patients receiving therapy for different types of malignancies. By using the study ICG dose and timing of injection, a broad range of pediatric malignant tumors consistently exhibited fluorescence compared to background tissue. These tumors included liver tumors, osteosarcomas, non-rhabdomyosarcomas, rhabdomyosarcomas, neuroblastomas, Ewing sarcoma, germ cells tumors, chondroblastoma, solid pseudopapillary neoplasms of the pancreas, lymphoma, and myoepithelial carcinoma. The sensitivity and specificity of ICG in identifying malignant tumor tissue intraoperatively were 88 and 77%, respectively. Moreover, the use of ICG in this study resulted in identifying 4 (6%) malignant lesions that would not have been identified by the standard of care.

Iridium systems examine large surfaces in real time with overlay of NIR images over standard white light visualization of the surgical field. Therefore, this system enables real-time fluorescence-guided tumor resection that allows the surgeon to maintain the principles of safe dissection without eliminating white light. At a dose less than the maximum FDA-approved pediatric dose, ICG accumulates in most pediatric solid tumors and is cleared from normal tissue within 24 h after injection. This was evident by the higher emission of photons from the tumor than adjacent organs, resulting in NIR imaging contrast demarcation between background tissue and most

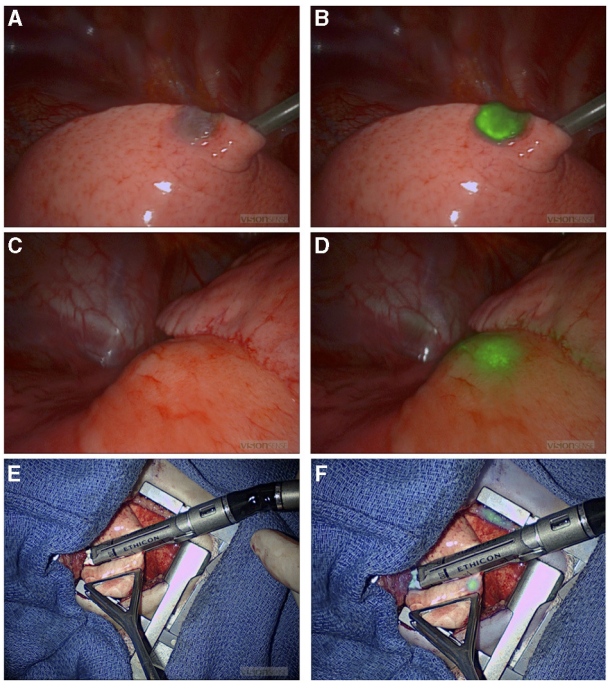

FIGURE 1 | NIR-guided localization of pulmonary metastases for three thoracoscopic resections from a hepatic primary tumor. **(A,B)** NIR localization of a superficial nodule. This nodule was seen with both standard of care white light **(A)** and NIR **(B)**. NIR, near infrared. **(C,D)** NIR localization of a 2 cm nodule seen on the preoperative CT scan, but not visible when seen by standard of care white light **(C)**. **(D)** The same deep nodule was localized with NIR. NIR, near infrared; CT, computed tomography. **(E,F)** A small 0.2 cm nodule not localized with preoperative CT scan or with standard of care white light/tactile feedback **(E)**. **(F)** The same nodule localized by NIR. NIR, near infrared.

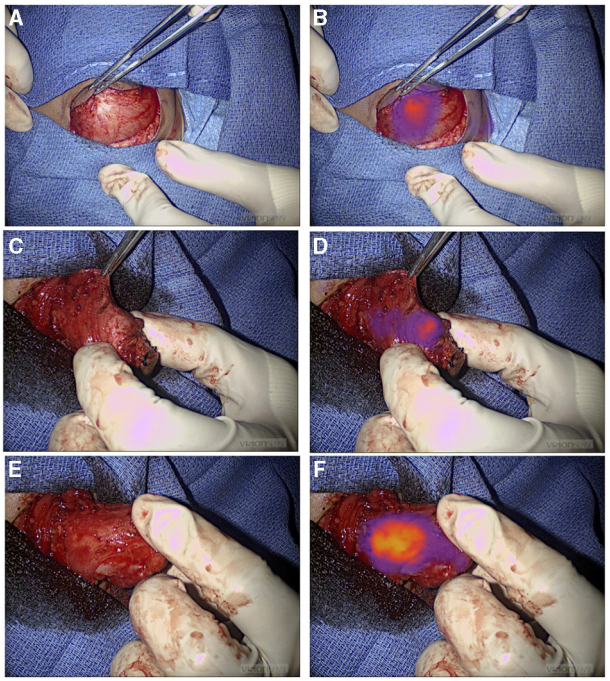

FIGURE 2 | NIR guide localization of margin extension. **(A,B)** Initial view of a chest wall myoepithelial carcinoma with white light **(A)** and NIR **(B)**. NIR, near infrared. **(C–F)** Medial extension of the myoepithelial carcinoma was not appreciated with standard of care white light and tactile feedback **(C)**, but it was recognized by NIR **(D)**. Medial extension of the tumor was further localized with NIR after medial dissection proceeded **(E)** and **(F)**. NIR, near infrared.

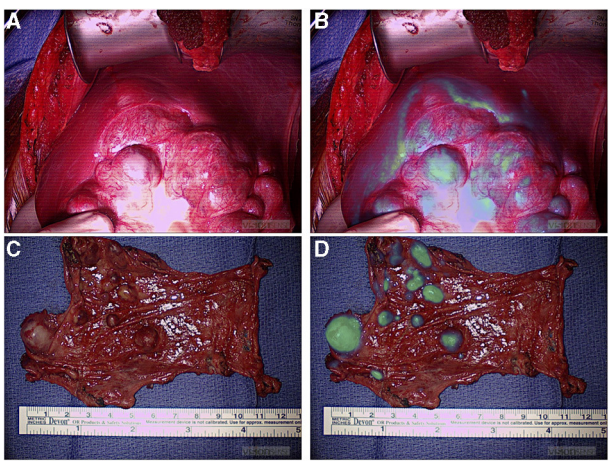

FIGURE 3 | NIR localization of primary and metastatic liver tumors. **(A,B)** White light **(A)** and NIR **(B)** view of a primary liver hepatoblastoma. **(C,D)** White light **(C)** and NIR **(D)** view of hepatocellular carcinoma peritoneal metastases. NIR, near infrared.

histology-confirmed tumors. The low specificity precludes strict interpretation of any fluorescence as tumor; hence, the risk of additional resection should be carefully weighed against the limitation of ICG-guided tumor bed assessment. Considering the high sensitivity of NIR imagery, the low specificity may not be a very significant limiting factor as the goal of most tumor surgeries is to avoid missing tumor deposits.

The more effective clearance of ICG in children from normal tissue may explain how optimal tumor-to-background ratio is achieved for NIR visualization of tumors with the ICG dose used in this study. Although, the incidence of background noise was high in open procedures likely as a result of ambient light contamination, this rarely limited the ability to delineate the tumor, as the source of background noise was mostly from organs and not directly adjacent to tumors such as bowel or skin tumors. Occasionally, organs adjacent to the tumor were the source of background noise and may have contributed to the negative fluorescence appearance of two ACTs, one of the two cases of osteosarcoma pulmonary metastasis, and one retroperitoneal metastatic lymph node relative to the intense signal from the kidney, diaphragm, and bowel, respectively. Proximity of the kidney did not preclude the optimal fluorescence of all included primary adrenal NBs (four patients). Moreover, ACT may require a different dosing or timing of ICG injection specific to this tumor biology for optimal imaging (22, 27). Measures to decrease ambient light contamination of the NIR field may mitigate background noise; however, it is unknown if further

reduction in the ICG dose would reduce background noise without decreasing tumor signal and potentially diminishing the tumor-to-background ratio for some tumors. Another limitation of NIR fluorescence is tissue attenuation, which reduces photon count and precludes tissue penetration beyond a depth of 2 cm. The limitation of depth of penetration can potentially be resolved by optimizing filtering to reduce background noise and increase camera integration time. Two patients with small (<0.5 cm), subpleural pulmonary metastatic nodules required simultaneous guide-wire localization, as NIR imaging could not detect deep small lesions; therefore, it is advisable to use an alternative localization technique in similar scenarios and not rely merely on NIR imagery for small subpleural lesions.

Most tumor resections were amenable to localization with standard of care white light visualization and tactile feedback (94%). During four tumor resections (6%), ICG fluorescence provided surgical guidance to localize tumors otherwise not adequately localized by the standard of care. Three of these resections were pulmonary metastases from hepatic primary tumors appreciated only with ICG guidance. Although, one of these lesions was a 2 cm nodule seen on the preoperative CT scan, the depth of this nodule was 2.3 cm from the pleural surface, and it was not seen by intraoperative white light. The other two nodules were subcentimetric and neither was seen on the preoperative CT scan or with intraoperative standard of care. The utility of ICG hepatic tumor localization has been confirmed by previous studies (28, 29), which is in keeping with our findings that liver primary and metastatic deposits were consistently fluorescent (**Figure 3**). Also, ICG guidance helped identify a medial tumor extension of myoepithelial carcinoma of the chest wall otherwise not differentiated from adjacent normal muscle. This enabled achieving complete resection; however, it may not be possible to conclude on the utility of fluorescence-guided identification of tumor margins outside of prospective trials. We are currently prospectively examining the utility of ICG-mediated NIR imagery to discern tumor margins, identify residual disease, and study ICG uptake in pretreated tumors (30).

CONCLUSION

ICG-guided tumor localization is a feasible adjunct for most pediatric solid tumors at a relatively lower dose than what is recommended in preclinical studies and adult clinical trials. ICG is highly sensitive for tumor tissue but its specificity is low. For deep and small lesions, an alternative localization technique needs to be used simultaneously that helps in cases where NIR cannot penetrate deep enough to identify a lesion. Background noise is less during the MIS approach, and optimal control of ambient light contamination may help mitigate this issue.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by St. Jude Institutional Review Boards (IRBs), St. Jude Children's Research Hospital, Memphis, USA. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

All authors took part in writing the manuscript, reviewing it, and revising its intellectual and technical content. All authors assume responsibility and accountability for the results.

ACKNOWLEDGMENTS

Thanks to Vani Shanker for editing of the manuscript.

REFERENCES

1. Kennedy GT, Newton A, Predina J, Singhal S. Intraoperative near-infrared imaging of mesothelioma. *Transl Lung Cancer Res.* (2017) 6:279–84. doi: 10.21037/tlcr.2017.05.01
2. Predina JD, Newton AD, Corbett C, Xia L, Shin M, Sulfyok LF, et al. A clinical trial of tumorglow(R) to identify residual disease during pleurectomy and decortication. *Ann Thorac Surg.* (2019) 107:224–32. doi: 10.1016/j.athoracsur.2018.06.015
3. Holt D, Okusanya O, Judy R, Venegas O, Jiang J, DeJesus E, et al. Intraoperative near-infrared imaging can distinguish cancer from normal tissue but not inflammation. *PLoS ONE.* (2014) 9:e103342. doi: 10.1371/journal.pone.0103342 eCollection 2014.
4. Keating J, Tchou J, Okusanya O, Fisher C, Batiste R, Jiang J, et al. Identification of breast cancer margins using intraoperative near-infrared imaging. *J Surg Oncol.* (2016) 113:508–14. doi: 10.1002/jso.24167
5. Jeon JW, Cho SS, Nag S, Buch L, Pierce J, Su YS, et al. Near-infrared optical contrast of skull base tumors during endoscopic endonasal surgery. *Oper Neurosurg (Hagerstown).* (2019) 17:32–42. doi: 10.1093/ons/opy213
6. Jiang JX, Keating JJ, Jesus EM, Judy RP, Madajewski B, Venegas O, et al. Optimization of the enhanced permeability and retention effect for near-infrared imaging of solid tumors with indocyanine green. *Am J Nucl Med Mol Imaging.* (2015) 5:390–400.
7. Keating J, Judy R, Newton A, Singhal S. Near-infrared operating lamp for intraoperative molecular imaging of a mediastinal tumor. *BMC Med Imaging.* (2016) 16:15. doi: 10.1186/s12880-016-0120-5
8. Keating J, Newton A, Venegas O, Nims S, Zeh R, Predina J, et al. Near-infrared intraoperative molecular imaging can locate metastases to the lung. *Ann Thorac Surg.* (2017) 103:390–8. doi: 10.1016/j.athoracsur.2016.08.079
9. Kim GY, Bae KS, Noh GJ, Min WK. Estimation of indocyanine green elimination rate constant k and retention rate at 15 min using patient age, weight, bilirubin, and albumin. *J Hepatobiliary Pancreat Surg.* (2009) 16:521–8. doi: 10.1007/s00534-009-0097-3
10. Kimura T, Muguruma N, Ito S, Okamura S, Imoto Y, Miyamoto H, et al. Infrared fluorescence endoscopy for the diagnosis of superficial gastric tumors. *Gastrointest Endosc.* (2007) 66:37–43. doi: 10.1016/j.gie.2007.01.009
11. Kitagawa N, Shinkai M, Mochizuki K, Usui H, Miyagi H, Nakamura K, et al. Navigation using indocyanine green fluorescence imaging for

hepatoblastoma pulmonary metastases surgery. *Pediatr Surg Int.* (2015) 31:407–11. doi: 10.1007/s00383-015-3679-y
12. Lee JY, Thawani JP, Pierce J, Zeh R, Martinez-Lage M, Chanin M, et al. Intraoperative near-infrared optical imaging can localize gadolinium-enhancing gliomas during surgery. *Neurosurgery.* (2016) 79:856–71. doi: 10.1227/NEU.0000000000001450
13. Lee JYK, Pierce JT, Thawani JP, Zeh R, Nie S, Martinez-Lage M, et al. Near-infrared fluorescent image-guided surgery for intracranial meningioma. *J Neurosurg.* (2018) 128:380–90. doi: 10.3171/2016.10.JNS161636
14. Lee JYK, Pierce JT, Zeh R, Cho SS, Salinas R, Nie S, et al. Intraoperative near-infrared optical contrast can localize brain metastases. *World Neurosurg.* (2017) 106:120–30. doi: 10.1016/j.wneu.2017.06.128
15. Mohs AM, Mancini MC, Singhal S, Provenzale JM, Leyland-Jones B, Wang MD, et al. Hand-held spectroscopic device for in vivo and intraoperative tumor detection: contrast enhancement, detection sensitivity, and tissue penetration. *Anal Chem.* (2010) 82:9058–65. doi: 10.1021/ac102058k
16. Newton AD, Kennedy GT, Predina JD, Low PS, Singhal S. Intraoperative molecular imaging to identify lung adenocarcinomas. *J Thorac Dis.* (2016) 8:S697–704. doi: 10.21037/jtd.2016.09.50
17. Newton AD, Predina JD, Nie S, Low PS, Singhal S. Intraoperative fluorescence imaging in thoracic surgery. *J Surg Oncol.* (2018) 118:344–55. doi: 10.1002/jso.25149
18. Okusanya OT, Madajewski B, Segal E, Judy BF, Venegas OG, Judy RP, et al. Small portable interchangeable imager of fluorescence for fluorescence guided surgery and research. *Technol Cancer Res Treat.* (2015) 14:213–20. doi: 10.7785/tcrt.2012.500400
19. Xia L, Zeh R, Mizelle J, Newton A, Predina J, Nie S, et al. Near-infrared intraoperative molecular imaging can identify metastatic lymph nodes in prostate cancer. *Urology.* (2017) 106:133–8. doi: 10.1016/j.urology.2017.04.020
20. Yamamichi T, Oue T, Yonekura T, Owari M, Nakahata K, Umeda S, et al. Clinical application of indocyanine green (ICG) fluorescent imaging of hepatoblastoma. *J Pediatr Surg.* (2015) 50:833–6. doi: 10.1016/j.jpedsurg.2015.01.014
21. Zeh R, Sheikh S, Xia L, Pierce J, Newton A, Predina J, et al. The second window ICG technique demonstrates a broad plateau period for near infrared fluorescence tumor contrast in glioblastoma. *PLoS One.* (2017) 12:e0182034. doi: 10.1371/journal.pone.0182034 eCollection 2017.
22. Newton AD, Predina JD, Corbett CJ, Frenzel-Sulyok LG, Xia L, Petersson EJ, et al. Optimization of second window indocyanine green for intraoperative near-infrared imaging of thoracic malignancy. *J Am Coll Surg.* (2019) 228:188–97. doi: 10.1016/j.jamcollsurg.2018.11.003
23. Fineman MS, Maguire JI, Fineman SW, Benson WE. Safety of indocyanine green angiography during pregnancy: a survey of the retina, macula, and vitreous societies. *Arch Ophthalmol.* (2001) 119:353–5. doi: 10.1001/archopht.119.3.353
24. Yamada Y. OhnoM, Fujino A, et al. Fluorescence-guided surgery for hepatoblastoma with indocyanine green. *Cancers (Basel).* (2019) 11:1215. doi: 10.3390/cancers11081215
25. Takahashi N, Yamada Y, Hoshino K, et al. Living donor liver re-transplantation for recurrent hepatoblastoma in the liver graft following complete eradication of peritoneal metastases under indocyanine green fluorescence imaging. *Cancers (Basel).* (2019) 11:730. doi: 10.3390/cancers11050730
26. Evans WE, Relling MV, de Graaf S, Rodman JH, Pieper JA, Christensen ML, et al. Hepatic drug clearance in children: studies with indocyanine green as a model substrate. *J Pharm Sci.* (1989) 78:452–6. doi: 10.1002/jps.2600780605
27. Kahramangil B, Kose E, Berber E. Characterization of fluorescence patterns exhibited by different adrenal tumors: Determining the indications for indocyanine green use in adrenalectomy. *Surgery.* (2018) 164:972–7. doi: 10.1016/j.surg.2018.06.012
28. Kawakita N, Takizawa H, Sawada T, Matsumoto D, Tsuboi M, Toba H, et al. Indocyanine green fluorescence imaging for resection of pulmonary metastasis of hepatocellular carcinoma. *J Thorac Dis.* (2019) 11:944–9. doi: 10.21037/jtd.2019.01.107
29. Purich K, Dang JT, Poonja A, Sun WYL, Bigam D, Birch D, et al. Intraoperative fluorescence imaging with indocyanine green in hepatic resection for malignancy: a systematic review and meta-analysis of diagnostic test accuracy studies. *Surg Endosc.* (2020) 34:2891–903. doi: 10.1007/s00464-020-07543-2
30. Indocyanine Green (ICG) Guided Tumor Resection. Available online at: https://clinicaltrials.gov/ct2/show/NCT04084067

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The reviewer TL declared a past co-authorship with one of the authors AD to the handling editor.

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frontiers in Surgery

CASE REPORT
published: 12 October 2021
doi: 10.3389/fsurg.2021.746700

Case Report: Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy Application in Intraperitoneally Disseminated Inflammatory Myofibroblastic Tumor and in the Youngest Patient in the World: New Indication and Modification of Technique

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Introduction: Peritoneal metastases occur in cancers that spread to the peritoneal cavity and indicate the advanced stage of the disease. In children they are mainly seen in sarcomas, Gastrointestinal Stromal Tumors and primary disseminated ovarian tumors. Inflammatory Myofibroblastic Tumor (IMT) is a very rare lesion, characterized by an unpredictable clinical course. The absorption of chemotherapeutic agents through the peritoneal-plasma barrier (PPB) is minimized, thus HIPEC procedure limits the systemic exposure to chemotherapy and permits the administration of its higher doses. The main purpose of HIPEC is to remove the visible macroscopic disease in order to achieve complete cytoreduction (CRS).

HIPEC Procedure in Children: Several papers deal with the CRS and HIPEC in children and adolescents, however pediatric experience is still limited. Thus far, the HIPEC procedure has been carried out on patients over 2 years old. The most common indication for the surgery and the best outcome was experienced by patients with desmoplastic small round cell tumor (DSRCT). Most patients received intraperitoneal cisplatin.

HIPEC Modification: A 5-month-old infant was admitted to the Department of Pediatric Oncology due to the abdominal distention and blood in the stool. The Computed Tomography (CT) revealed a solid-cystic mass in the right abdominal area. The primary tumor and numerous peritoneal metastasis were removed and

OPEN ACCESS

Edited by:
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Giannina Gaslini Institute (IRCCS), Italy

Reviewed by:
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University General Hospital Attikon, Greece
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Specialty section:
This article was submitted to Pediatric Surgery, a section of the journal Frontiers in Surgery

Received: *24 July 2021*
Accepted: *25 August 2021*
Published: *12 October 2021*

Citation:
Garnier H, Murawski M, Jastrzebski T, Pawinska-Wasikowska K, Balwierz W, Sinacka K, Gorecki W, Izycka-Swieszewska E and Czauderna P (2021) Case Report: Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy Application in Intraperitoneally Disseminated Inflammatory Myofibroblastic Tumor and in the Youngest Patient in the World: New Indication and Modification of Technique. Front. Surg. 8:746700. doi: 10.3389/fsurg.2021.746700

the Inflammatory Myofibroblastic Tumor (IMT) was diagnosed. The patient underwent subsequently CRS and modified HIPEC procedure. To avoid overheating of the infant, the intraperitoneal normothermic chemoperfusion was performed. Due to the low body weight a modified dosage of intraperitoneal doxorubicin was used. The child underwent standard postoperative chemotherapy and received crizotinib therapy. At 12 months follow-up since treatment completion the patient remains in complete remission. To our knowledge this is the youngest patient, the only infant and the first pediatric patient with IMT who underwent the modified HIPEC procedure in the world.

Conclusions: CRS and HIPEC is technically possible also in infants. For its safe course patients selection and technique modification are necessary. Use of HIPEC should be also considered in intraperitoneally disseminated IMT. A complete cytoreductive surgery as the first HIPEC step seems to be the key factor in survival.

Keywords: HIPEC (heated intraperitoneal chemotherapy), cytoreductive surgery, inflammatory myofibroblastic tumor, pediatric oncology, oncological surgical treatment

INTRODUCTION

Frequency of peritoneal metastasis of cancer or sarcoma in children still remains unknown. Peritoneal involvement is mainly seen in sarcomas (i.e., Desmoplastic Small Round Cell Tumor DSRCT, rhabdomyosarcoma, leiomyosarcoma, liposarcoma, etc.), GastroIntestinal Stromal Tumors (GIST), primary disseminated ovarian tumors (i.e., yolk sac tumor, Sertoli and Leydig cell tumors, ovarian carcinomas, etc.) (1–3). Peritoneal metastases involve cancers that spread to the peritoneal cavity and usually indicate an advanced stage of the disease.

Inflammatory Myofibroblastic Tumor (IMT), also called inflammatory pseudotumor, is a very rare pulmonary or extra-pulmonary lesion, characterized by an unpredictable clinical course. It can be benign, malignantly transformed, recurrent, or even metastasize. These tumors are very difficult to distinguish from other neoplasms and a detailed histologic analysis is required to establish the diagnosis. The pathogenesis of this disease remains unknown, but some IMTs have altered anaplastic lymphoma kinase (ALK) expression mostly resulting from rearrangement of the ALK gene and its fusion with other genes such as: TPM3-ALK, TPM4-ALK, and CLTC-ALK (4). These tumors consist of spindle-shaped myofibroblastic cells accompanied by inflammatory infiltration of plasma cells, lymphocytes, and eosinophils. IMT is characterized by a low mitotic index, no atypical division figures, necrosis, nuclear atypia, and above all, it seems not to spread through blood vessels (5). IMT can be a result of genetic mutation, or secondary to infectious or autoimmune disease. The treatment of choice is surgical resection of the lesion and subsequent chemo or radiotherapy, however due to the rare nature of IMT, proper guidelines have yet to be established. An aggressive surgical management is usually necessary due to the lack of other effective treatment. Due to the fact that the biology of myofibroblastic hyperplasia remains unpredictable, further observation of patients after surgery is necessary (6, 7).

The first detailed description of cytoreductive procedures within the peritoneum are found in Sugarbaker's work based on adults (8). With this early promising data, the interest promptly spread throughout the medical world in hope of finding better outcomes for oncological patients. Within the years several studies in animals demonstrated prolonged survival in groups receiving hyperthermic intraperitoneal chemotherapy (HIPEC) (9, 10). The absorption of chemotherapeutic agents through the peritoneal-plasma barrier (PPB) is minimized, thus HIPEC procedure limits the systemic exposure to chemotherapy and permits the administration of its higher doses. The main purpose of HIPEC is to remove the visible macroscopic disease with complete cytoreduction (CRS) and exposed the remnant lesions to intraperitoneal chemotherapy.

HIPEC AND CYTOREDUCTIVE SURGERY IN CHILDREN

The possibility of complete (CR0) or near complete (CR1) cytoreduction is of key importance in selecting a patient for the CRS and HIPEC procedure. Patient's survival depends primarily on the completeness of cytoreduction measured by the CR Scale (**Figure 1**). The inability to obtain macroscopic clearance at resection (CR0 or CR1) may result in a decision to withdraw from the HIPEC procedure and initiate palliative treatment (12). Other important eligibility criterion is lack of distant metastases. Hayes-Jordan et al. (13) proved that no disease outside the abdomen at the time of surgery ensures the best outcome (disease-free interval 37.9 vs. 14.3 months). Otherwise, liver metastases do not exclude patients from the CRS-HIPEC procedure providing it is possible to either resect them at the time of surgery or treat them with radiation, or radiofrequency ablation (14, 15). Normal kidney function also seems to play a crucial role in qualifying the child for the procedure. According to the Owusu-Agyemang et al. study (16), to avoid renal toxicity during the CRS-HIPEC procedure, especially with cisplatin, it is important

Cytoreduction Score	Definition
CR0	Resection of all gross disease, no unresectable tumor left
CR1	Resection of all gross disease, <2,5mm unresectable tumor left
CR2	2,5mm – 25mm unresectable tumor left
CR3	>25mm unresectable tumor left

FIGURE 1 | CR Scale. Completeness of CRS (11).

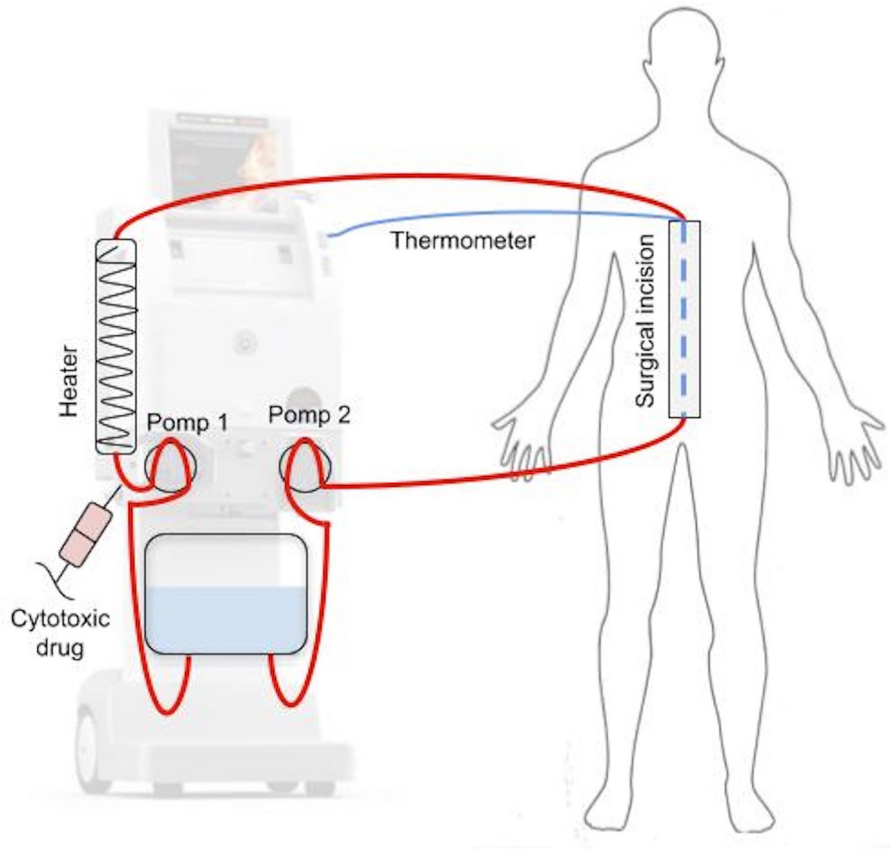

FIGURE 2 | Schematic overview of HIPEC.

to maintain the urine output at an average of 3 ml/kg/h and the fluid administration must oscillate at an average rate of 9 ml/kg/h. The relative selecting criteria for CRS+HIPEC are as follows: a minimum interval of 4 weeks from the last radiotherapy or chemotherapy and an interval of more than 4 months from the last HIPEC procedure, life expectancy of more than 6 weeks, and normal liver function (17). It also seems that CRS+HIPEC is more effective and increases the survival rate of children with stable disease or partial remission after prior chemical treatment (12).

The HIPEC and CRS procedure can be performed in two ways: opened (Coliseum) and closed technique (18). A significant advantage of the open technique is that it allows a uniform drug distribution within the peritoneal cavity. The disadvantage of this technique, however, is the heat loss of the perfusion fluid and the potential risk of contamination of the operating field. The closed technique, contrarily, is associated with uneven distribution of the chemotherapeutics but eliminates exposure of the operating team to the cytotoxic drugs. Furthermore, many authors have observed that it provides more stable intraoperative conditions, making it the most relevant choice for pediatric patients (19, 20). Lotti et al. (21) in their study drew attention to the usage of laparoscopy during the HIPEC procedure. It combines the advantages of both, open and closed techniques and could be an interesting alternative for children.

During HIPEC (**Figure 2**), after CRS phase, four drains are inserted into the peritoneal cavity: two delivering and two receiving the cytotoxic drug. Each of them is equipped with a

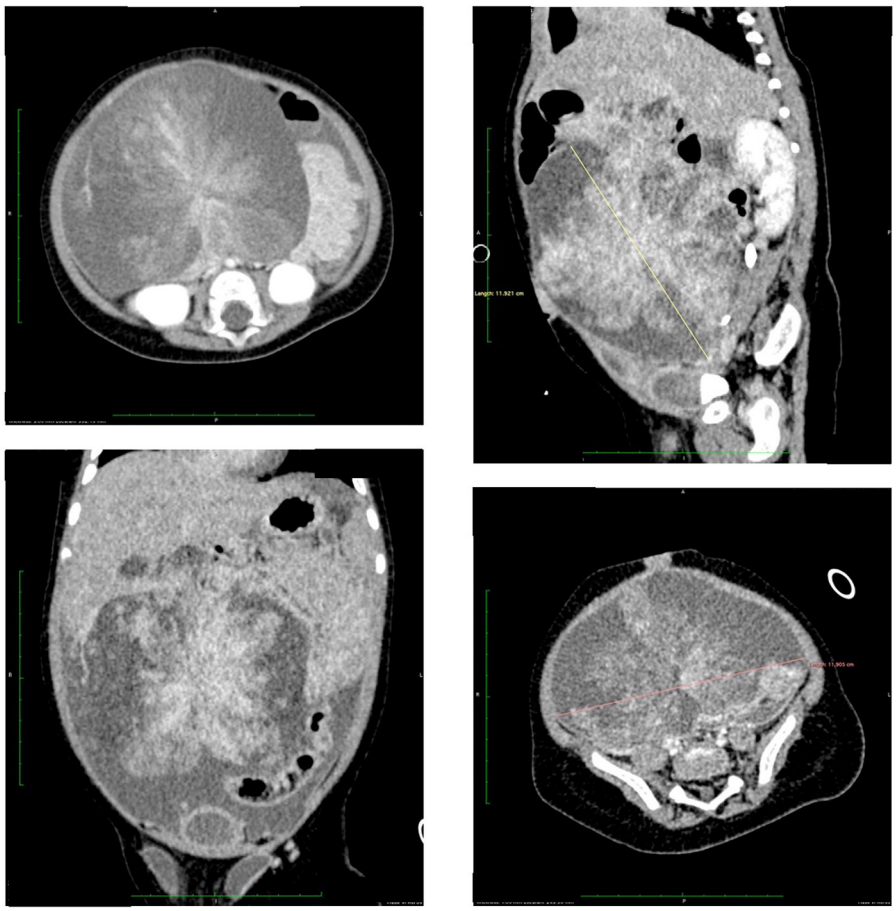

FIGURE 3 | Pre-operative CT scan: a solid-cystic mass (113 × 98 × 103 mm) in the right abdominal area.

thermometer to measure the temperature of the fluid entering and exiting the peritoneal cavity. Additionally, the temperature is usually measured in the sub-diaphragmic area and in the pelvis. Central temperature is measured with a temperature sensor located either in the esophagus or in the pulmonary artery. After insertion of the drains, perfusion fluid is administrated, usually Ringer's lactate, sodium chloride 0.9 or 5% glucose solution, depending on the anticancer drug used. The volume of fluid administrated ranges from 0.5 to 4 l and it is heated to 41–45°C. When the target temperature is reached, cytotoxic drugs are administrated. The perfusion time ranges between 30 and 90 min. After this period, the cytotoxic drugs are removed, and ∼3 l of clean perfusion fluid is administrated to rinse the peritoneal cavity. The duration of CRS + HIPEC ranges usually from 4 to 10 h (22, 23).

Intraperitoneal administration of chemotherapy maximizes the chemotherapeutic dose delivered to peritoneal lesions while minimizing systemic toxicity. The most commonly used intraperitoneal agents are cisplatin (100 mg/m^2) and doxorubicin (30 mg/m^2). However, use of other cytostatics, such as mitomycin C, oxaliplatin, carboplatin, 5-FU, or taxanes has also been described (24).

A CASE REPORT AND HIPEC PROCEDURE MODIFICATION

A 5-month-old infant presented to the emergency department due to the abdominal distention and blood in the stool. The Computed Tomography (CT) revealed a solid-cystic mass (113 × 98 × 103 mm) in the right abdominal area (**Figure 3**). In laboratory tests an increased C-reactive protein level was observed (40.9 mg/dL). Other parameters (blood count, asparate transaminase, alanine transaminase, alpha-fetoprotein, lactate dehydrogenase, neuron-specific enolase) were all within the age norm. Due to the unknown character of the tumor, the child underwent laparotomy elsewhere. A cecum tumor and numerous peritoneal metastasis were found intraoperatively. Primary tumor with cecum and all visible metastasis (in peritoneum and greater omentum) were removed and ileo-colonic anastomosis was performed. Postoperative course was uneventful and the child was discharged from the hospital on the day 11 after surgery without complications. Pathological examination revealed a non-RMS neoplasm—ALK1-positive Inflammatory Myofibroblastic Tumor (IMT). Two weeks after the surgery, the control MRI did not reveal any pathological lesions. Due to the presence of

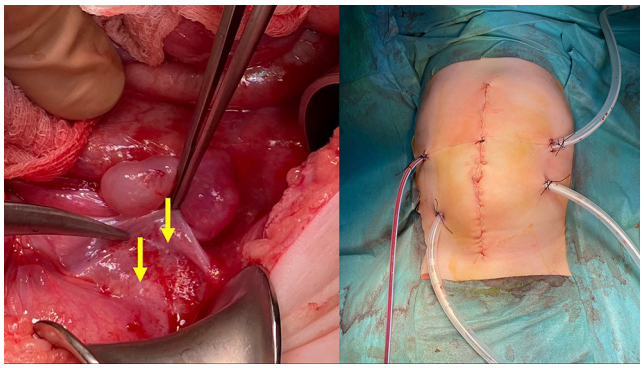

FIGURE 4 | On the left—intraoperative image: pelvic peritoneum with tumor implants (yellow arrows). On the right—abdomen during HIPEC.

numerous intraperitoneal metastases during the first surgery the patient was qualified to HIPEC procedure without neoadjuvant chemotherapy. An exploratory laparotomy was performed before to proceed with HIPEC. Several peritoneal metastasis were removed from vesico-uterine pouch, pouch of Douglas and total pelvic peritonectomy was performed (**Figure 4**). At the end of the CRS, the CR was complete (CR0) (**Figure 1**). After the CRS procedure the patient underwent subsequently modified HIPEC procedure. To avoid overheating of the infant, the intraperitoneal normothermic chemoperfusion was performed in 30 min. Due to the low weight of the infant a modified dosage (9.2 mg) of intraperitoneal Doxorubicin was used. The child underwent standard postoperative chemotherapy (CWS-Guidance 2014) and received crizotinib therapy. At 12 months follow-up since treatment completion the patient remains in complete remission. To our knowledge this is the youngest patient, the only infant and the only pediatric patient with IMT who underwent the modified HIPEC procedure in the world.

DISCUSSION

The first pediatric reports on CRS and HIPEC was presented by Hayes-Jordan et al. in 2015 and 2018 (13, 25). According to her study CRS + HIPEC may be the most effective in children with desmoplastic small round cell tumor (DSRCT), although other histology was also admissible. According to our knowledge there are no pediatric IMT cases treated with HIPEC reported in the literature. The gold standard for IMT is surgical treatment, although chemotherapy and radiotherapy are feasible alternatives to surgery. Tao et al. (26) presented a case successfully additionally treated with non-steroid anti-inflammatory drugs (diclofenac sodium). Steroids have also been reported to be effective, especially for IMT containing IgG4SD features (27). On the other hand, the clinical trial on crizotinib administration combined with surgical treatment for ALK-positive patients resulted in complete remission in most of the cases (28). The European pediatric Soft Tissue Sarcoma Study Group (EpSSG) has recently summarized their experience in treating IMT proving its high response to chemotherapy (especially vinblastine and low-dose methotrexate) and suggested the usage of targeted inhibitors in the standard of care (29). The principal problem in the treatment of peritoneal tumors with neoadjuvant or adjuvant chemotherapy is the limited drug absorption throughout the physiological peritoneal plasma barrier (30). In such cases local application of cytotoxic drugs seems to play an important role. Intraperitoneal chemotherapy ensures a high concentration of the drug in the peritoneal cavity and reduces its systemic side effects. It should be emphasized that the macroscopic excision of all visible lesions (CRS) is crucial for the positive effect of the therapy due to the limited penetration of cytotoxic drugs into the tissues (∼1 mm) (31). The time from surgery to administration of peritoneal chemotherapy is also important. HIPEC procedure in combination with CRS ensures better penetration of the drug (before the healing processes and formation of fibrin and adhesions start). Due to all of that, in the presented case CRC+HIPEC combined with postoperative chemotherapy and crizotinib seemed to be the best treatment option.

Identifying appropriate dosing regimens for the treatment of neonates and infants with cancer is a significant challenge in pediatric oncology. Most anti-cancer drugs given to children are dosed using only body surface area (BSA). However, infant's development differs significantly from older children. Thus, the cytotoxic drugs dosage should be different. In 2017 Balis et al. (32) described a modified infant chemotherapy dosing, calculated using not only BSA, but also developmental milestones. Following the above recommendations the dosage of Doxorubicin for the presented case was calculated.

Since abdominal location of IMT and its peritoneal spread is very rare and there are no more cases treated with HIPEC described in the literature no definitive conclusions can be made. Further studies involving larger patients groups are needed.

CONCLUSIONS

CRS and HIPEC is technically possible also in infants. For its safe course patients selection and technique modification are necessary. In the world literature the best HIPEC outcome was experienced in the treatment of DSRCT, but it should be also considered in intraperitoneally disseminated IMT. A complete cytoreductive surgery preceding HIPEC directly seems to be the key factor in survival.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

Written informed consent was obtained from the participant's next of kin for the publication of this case report. Written informed consent was obtained from the participant's next of kin for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

HG, MM, TJ, KP-W, WB, KS, WG, EI-S, and PC: substantial contributions to the conception or design of the work, the acquisition, analysis, or interpretation of data for the work, provide approval for publication of the content, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. HG, TJ, and PC: drafting the work or revising it critically for important intellectual content. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Kaste SC, Marina N, Frryrear R, Hedlund GL, Jones L, Poe D, et al. Peritoneal metastases in children with cancer. *Cancer*. (1998) 83:385–90. doi: 10.1002/(SICI)1097-0142(19980715)83:2<385::AID-CNCR25>3.0.CO;2-O
2. James KM, Boogue COO, Murphy AJ, Navarro OM. Peritoneal malignancy in children: a pictorial review. *Can Assoc Radiol J*. (2016) 67:402–8. doi: 10.1016/j.carj.2016.03.003
3. Moulik NR, Vaidya S, Mandeville H, Chisholm JC. Managing peritoneal involvement in children and young people with rhabdomyosarcoma: a single-center experience from the United Kingdom. *Pediatr Blood Cancer*. (2019) 66:e27805. doi: 10.1002/pbc.27805
4. Hisaoka M, Shimajiri S, Matsuki Y, Meis-Kindblom JM, Kindblom L-G, Li X-Q, et al. Inflammatory myofibroblastic tumor with predominant anaplastic lymphoma kinase-positive cells lacking a myofibroblastic phenotype. *Pathol Int*. (2003) 53:376–81. doi: 10.1046/j.1440-1827.2003.01484.x
5. Li H, Wang D-L, Liu X-W, Geng Z-J, Xie C-M. MRI characterization of inflammatory myofibroblastic tumors in the maxillofacial region. *Diagn Interv Radiol*. (2014) 20:310–5. doi: 10.5152/dir.2014.13348
6. Dalton BGA, Thomas PG, Sharp NE, Manalang MA, Fisher JE, Moir CRR, et al. Inflammatory myofibroblastic tumors in children. *J Pediatr Surg*. (2016) 51:541–4. doi: 10.1016/j.jpedsurg.2015.11.015
7. Palaskar S, Koshti S, Maralingannavar M, Bartake A. Inflammatory myofibroblastic tumor. *Contemp Clin Dent*. (2011) 2:274–7. doi: 10.4103/0976-237X.91787
8. Sugarbaker HP. Peritonectomy procedures. *Ann Surg*. (1995) 221:29–42. doi: 10.1097/00000658-199501000-00004
9. Koga S, Hamazoe R, Maeta M, Shimizu N, Kanayama H, Osaki Y. Treatment of implanted peritoneal cancer in rats by continuous hyperthermic peritoneal perfusion in combination with an anticancer drug. *Cancer Res*. (1984) 44:1840–2.
10. Miailhe G, Arfi A, Mirshahi M, Eveno C, Pocard M, Touboul C. A new animal model for hyperthermic intraperitoneal chemotherapy (HIPEC) in tumor-bearing mice in the treatment of peritoneal carcinomatosis of ovarian origin. *J Visc Surg*. (2018) 55:183–9. doi: 10.1016/j.jviscsurg.2017.10.008
11. Fuzun M, Sokmen S, Terzi C, Canda AE. Cytoreductive approach to peritoneal carcinomatosis originated from colorectal cancer: Turkish experience. *Acta Chir Iugosl*. (2006) 53:17–21. doi: 10.2298/ACI0602017F
12. Zmora O, Hayes-Jordan A, Nissan A, Kventsel I, Newmann Y, Itskovsky K, et al. Cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) for disseminated intra-abdominal malignancies in children-a single-institution experience. *J Pediatr Surg*. (2018) 53:1381–6. doi: 10.1016/j.jpedsurg.2017.09.014
13. Hayes-Jordan A, Green H, Lin H, Owusu-Agyemang P, Mejia R, Okhuysen-Cawley R, et al. Cytoreductive surgery and Hyperthermic Intraperitoneal Chemotherapy (HIPEC) for children, adolescents, and young adults: the first 50 cases. *Ann Surg Oncol*. (2015) 2:1726–32. doi: 10.1245/s10434-014-4289-y
14. Lo Dico R, Faron M, Yonemura Y, Glehen O, Pocard M, Sardi A, et al. Combined liver resection and cytoreductive surgery with HIPEC for metastatic colorectal cancer: results of a worldwide analysis of 565 patients from the Peritoneal Surface Oncology Group International (PSOGI). *Eur J Surg Oncol*. (2021) 47:89–100. doi: 10.1016/j.ejso.2020.07.038
15. Cracco A, Roy M, Simpfendorfer CH. Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy combined with two-stage hepatectomy for multiple and bilobar desmoplastic small round cell tumor liver metastases. *J Gastrointest Oncol*. (2017) 8:E60–4. doi: 10.21037/jgo.2017.07.07
16. Owusu-Agyemang P, Arunkumar R, Green H, Hurst D, Landoski K, Hayes-Jordan A. Anesthetic management and renal function in pediatric patients undergoing cytoreductive surgery with continuous hyperthermic intraperitoneal chemotherapy (HIPEC) with cisplatin. *Ann Surg Oncol*. (2012) 19:2652–6. doi: 10.1245/s10434-012-2319-1
17. Votanopoulos KI, Ihemelandu C, Shen P, Stewart JH, Russell GB, Levine EA. Outcomes of repeat cytoreductive surgery with hyperthermic intraperitoneal chemotherapy for the treatment of peritoneal surface malignancy. *J Am Coll Surg*. (2012) 215:412–7. doi: 10.1016/j.jamcollsurg.2012.04.023
18. Leiting JL, Cloyd JM, Ahmed A, Fournier K, Lee AJ, Dessureault S, et al. Comparison of open and closed hyperthermic intraperitoneal chemotherapy: results from the United States hyperthermic intraperitoneal chemotherapy collaborative. *World J Gastrointest Oncol*. (2020) 12:756–67. doi: 10.4251/wjgo.v12.i7.756
19. Halkia E, Tsochrinis A, Vassiliadou DT, Pavlakou A, Vaxevanidou A, Datsis A, et al. (2015). Peritoneal carcinomatosis: intraoperative parameters in open (coliseum) versus closed abdomen HIPEC. *Inter J Surg Oncol*. (2015) 2015:610597. doi: 10.1155/2015/610597
20. Rodriguez Silva C, Moreno Ruiz FJ, Bellido Estevez I, Carraasco Campos J, Titos Garcia A, Ruiz Lopez M, et al. Are there intra-operative hemodynamic differences between the Coliseum and closed HIPEC techniques in the treatment of peritoneal metastasis? A retrospective cohort study. *World J Surg Oncol*. (2017) 15:51. doi: 10.1186/s12957-017-1119-2
21. Lotti M, Giulii Capponi M, Piazzalunga D, Poiasina E, Pisano M, Manfredi R, et al. Laparoscopic HIPEC: a bridge between open and closed-techniques. *J Minim Access Surg*. (2016) 12:86–9. doi: 10.4103/0972-9941.158965
22. Gonzalez-Moreno S, Gonzalez-Bayon LA, Ortega-Perez G. Hyperthermic intraperitoneal chemotherapy: rationale and technique. *World J Gastrointest Oncol*. (2010) 2:68–75. doi: 10.4251/wjgo.v2.i2.68
23. Valle SJ, Alzahrani NA, Liauw W, Sugarbaker PH, Bhatt A, Morris DL. Hyperthermic Intraperitoneal Chemotherapy (HIPEC) methodology, drugs and bidirectional chemotherapy. *Indian J Surg Oncol*. (2016) 7:152–9. doi: 10.1007/s13193-016-0498-0
24. Goodman MD, McPartland S, Detelich D, Wasif Saif M. Chemotherapy for intraperitoneal use: a review of hyperthermic intraperitoneal chemotherapy and early post-operative intraperitoneal chemotherapy. *J Gastrointest Oncol*. (2016) 7:45–57. doi: 10.3978/j.issn.2078-6891.2015.111
25. Hayes-Jordan AA, Coakley BA, Green HL, Xiao LC, Fournier KF, Herzog CE, et al. Desmoplastic small round cell tumor treated with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy: results of a phase 2 trial. *Ann Surg Oncol*. (2018) 25:872–7. doi: 10.1245/s10434-018-6333-9
26. Tao YL, Wang ZJ, Han JG, Wei P. Inflammatory myofibroblastic tumor successfully treated with chemotherapy and nonsteroidals: a case report. *World Gastroenterol J*. (2012) 18:7100–3. doi: 10.3748/wjg.v18.i47.7100
27. Saab ST, Hornick JL, Fletcher CD, Olson SJ, Coffin CM. IgG4 plasma cells in inflammatory myofibroblastic tumor: inflammatory marker or pathogenic link? *Mod Pathol*. (2011) 24:606–12. doi: 10.1038/modpathol.2010.226
28. Butrynski JE, D'Adamo DR, Hornick JL, Dal Cin P, Antonescu CR, Jhanwar SC, et al. Crizotinib in ALK-rearranged inflammatory myofibroblastic tumor. *N Engl Med J*. (2010) 363:1727–33. doi: 10.1056/NEJMoa1007056
29. Casanova M, Brennan B, Alaggio R, Kelsey A, Orbaach D, van Noesel MM, et al. Inflammatory myofibroblastic tumor: the experience of the European pediatric Soft Tissue Sarcoma Study Group (EpSSG). *Eur Cancer J*. (2020) 127:123–9. doi: 10.1016/j.ejca.2019.12.021

30. Jacquet P, Sugarbaker PH. Peritoneal-plasma barrier. *Cancer Treat Res*. (1996) 82:53–63. doi: 10.1007/978-1-4613-1247-5_4
31. Mikolajczyk A, Khosrawipour T, Martino A, Kulas J, Pieczka M, Zacharski M, et al. Enabling Microparticle Imprinting to Achieve Penetration and Local Endurance in the Peritoneum via High-Intensity Ultrasound (HIUS) for the Treatment of Peritoneal Metastasis. *Int J Surg Oncol*. (2020) 9679385. doi: 10.1155/2020/9679385
32. Balis FM, Womer RB, Berg S, Winick N, Adamson PC, Fox E, et al. Dosing anticancer drugs in infants: current approach and recommendations from the Children's Oncology Group's Chemotherapy Standardization Task Force. *Pediatr Blood Cancer*. (2017) 91:56–67. doi: 10.1002/pbc.26636

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frontiers in Pediatrics

SYSTEMATIC REVIEW
published: 13 October 2021
doi: 10.3389/fped.2021.706800

Roles of Surgery in the Treatment of Patients With High-Risk Neuroblastoma in the Children Oncology Group Study: A Systematic Review and Meta-Analysis

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Purpose: Neuroblastoma is the most common extracranial solid tumor in children, and most patients are at high risk when they are initially diagnosed. The roles of surgery and induction chemotherapy in patients with high-risk neuroblastoma have been a subject of much controversy and debate. The objective of the current study was to assess the roles of surgery in high-risk neuroblastoma.

Method: The review protocol was prospectively registered (PROSPEROID: CRD42021253961). The PubMed, Embase, Cochrane, and CNKI databases were searched from inception to January 2020 with no restrictions on language or publication date. Clinical studies comparing the outcomes of different surgical ranges for the treatment of high-risk neuroblastoma were analyzed. The Mantel–Haenszel method and a random effects model was utilized to calculate the hazard ratio (95% CI).

Results: Fourteen studies that assessed 1,915 subjects met the full inclusion criteria. Compared with the gross tumor resection (GTR) group, complete tumor resection (CTR) did not significantly improve the 5-year EFS [$p = 1.0$; HR = 0.95 (95% CI, 0.87–1.05); $I^2 = 0\%$], and the 5-year OS [$p = 0.76$; HR = 1.08 (95% CI, 0.80–1.46); $I^2 = 0\%$] of patients. GTR or CTR resection had significantly better 5-year OS [p = 0.45; HR = 0.56 (95% CI, 0.43–0.72); $I^2 = 0\%$] and 5-year EFS [$p = 0.15$; HR = 0.80 (95% CI, 0.71–0.90); $I^2 = 31\%$] than subtotal tumor resection (STR) or biopsy only; however, both CTR or GTR showed a trend for more intra and post-operative complications compared with the STR or biopsy only [$p = 0.37$; OR = 1.54 (95% CI, 1.08–2.20); $I^2 = 0\%$]. The EFS of the patients who underwent GTR or CTR at the time of diagnosis and after induction chemotherapy were similar [$p = 0.24$; HR = 1.53 (95% CI, 0.84–2.77); $I^2 = 29\%$].

Conclusion: For patients with high-risk neuroblastoma, complete tumor resection and gross tumor resection of the primary tumor were related to improved survival, with very limited effects on reducing intraoperative and postoperative complications. It is

OPEN ACCESS

Edited by:
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Giannina Gaslini Institute (IRCCS), Italy

Reviewed by:
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Dalhousie University, Canada
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Specialty section:
This article was submitted to Pediatric Surgery, a section of the journal Frontiers in Pediatrics

Received: *08 May 2021*
Accepted: *03 September 2021*
Published: *13 October 2021*

Citation:
Qi Y and Zhan J (2021) Roles of Surgery in the Treatment of Patients With High-Risk Neuroblastoma in the Children Oncology Group Study: A Systematic Review and Meta-Analysis. Front. Pediatr. 9:706800. doi: 10.3389/fped.2021.706800

necessary to design strong chemotherapy regimens to improve the survival rate of advanced patients.

Systematic Review Registration: https://www.crd.york.ac.uk/PROSPERO/, PROSPEROID [CRD42021253961].

Keywords: high-risk neuroblastoma, resection, induction chemotherapy, meta-analysis, surgery

INTRODUCTION

Neuroblastoma originating from the adrenal medulla or sympathetic ganglion is the most common extracranial solid tumor in children. It is most common in the abdomen (75%), followed by the mediastinum (20%) and the neck (5%) (1). The Children Oncology Group (COG) classified neuroblastoma patients as low-risk, medium-risk, or critical based on age at diagnosis, biological characteristics of the tumor including MYCN status and genomic segmental aberrations, International Neuroblastoma Pathology Classification (INPC), tumor DNA index, and tumor stage as defined by the International Neuroblastoma Staging System (INSS) (2). Notably, however, groups from different regions of the world do not use a consistent approach to classify patient risk. Investigators of major national and international cooperative groups from North America (COG), Europe (SIOPEN-R-NET), Germany (GPOH), and Japan (JANB/JINCS) developed the International Neuroblastoma Risk Group (INRG) classification and staging systems (INRGSS) using data from over 8,000 neuroblastoma patients internationally. The INRG classification system assigns neuroblastoma patients to 1 of 16 pretreatment risk groups based on INRGSS, age, histologic category, grade of tumor differentiation, MYCN amplification, and 11q aberration. Children with high-risk neuroblastoma account for approximately half of all patients diagnosed with neuroblastoma. Combined studies indicate that long-term survival rates of children with high-risk neuroblastoma are currently ∼40–50% (3). Current therapy for high-risk neuroblastoma (HRNB) consists of combinations of intensive multi-agent induction chemotherapy, surgery, radiation, myeloablative consolidation therapy with stem cell rescue and transplantation, 13-cis retinoic acid, and immunotherapy (4). The prognosis of children in the high-risk group is poor, and the long-term disease-free survival rate is <50%. There is an urgent need to improve the prognosis of neuroblastoma, especially in refractory or high-risk patients, and increase the tumor resection rate and reduce the risks associated with surgery (5).

Because of the small number of published studies and their heterogeneity, many systematic reviews have reported inconsistent results. The current investigation was an updated meta-analysis on the survival rate and complications associated with surgery for neuroblastoma. More specifically, a systematic review and meta-analysis was performed to identify the effects of complete tumor resection (CTR), gross tumor resection (GTR), subtotal tumor resection (STR), and biopsy only (BX) on overall survival (OS), event-free survival (EFS), and complications. Whether induction chemotherapy has any positive effects on survival rates was also investigated.

MATERIALS AND METHODS

Search Strategy

The review protocol was prospectively registered (PROSPEROID: CRD42021253961). A systematic review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline (6). The search was conducted in the major electronic databases of PubMed, Embase, and CNKI, we had no restrictions on the language of the article.

Taking PubMed as the example, the keyword of the search was "(((surgery[Title/Abstract]) OR (resection[Title/Abstract])) AND (neuroblastoma[Title/Abstract]))NOT(olfactory)"; "Neuroblastoma/surgery"[Mesh]; (neuroblastoma[Title]) AND (surgical[Title]), (surgery[Title/Abstract]) AND ((induction chemotherapy) OR (neoadjuvant chemotherapy)).

When several studies reported findings for the same patients, the most recent or most complete study was chosen.

Inclusion Criteria

Studies were included on the basis of the following criteria: (1) patients who were diagnosed as high risk of NB according to Children's Oncology Group (COG) risk stratification; (2) the patients underwent surgery, or only underwent pathological puncture, or received induction chemotherapy before the operation; (3) the scope of the operation was determined; (4) the follow-up period of the patient was at least 5 years, and the OS or EFS of the patient was involved; and (5) there is a record of the number of intraoperative or postoperative complications.

We excluded some articles in which the follow-up time was not enough and the survival rate was calculated by combining patients with GTR and patients with STR or biopsy only. The study would be excluded if the percentage of the tumor removal was not clearly stated in the article. A few of the studies, including patients with low or mediate risk neuroblastoma, also were excluded (**Figure 1**).

Definitions

For the meta-analysis, we collected those who carried out CTR (complete tumor resection), GTR (gross tumor resection), STR (subtotal tumor resection), or BX (biopsy only), and those who accepted the induction chemotherapy before the operation. "CTR" represents macroscopic total removal of all visible tumor and nearby abnormal lymph nodes, "GTR" represents resection of tumor leaving a minimal macroscopic residue or removal of more than 90 or 95% of the visible tumor, "STR" represents removal of more than 50% but <90 or 95% of the visible tumor. "induction chemotherapy" means that systemic chemotherapy

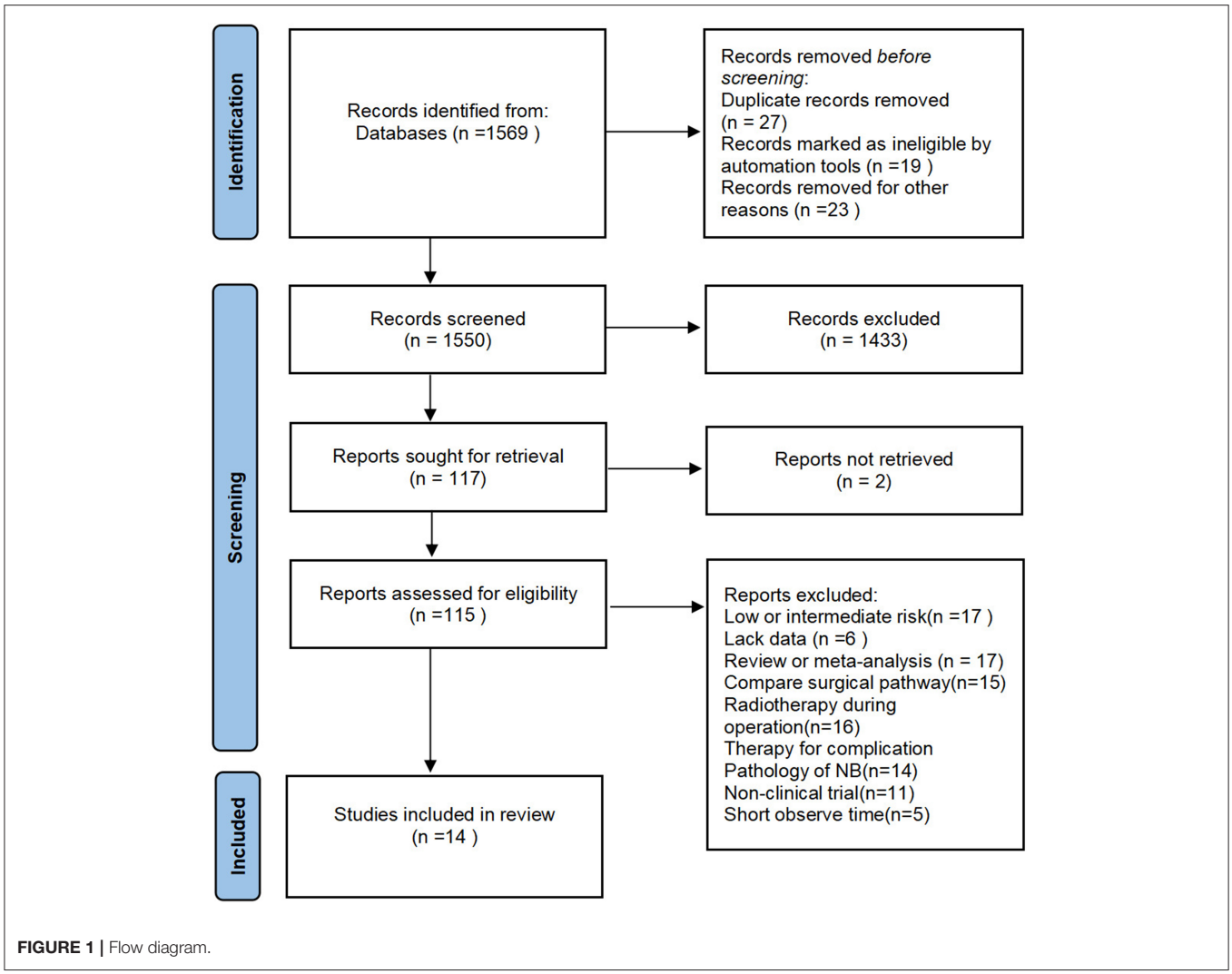

FIGURE 1 | Flow diagram.

prior to local treatment, such as surgery or radiotherapy, is intended to shrink the mass and kill invisible metastatic cells early enough to facilitate subsequent surgery, radiotherapy, and so on (7).

Data Extraction

We reviewed all titles and abstracts to determine eligibility and retrieve articles. The following information was extracted according to a fixed protocol: study design, geographical location, stage, sample size, group number, number of complications (**Table 1**). The long-term survival rate was defined by the 5-year OS and EFS.

Validity Assessment

The quality of included studies was accessed independently by using the Newcastle–Ottawa Quality Assessment Scale. The scale was comprised of three factors: patient selection, comparability of the study groups, and assessment of outcome. A score of 1 was awarded for each item if the standard was completely met, a score of 0.5 was awarded if the standard was partially met, and a score of 0 was awarded if it was not met or if it was unclear whether it was met. The total score for each study was then calculated, a score of >6 indicated a high-quality study, a score of ≥ 3 and ≤ 6 indicated a median-quality study, while a score of ≥ 0 and ≤ 2 indicated a low-quality study (21).

Statistical Analysis

Hazard ratios (HRs) with 95% CIs were calculated according to calculate lnHR and its variance by survival curves. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated based on the reported numbers of patients and events. The significance of the pooled OR/HR was evaluated by a Z-test, and a p-value of <0.05 was considered significant. Statistical heterogeneity among studies was evaluated by I^2 and Q statistics. I^2 values of $<50\%$ correspond to low levels of heterogeneity sensitivity and subgroup analyses were used to explore potential causes of heterogeneity (22). A p-value of <0.05 was considered significant for heterogeneity. Publication bias was assessed with funnel plots (23).

TABLE 1 | Characteristics of included studies.

References	Country	Stage	Sample size	Group number		No. of complication
Vollemer et al. (8)	Germany	IV[a]:40	40	50–90%/BX[b]:11 100/>90%:29		50–90%/BX:2 100/>90%:15
Adkins et al. (9)	USA	II:1 III:72 IV:466	539	Initial surgery 100%:120; >95%:60<95%:51;BX:213	Best surgery 100%:210>95%: 115<95%:74;BX:69	Best surgery 100%:60>95%:44 <95%:27
Englum et al. (10)	USA	NA[c]	87	100%:33;>90%: 23<90%:21;BX:7		
von Allmen (24)	USA	NA	220	100%/>90%:154<90%/BX:66		100%/>90%:37 <90%/BX:13
			84	100%/>90%:62 <90%/BX:22		
Li et al. (11)	China	NA	96	100%:39;>90%:23<90%:10; BX:24		
Castel et al. (12)	Spain	IV:98	98	Initial surgery 100%:4; >90%:1 <90%:1;BX:74	Best surgery 100%:39;>90%:21 <90%:11;BX:5	
Mcgregor et al. (13)	USA	NA	124	>95%:7<95%:3;BX:114	>95%:83<95%:5; <50%:9	
Simon et al. (14)	Germany	IV:278	278	Initial surgery 100%:17;>90%:2 <90%:12; BX:246	Best surgery 100%:152;>90%:68 <90%:17;BX:37	
De Ioris et al. (15)	Italy	NA	58	>95%/100%:45 <95%/BX:13		
Yeung et al. (16)	China	IV:34	34	100%:24;>95%:6 <95%:4		
Koh et al. (17)	China	IV:19	19	100%:9;>95%: <95%:5		
von Allmen et al. (18)	USA	NA	76	100%:48;>90%:12 50–90%:10;BX:6		
Salim et al. (19)	UK	III:13 IV:56	63	>95%:21;<95%:19 BX:23		
Tsuchida et al. (20)	Japan	IV:102	102	100%/<100%:75 BX:10		

[a] *Staging on the basis of International Neuroblastoma Staging System (INSS) criteria.*
[b] *The percentage represents the degree of tumor resection.*
BX, biopsy only.
NA, not available.

RESULTS

Fourteen studies that assessed 1,915 subjects were included in the meta-analysis. The sample size ranged from 40 to 539 issues (**Table 1**). All of the studies were published in or after 1992. Their validity scores are shown in **Table 2**. Seven articles are of high quality, nine articles are of medium quality, and low-quality articles were not included in this meta-analysis. von Allmen et al. (24) analyzed two groups of patients, one of these groups were determined by local surgeons' assessment, the other was determined by imaging central review. Adkins et al. (9), Castel et al. (12), and Simon et al. (14) separately recorded the survival rate of patients with the initial operation at diagnosis and delayed operation after induction chemotherapy.

Meta-Analysis Findings

Compared with the gross tumor resection (GTR) group, complete tumor resection (CTR) did not significantly improve the 5-year EFS [$p = 1.0$; HR = 0.95 (95% CI, 0.87–1.05); $I^2 = 0\%$] (**Figure 2**) and 5-year OS [$p = 0.76$; HR = 1.08 (95% CI, 0.80–1.46); $I^2 = 0\%$] of the patients (**Figure 3**). GTR or CTR resection had significantly better 5-year OS [$p = 0.45$; HR = 0.56 (95% CI, 0.43–0.72); $I^2 = 0\%$] (**Figure 4**) and 5-year EFS [$p = 0.15$; HR = 0.80 (95% CI, 0.71–0.90); $I^2 = 31\%$] (**Figure 5**) than subtotal tumor resection (STR) or biopsy only; however, both CTR or GTR showed a trend for more intra and post-operative complications compared with the STR or biopsy only [$p = 0.37$; OR = 1.54 (95% CI, 1.08–2.20); $I^2 = 0\%$](**Figure 6**). The EFS of

TABLE 2 | The score of included studies.

References	Selection	Comparability	Outcome	Total
Vollemer (8)	2.5	0	2.5	5
Adkins et al. (9)	3	1	2.5	6.5
Englum et al. (10)	3	1	2.5	6.5
von Allmen (24)	3	1	2.5	6.5
Li et al. (11)	3	1	2	6
Castel et al. (12)	2.5	0	2.5	5
Mcgregor et al. (13)	3	1	2.5	6.5
Simon et al. (14)	3	1	2.5	6.5
De Ioris et al. (15)	3	1	2.5	6.5
Yeung et al. (16)	3	1	2	6
Koh et al. (17)	2.5	1	2.5	6
von Allmen et al. (18)	3	1	2	6
Tsuchida et al. (20)	2.5	1	2	5.5
Salim et al. (19)	2.5	1	2.5	6

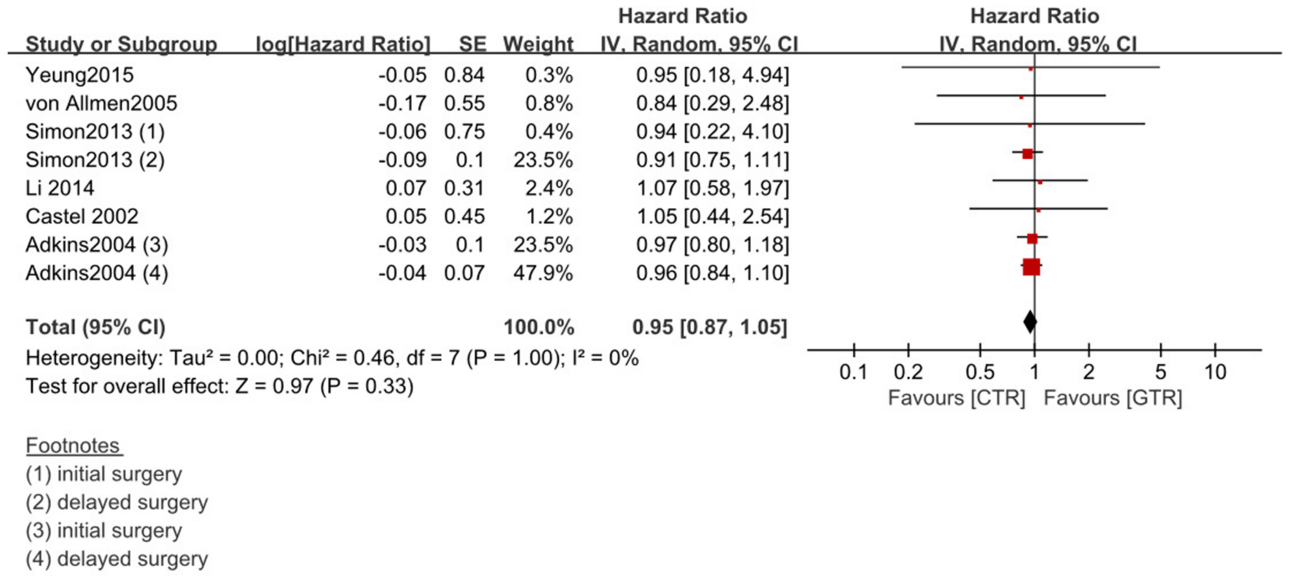

FIGURE 2 | Effect of complete tumor resection (CTR) vs. gross tumor resection (GTR) on 5-year event-free survival (EFS) in high-risk neuroblastoma patients. Weights are from Mantel–Haenszel random effects analysis. Hazard ratios are shown with 95% confidence intervals.

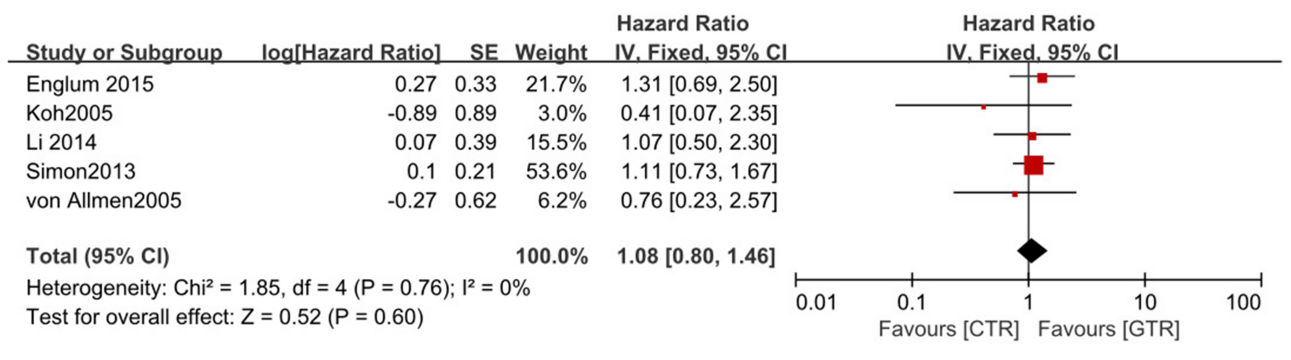

FIGURE 3 | Effect of CTR vs. GTR on 5-year overall survival (OS) in high-risk neuroblastoma patients. Weights are from Mantel–Haenszel random effects analysis. Hazard ratios are shown with 95% confidence intervals.

the patients who underwent GTR or CTR at the time of diagnosis and after induction chemotherapy were similar [$p = 0.24$; HR = 1.53 (95% CI, 0.84–2.77); $I^2 = 29\%$] (**Supplementary Figure 1**).

Subgroup Analysis

We repeated the meta-analyses on the basis of year (>2010 or <2010) and quality (high or moderate; **Table 3**), and the result is consistent with that of the primary meta-analysis.

Publication Bias

In the funnel diagram of the GTR or CTR vs. STR or biospy only (**Supplementary Figure 2**) group and CTR vs. GTR group (**Supplementary Figure 3**), grouping of studies at the apex of the plot suggested that larger studies with higher patient numbers are more likely to have been included. The lack of studies gathered at the base of the plot suggests a paucity of publications of smaller sample size.

DISCUSSION

Neuroblastoma is the most common extracranial solid tumor in children, and most patients are at high risk when they are initially diagnosed. The roles of surgery and induction chemotherapy in patients with high-risk neuroblastoma have been a subject of much controversy and debate. The objective of the current study was to assess the roles of surgery in high-risk neuroblastoma.

Effects Between Gross Tumor Resection and Complete Tumor Resection on Event-Free Survival and Overall Survival

A systematic review by Zwaveling et al. (25) investigated the current status of surgical treatment of neuroblastoma. Of the 20 studies included in their analysis, only 4 explicitly compared

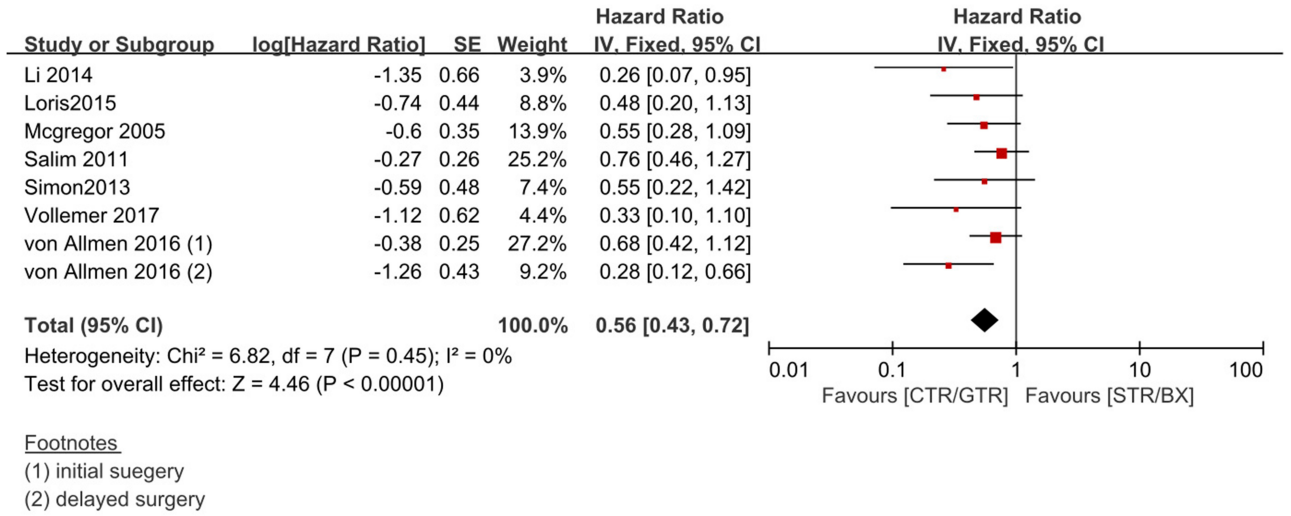

FIGURE 4 | Effect of CTR/GTR vs. subtotal tumor resection (STR)/biopsy only (BX) on 5-year OS in high-risk neuroblastoma patients. Weights are from Mantel–Haenszel random effects analysis. Hazard ratios are shown with 95% confidence interval.

survival in patients who underwent CTR with survival in patients who underwent GTR. In 2 of these studies, CTR yielded more favorable results than GTR, whereas in the other 2 there were no significant differences in survival. The authors concluded that a true comparison of the effects of surgery on survival based on previous studies was severely hampered. In the current meta-analysis, seven studies compared survival rates in GTR and CTR groups. Li et al. (11) and Simon et al. (14) reported that survival was similar in the CTR and GTR groups. Although, Adkins et al. (9), Castel et al. (12), Yeung et al. (16), Koh et al. (17), and von Allmen et al. (24) did not perform statistical comparisons between CTR and GTR groups, they all followed up the two groups of patients postoperatively and plotted survival curves. Compared with GTR, in addition to an increased extent of resection, lymph node dissection around the primary site or even primary organ resection achieved the purpose of complete resection in some groups. In the current meta-analysis CTR had little effect on the survival rate of neuroblastoma patients compared with GTR. Therefore, it is not necessary to pursue complete resection with lymph node dissection or removal of the primary organ.

Effects Between Gross Tumor Resection/Complete Tumor Resection and Subtotal Tumor Resectioon/Biopsy Only on Event-Free Survival and Overall Survival

Nine studies in the current meta-analysis compared OS and EFS in patients treated between CTR/GTR and STR/biopsy only, but the results were not consistent. Vollemer et al. (8) reported that children who underwent GTR or CTR have significantly better OS and EFS than children who underwent partial resection. Englum et al. (10) and Li et al. (11) reported clear trends toward improved OS associated with CTR. von Allmen et al. (24) reported that >90% resection was associated with better EFS than <90% resection. McGregor et al. (13), De Loris et al. (15), von Allmen et al. (18), and Salim et al. (19) reported that the extent of best operation had no significant effect on EFS or OS. The reason for the difference in these four studies may be that the MYCN gene—which promotes tumor cell proliferation and inhibits apoptosis and differentiation—is evidently closely related to neuroblastoma occurrence and development, possibly limiting conclusions pertaining to these outcomes. Last, the location of the primary tumor, the level of experience of the surgeon, and the treatment compliance of the patient after the operation affects recovery. Although the above-described studies did not support CTR or GTR, the results of the current meta-analysis were mainly positive.

Intraoperative and Postoperative Complications

In the present analysis, five reports described intraoperative and postoperative complications. Unexpectedly, Vollemer et al. (8), Adkins et al. (9), and Salim et al. (19) reported that complications were unrelated to the extent of resection. In the current meta-analysis, CTR and GTR were associated with increased complications, possibly because in many situations complete resection may have been abandoned after one or more complications occurred during the operation or after the initial operation.

Induction Chemotherapy

Induction chemotherapy is now thought to make surgery easier. The most commonly used induction chemotherapeutic regimen (developed at the Memorial Sloan-Kettering Cancer Center) includes dose-intensive cycles of cisplatin and etoposide alternating with vincristine, doxorubicin, and cyclophosphamide. COG investigators added topotecan to this induction regimen on the basis of data indicating anti-neuroblastoma activity in cases of relapse. European protocols have utilized OPEC/COJEC regimens, which include vincristine,

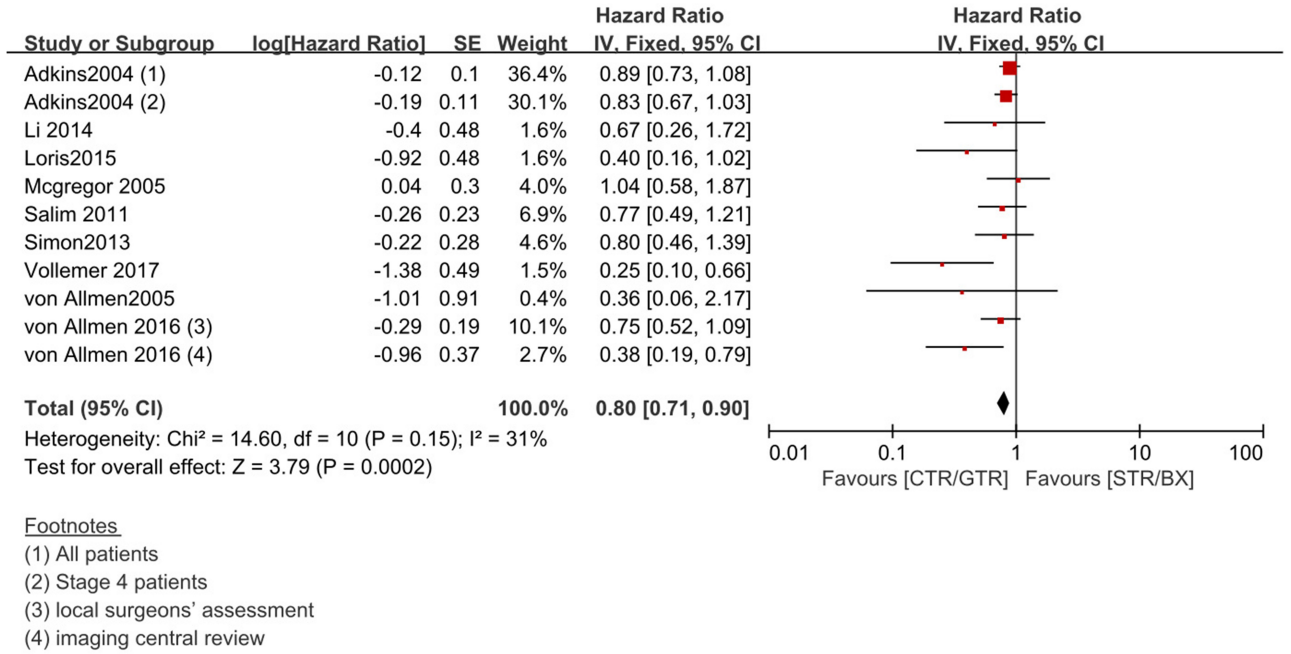

FIGURE 5 | Effect of CTR/GTR vs. GTR/BX on 5-year EFS in high-risk neuroblastoma patients. Weights are from Mantel–Haenszel random effects analysis. Hazard ratios are shown with 95% confidence intervals.

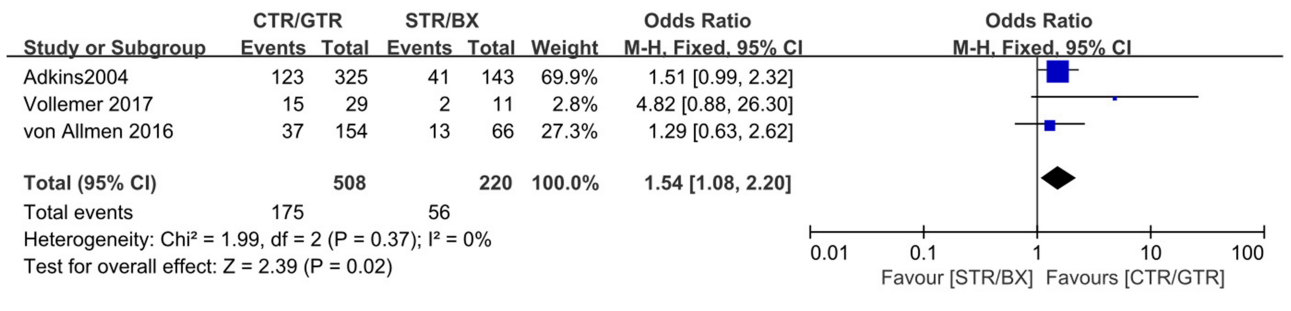

FIGURE 6 | Complications of CTR/GTR vs. STR/BX during or after surgery in high-risk neuroblastoma patients. Weights are from Mantel–Haenszel random effects analysis. Odds ratios are shown with 95% confidence intervals.

cisplatin, etoposide, and cyclophosphamide in OPEC, with additional carboplatin for COJEC (26). In the current meta-analysis, three studies mentioned the prognosis of induction chemotherapy. McGregor et al. (13) reported that patients who underwent GTR or CTR at the time of diagnosis had higher predicted 5-year survival than patients who had GTR or CTR after induction chemotherapy. Survival rates were also compared in 17 patients with primary surgical resection and 75 patients with delayed surgical resection by Tsuchida et al. (20), but there was no statistically significant difference in survival rate between these two groups. Adkins et al. (9) and Simon et al. (14) reported the survival rates of patients who underwent complete resection before and after chemotherapy, but they did not conduct statistical analysis. In the current analysis there was no significant difference in EFS between patients who underwent initial surgery and those in whom surgery was delayed. It is therefore necessary to design strong chemotherapy regimens to improve the survival rate of advanced patients.

Association With Other Studies

Previous meta-analyses have drawn various conclusions depending on the types of control interventions used for comparison. Two of them are about the surgery method for NB. A systematic review by Yang et al. (27) published in 2018 included 18 studies. Although he also showed that the pooled effects of gross resection were significantly superior to other surgical options, the classification of the scope of operation was too general. In these included studies, the definition of gross tumor resection was different, and it had no effect on the postoperative and intraoperative complication rate. What is more, another study by Mullassery et al. (28) included 15 studies; the subjects of Mullassery are patients with stages

TABLE 3 | Subgroup analysis assessing hazard ratio (HR).

Subgroups		EFS:CTR vs. GTR	OS:CTR vs. >GTR	EFS:CTR/GTR vs. STR/BX	OS:CTR/GTR vs. STR/BX
Years	>2010	HR = 0.93 [0.77, 1.12] I^2 =0% P = 0.97	HR = 1.15 [0.83, 1.57] I^2 = 0% P = 0.89	HR = 0.65 [0.52, 0.81] I^2 = 29% P = 0.2	HR = 0.56 [0.43, 0.74] I^2 = 12 P = 0.34
	<2010	HR = 0.96 [0.86,1.08] I^2 = 0% P = 0.99	HR = 0.62 [0.23,1.69] I^2 = 0% P = 0.57	HR = 0.86 [0.75,0.99] I^2 = 0% P = 0.68	Only one study
Quality	High	HR = 0.95 [0.86, 1.05] I^2 = 0% P = 0.97	HR = 1.6 [0.82, 1.64] I^2 = 0% P = 0.66	HR = 0.82 [0.72, 0.93] I^2 = 25% P = 0.24	HR = 0.54 [0.72, 0.93] I^2 = 25% P = 0.24
	Moderate	HR = 1.02 [0.66, 1.58] I^2 = 0% P = 0.98	HR = 0.88 [0.48, 1.61] I^2 = 0% P = 0.59	HR = 0.62 [0.43, 0.90] I^2 = 36% P = 0.2	HR = 0.60 [0.39, 0.94] I^2 = 42% P = 0.18

III and IV of NB, and there are some patients with a low and moderate risk of NB, so the results deviated greatly. Even so, this study drew a conclusion that a clear survival benefit is shown for GTR or CTR over STR in stage 3 NBL only. Though some advantages can be demonstrated for GTR as defined by DFS in stage 4 NBL, GTR did not significantly improve OS in stage 4 disease. Considering the observed heterogeneity, this can be considered to be approximately beneficial to wider resection. Therefore, both articles have come to a similar conclusion with this meta-analysis, that is, removal of all tumors as far as possible can effectively improve the survival rate of patients. We also summarized the commonly accepted induction chemotherapy regimens to provide a more detailed reference for doctors to treat high-risk patients in the future.

Limitations

The present meta-analysis had some limitations. A more precise analysis could have been conducted if individual patient data were available, enabling adjustment for age, sex, ethnicity, and geographical location. Different research institutions administer different chemotherapeutic drugs to patients; there is no unified standard for the evaluation of surgical tolerance, and biological heterogeneity affects clinical results. Immunotherapy and myeloablative therapy followed by autologous stem cell transplantation have yielded improved outcomes in collaborative trials, so larger and higher-quality trials are needed to confirm these conclusions.

SUMMARY

For patients with high-risk neuroblastoma, complete tumor resection and gross tumor resection of the primary tumor were related to improved survival, with very limited effects on intraoperative and postoperative complications. It is necessary to design strong chemotherapy regimens to improve the survival rate of advanced patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

YQ contributed to the conception, design of the study, and drafting of the article. JZ contributed to revising the article critically for important intellectual content and contributed to the final approval of the version to be submitted. All authors contributed to the article and approved the submitted version.

FUNDING

This study was funded by the Tianjin Health Bureau special grant (grant number: 14KG129) and the Tianjin Children's Hospital special grant (grant number: Y2020002).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.706800/full#supplementary-material

Supplementary Figure 1 | EFS of the patients who underwent CTR or GTR tumor resection at the time of diagnosis and the patients who had CTR or GTR tumor resection after induced chemotherapy.

Supplementary Figure 2 | Funnel plot for **(A)** 5-year OS in the comparison between CTR/GTR vs. STR/BX **(B)** 5-year EFS in the comparison between CTR/GTR vs. STR/BX tumor resection.

Supplementary Figure 3 | Funnel plot for **(A)** 5-year OS in the comparison between CTR or GTR **(B)** 5-year EFS in the comparison between CTR or GTR.

REFERENCES

1. Al-Shammari NF, Redha E, Al Hajeri MH. Cervical neonatal neuroblastoma with recurrent SVT. *Gulf J Oncol.* (2009) 6:45–57. PMID: 20194085.
2. Monclair T, Brodeur GM, Ambros PF, Brisse HJ, Cecchetto G, Holmes K, et al. The International Neuroblastoma Risk Group (INRG) staging system: an INRG Task Force report. *J Clin Oncol.* (2009) 27:298–303. doi: 10.1200/JCO.2008.16.6876
3. Whittle SB, Smith V, Doherty E, Zhao S, McCarty S, Zage PE. Overview and recent advances in the treatment of neuroblastoma. *Expert Rev Anticancer Ther.* (2017) 17:369–86. doi: 10.1080/14737140.2017.1285230
4. Rojas Y, Jaramillo S, Lyons K, Mahmood N, Wu MF, Liu H, et al. The optimal timing of surgical resection in high-risk neuroblastoma. *J Pediatr Surg.* (2016) 51:1665–9. doi: 10.1016/j.jpedsurg.2016.05.021
5. Pandey GK, Kanduri C. Long noncoding RNAs and neuroblastoma. *Oncotarget.* (2015) 6:18265–75. doi: 10.18632/oncotarget.4251
6. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* (2009) 6:e1000097. doi: 10.1371/journal.pmed.1000097
7. Rich BS, McEvoy MP, Kelly NE, Oh E, Abramson SJ, Price AP, et al. Resectability and operative morbidity after chemotherapy in neuroblastoma patients with encasement of major visceral arteries. *J Pediatr Surg.* (2011) 46:103–7. doi: 10.1016/j.jpedsurg.2010.09.075
8. Vollmer K, Gfroerer S, Theilen TM, Bochennek K, Klingebiel T, Rolle U, et al. Radical Surgery Improves Survival in Patients with Stage 4 Neuroblastoma. *World J Surg.* (2018) 42:1877–84. doi: 10.1007/s00268-017-4340-9
9. Adkins ES, Sawin R, Gerbing RB, London WB, Matthay KK, Haase GM. Efficacy of complete resection for high-risk neuroblastoma: a Children's Cancer Group study. *J Pediatr Surg.* (2004) 39:931–6. doi: 10.1016/j.jpedsurg.2004.02.041
10. Englum BR, Rialon KL, Speicher PJ, Gulack B, Driscoll TA, Kreissman SG, et al. Value of surgical resection in children with high-risk neuroblastoma. *Pediatr Blood Cancer.* (2015) 62:1529–35. doi: 10.1002/pbc.25504
11. Li P, Liu J, Zhen Z, Liu Z, Gao Y, Zhu J, et al. Effect of surgical resection range on prognosis of stage 4 neuroblastoma. *Chin Clin Oncol.* (2014) 24:1568–72. doi: 10.3969/j.issn.1000-8179.20141222
12. Castel V, Tovar JA, Costa E, Cuadros J, Ruiz A, Rollan V, et al. The role of surgery in stage IV neuroblastoma. *J Pediatr Surg.* (2002) 37:1574–8. doi: 10.1053/jpsu.2002.36187
13. McGregor LM, Rao BN, Davidoff AM, Billups CA, Hongeng S, Santana VM, et al. The impact of early resection of primary neuroblastoma on the survival of children older than 1 year of age with stage 4 disease: the St. Jude Children's Research Hospital experience. *Cancer.* (2005) 104:2837–46. doi: 10.1002/cncr.21566
14. Simon T, Häberle B, Hero B, von Schweinitz D, Berthold F. Role of surgery in the treatment of patients with stage 4 neuroblastoma age 18 months or older at diagnosis. *J Clin Oncol.* (2013) 31:752–8. doi: 10.1200/JCO.2012.45.9339
15. De Ioris MA, Crocoli A, Contoli B, Garganese MC, Natali G, Tomà P, et al. Local control in metastatic neuroblastoma in children over 1 year of age. *BMC Cancer.* (2015) 15:79. doi: 10.1186/s12885-015-1082-7
16. Yeung F, Chung PH, Tam PK, Wong KK. Is complete resection of high-risk stage IV neuroblastoma associated with better survival? *J Pediatr Surg.* (2015) 50:2107–11. doi: 10.1016/j.jpedsurg.2015.08.038
17. Koh CC, Sheu JC, Liang DC, Chen SH, Liu HC. Complete surgical resection plus chemotherapy prolongs survival in children with stage 4 neuroblastoma. *Pediatr Surg Int.* (2005) 21:69–72. doi: 10.1007/s00383-004-1353-x
18. von Allmen D, Grupp S, Diller L, Marcus K, Ecklund K, Meyer J, et al. Aggressive surgical therapy and radiotherapy for patients with high-risk neuroblastoma treated with rapid sequence tandem transplant. *J Pediatr Surg.* (2005) 40:936–41. doi: 10.1016/j.jpedsurg.2005.03.008
19. Salim A, Mullassery D, Pizer B, McDowell HP, Losty PD. Neuroblastoma: a 20-year experience in a UK regional centre. *Pediatr Blood Cancer.* (2011) 57:1254–60. doi: 10.1002/pbc.23149
20. Tsuchida Y, Yokoyama J, Kaneko M, Uchino J, Iwafuchi M, Makino S, et al. Therapeutic significance of surgery in advanced neuroblastoma: a report from the study group of Japan. *J Pediatr Surg.* (1992) 27:616–22. doi: 10.1016/0022-3468(92)90461-f
21. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol.* (2010) 25:603–5. doi: 10.1007/s10654-010-9491-z
22. Egger M, Smith GD. Bias in location and selection of studies. *BMJ.* (1998) 316:61–6. doi: 10.1136/bmj.316.7124.61
23. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ.* (2003) 327:557–60. doi: 10.1136/bmj.327.7414.557
24. von Allmen D, Davidoff AM, London WB, Van Ryn C, Haas-Kogan DA, Kreissman SG, et al. Impact of extent of resection on local control and survival in patients from the COG A3973 study with high-risk neuroblastoma. *J Clin Oncol.* (2017) 35:208–16. doi: 10.1200/JCO.2016.67.2642
25. Zwaveling S, Tytgat GA, van der Zee DC, Wijnen MH, Heij HA. Is complete surgical resection of stage 4 neuroblastoma a prerequisite for optimal survival or may >95 % tumour resection suffice? *Pediatr Surg Int.* (2012) 28:953–9. doi: 10.1007/s00383-012-3109-3
26. Maris JM. Recent advances in neuroblastoma. *N Engl J Med.* (2010) 362:2202–11. doi: 10.1056/NEJMra0804577
27. Yang X, Chen J, Wang N, Liu Z, Li F, Zhou J, et al. Impact of extent of resection on survival in high-risk neuroblastoma: a systematic review and meta-analysis. *J Pediatr Surg.* (2019) 54:1487–94. doi: 10.1016/j.jpedsurg.2018.08.046
28. Mullassery D, Farrelly P, Losty PD. Does aggressive surgical resection improve survival in advanced stage 3 and 4 neuroblastoma? A systematic review and meta-analysis. *Pediatr Hematol Oncol.* (2014) 31:703–16. doi: 10.3109/08880018.2014.947009

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

ORIGINAL RESEARCH
published: 25 October 2021
doi: 10.3389/fped.2021.757377

Diagnostic Errors in Wilms' Tumors: Learning From Our Mistakes

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Specialty section:
This article was submitted to Pediatric Surgery, a section of the journal Frontiers in Pediatrics

Received: 12 August 2021
Accepted: 21 September 2021
Published: 25 October 2021

Citation:
de Carvalho LG, Kobayashi T, Cypriano MdS, Caran EMM, Lederman HM, Alves MTdS and Abib SdCV (2021) Diagnostic Errors in Wilms' Tumors: Learning From Our Mistakes. Front. Pediatr. 9:757377. doi: 10.3389/fped.2021.757377

Aim: This study aimed to analyze clinical characteristics and image findings in patients initially diagnosed with renal masses and treated on the Société Internationale d'Oncologie Pédiatrique (SIOP) 2001 protocol for Wilms tumor (WT) that eventually were diagnosed with different pathologies.

Methods: We reviewed the preoperative symptoms, laboratory tests, and images of patients who were initially treated for WT and proved to have other diagnoses. Data from these patients were compared to those of the last 10 patients with WT and the last 10 patients with neuroblastoma (NBL) treated at a single institution.

Results: From June 2001 to December 2020, we treated 299 patients with NBL and 194 with WT. Five patients treated with preoperative chemotherapy for WT were postoperatively diagnosed with NBL (one patient had bilateral renal masses and one with multifocal xanthogranulomatous pyelonephritis). Three underwent nephrectomy, two biopsies only, and one adrenalectomy due to intraoperative characteristics. Regarding clinical presentation, abdominal mass or swelling was very suggestive of WT ($p = 0.011$); pain, although very prevalent in the study group (67%), was not statistically significant, as well as intratumoral calcifications on computed tomography (CT) (67%). Urinary catecholamines were elevated in all patients mistreated for WT with the exception of the patient with pyelonephritis in which it was not collected.

Conclusion: Some pathologies can be misdiagnosed as WT, especially when they present unspecified symptoms and dubious images. Diagnostic accuracy was 98.1%, which highlights the quality of the multidisciplinary team. Abdominal mass or swelling is highly suggestive of WT, especially in the absence of intratumoral calcifications on CT. If possible, urinary catecholamines should be collected at presentation as they help in the differential diagnosis of NBL.

Keywords: Wilms, neuroblastoma, surgery, diagnostic errors, preoperative chemotherapy

INTRODUCTION

Wilms tumors (WT) and neuroblastomas are the most common diagnoses in children with palpable abdominal masses (1). Neuroblastoma is the fourth most common neoplasm in childhood, behind leukemias, brain tumors, and lymphomas, and is the most common extracranial solid tumor in children (2). It has the peak of incidence in the first year of life, and it is extremely rare after 5 years of age (3). Neuroblastomas originate from primitive ganglion cells from the neural crest that undergo transformation and migrate ventrally and caudally to form many tissues such as the branchial arches, thoracic vessels, sympathetic nervous system, and adrenal medulla. Due to this migration of neuroblasts in the embryonic period, neuroblastomas can be located in the abdomen, chest, neck, and pelvis and very rarely as an intrarenal tumor (4). On computed tomography (CT), the tumor typically is heterogeneous with calcifications seen in 80–90% of cases (5). Areas of necrosis are of low attenuation. The tumor morphology is often helpful, with the mass seen insinuating itself beneath the aorta and lifting it off the vertebral column. It tends to encase vessels and may lead to compression. Adjacent organs are usually displaced; although in more aggressive tumors, direct invasion of the psoas muscle or kidney can be seen. The latter can make distinguishing neuroblastoma from WT difficult.

WT is the most common renal neoplasm in children under 15 years of age and represents 95% of cases of renal masses in children. The peak incidence is between 2 and 4 years of age (6), and 95% of the cases are diagnosed before 5 years of age (7). Both neuroblastoma and WT can present with asymptomatic abdominal mass or pain and hypertension. WT can also present with hematuria (7). The diagnosis of neuroblastomas and WT is based on clinical history, physical examination, and laboratory tests and image. In suspected cases of neuroblastomas, urinary catecholamine should be collected (8), associated with a bone marrow aspirate and a primary tumor biopsy. Imaging exams assess the characteristics of the mass and the extent of the disease (9).

Ultrasonography is the main initial examination for the investigation of abdominal mass, allowing the identification of calcifications and tumor characteristics (9). In patients with suspected renal tumor, Doppler ultrasonography may demonstrate tumor extension to the renal vein and inferior vena cava. CT scan and magnetic resonance imaging (MRI) are performed to investigate the extension of the mass and its relationship with adjacent tissues (10).

The differential diagnosis between neuroblastoma, WT, and other kidney tumors is based on the patient's age, symptoms, and laboratory and imaging characteristics. In some cases, aspects of imaging studies are common among tumors, leading to a difficulty in preoperative diagnosis (8). There are some reports in the literature of xanthogranulomatous pyelonephritis presenting as WT (11).

Unlike the Children's Oncology Group (COG), which advocates the initial surgical approach and only after the diagnosis is confirmed is chemotherapy started, Société Internationale d'Oncologie Pédiatrique (SIOP) advocates preoperative (neoadjuvant) chemotherapy for WT, without an anatomopathological substrate, using only classic imaging findings (12) with a global diagnostic accuracy of 86% (13). When a case of neuroblastoma or non-Wilms kidney tumor is misdiagnosed by imaging studies as a WT, patients are treated with neoadjuvant chemotherapy not specific for the true histological type of the tumor, which can delay treatment and change the prognosis of the disease.

The aim of this study is to evaluate patients who received preoperative chemotherapy for WT and had different diagnosis.

MATERIALS AND METHODS

This is a retrospective study of children misdiagnosed with WTs, treated at the Pediatric Oncology Institute—GRAACC—Federal University of São Paulo, Brazil, from June 2001 to December 2020. The study was approved by the institutional review board (CEP #0808/13). All patients treated with preoperative chemotherapy for WT on the SIOP 2001 or SIOP 2016 (Umbrella) protocol whose pathology revealed a different diagnosis were included. Patients who had surgery as a first approach or were treated on other protocols were excluded. Initial symptoms, physical examination findings, laboratory tests, and imaging studies were retrospectively reviewed and compared to those of the last 10 patients treated for WT and neuroblastoma in the institution.

We use the main features of our imaging exam reports to perform data analysis. On ultrasound were heterogenicity, lobed outline, size, restriction of the lesion to the kidney, Doppler flowmetry, and laterality. In CT scan were heterogenicity, calcification, lymph node enlargement, capsule invasion, and size.

Statistical analysis was performed using the Fisher test and Student's t-test. In this study, an association was considered significant if the corresponding p-value was <0.05.

RESULTS

From June 2001 to December 2020, 299 cases of NBL and 194 of WT were treated at the Pediatric Oncology Institute—GRAACC—Federal University of São Paulo. Six patients received preoperative chemotherapy according to the SIOP protocol, and pathology proved to have other diagnoses. Five patients presented undifferentiated neuroblastoma as histopathological findings and one as xanthogranulomatous multifocal pyelonephritis. Diagnostic accuracy was of 98.1%.

Patient A after chemotherapy showed tumor growth with areas of necrosis. He underwent nephrectomy, adrenalectomy, splenectomy, and colectomy due to tumor invasion and died 2 years after surgery due to tumor progression despite the institution of neuroblastoma-directed therapy after the histological result. Patient B (**Figure 1**) had a bilateral tumor that showed a small reduction after chemotherapy. After two cycles of actinomycin and vincristine and one cycle of vincristine, biopsy was performed; due to the appearance of the tumor and due to the advanced stage of the disease, he

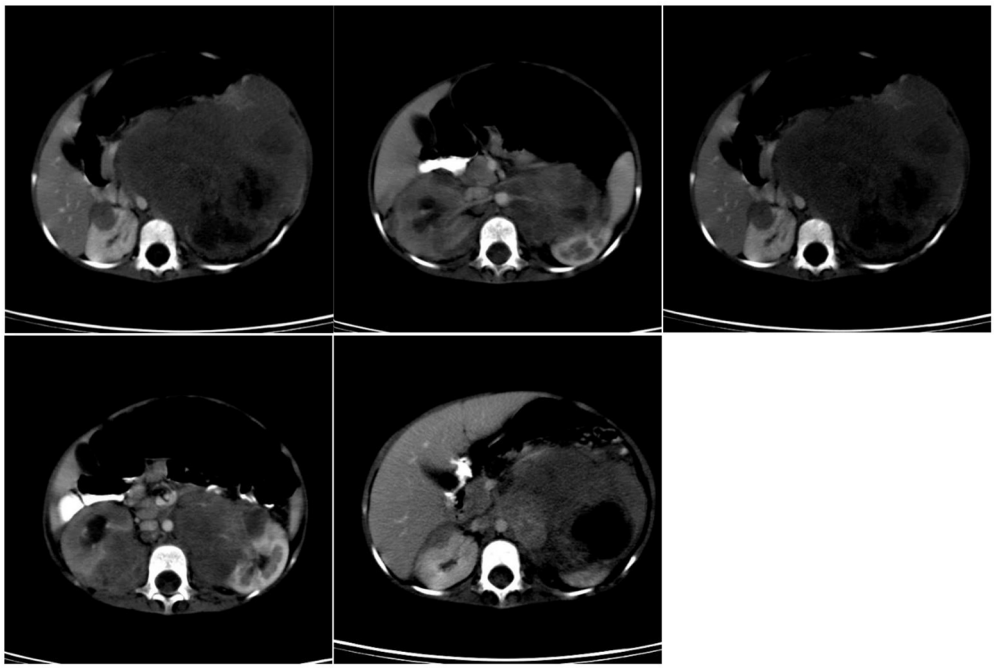

FIGURE 1 | Preoperative CT scan of patient B.

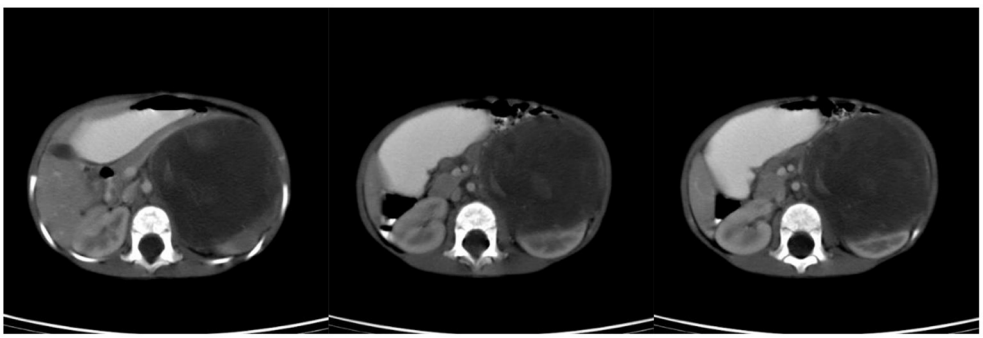

FIGURE 2 | Preoperative CT scan of patient C.

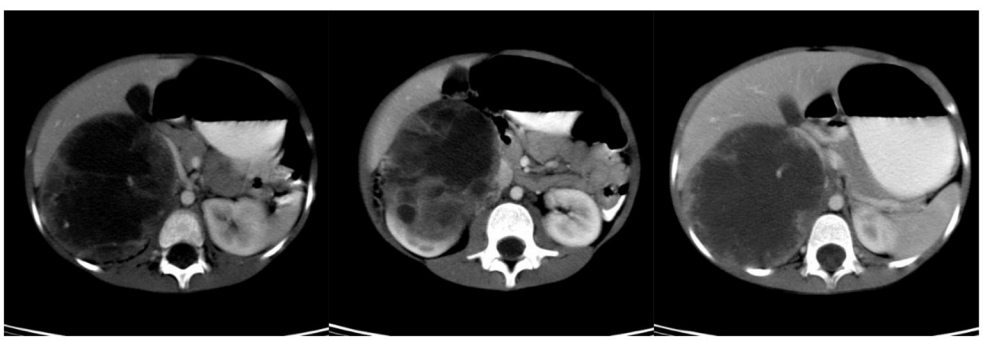

FIGURE 3 | Preoperative CT scan of patient E.

TABLE 1 | Comparison between the study group, the Wilms group, and the neuroblastoma group in relation to symptoms.

	Number of patients	*p*-value
Increase abdominal volume		
Study group	2	
Wilms group	10	**0.011***
Neuroblastoma group	1	0.247
Pain		
Study group	4	
Wilms group	2	0.061
Neuroblastoma group	7	0.889
Fever		
Study group	2	
Wilms group	1	0.247
Neuroblastoma group	1	0.247
Vomiting		
Study group	2	
Wilms group	2	0.55
Neuroblastoma group	4	0.789
Diarrhea		
Study group	0	
Wilms group	1	0.606
Neuroblastoma group	2	0.43
Weight loss		
Study group	0	
Wilms group	1	0.182
Neuroblastoma group	1	0.606
Constipation		
Study group	1	
Wilms group	1	0.696
Neuroblastoma group	3	0.55
Enterorrhagia		
Study group	1	
Wilms group	0	0.282
Neuroblastoma group	0	0.282
Hematuria		
Study group	0	
Wilms group	1	0.606
Neuroblastoma group	0	–
Adrenomegaly		
Study group	0	
Wilms group	0	–
Neuroblastoma group	1	0.606
Tremor		
Study group	0	
Wilms group	0	–
Neuroblastoma group	1	0.606

**Bold values means statistical significance ($p < 0.05$).*

TABLE 2 | Comparison between the study group, the Wilms group, and the neuroblastoma group in relation to laboratory tests.

		p-value
Hemoglobin (g/dl)		
Study group	10.7 (7.8–15.4)	
Wilms group	10.6 (8–13.5)	0.15
Neuroblastoma group	11.5 (8.9–14.4)	0.203
Leukocytes ($10^3/\mu l$)		
Study group	10.4 (5.1–17.4)	
Wilms group	11.8 (7.1–21.2)	0.297
Neuroblastoma group	9.4 (5.1–17.0)	0.134
Platelets (platelets/μl)		
Study group	459,317 (21,800–696,500)	
Wilms group	414,350 (170,000–630,000)	0.55
Neuroblastoma group	346,230 (62,000–710,000)	0.894
LDH (UI/L)		
Study group	716 (282–950)	
Wilms group	1,082 (966–1,082)	–
Neuroblastoma group	627 (57–2,364)	0.082
HVA (mg/24 h)		
Study group	58.8 (29.4–91.6)	
Wilms group	–	–
Neuroblastoma group	239 (18–1,269)	0.086
VMA (mg/24 h)		
Study group	29.2 (4.8–71.5)	
Wilms group	–	–
Neuroblastoma group	82.3 (6.8–231)	0.073

was considered irresecable and died due to disease progression despite chemotherapy treatment. Patient C (**Figure 2**) presented tumor regression with chemotherapy, undergoing nephrectomy and adrenalectomy, and is still undergoing treatment. Patient D showed no response to chemotherapy and underwent nephrectomy and adrenalectomy. He is currently off therapy for 14 years. Patient E (**Figure 3**) presented tumor growth with necrosis after chemotherapy and underwent only tumor biopsy and died 1 month after surgery because of disease progression. Patient F had Beckwith–Wiedemann syndrome and had a 50% tumor reduction with chemotherapy. Right nephrectomy was performed, but histopathological findings confirmed a xanthogranulomatous multifocal pyelonephritis.

The identified patients showed smaller abdominal volume, when compared to the 10 WT patient controls ($p = 0.011$). In relation to the neuroblastoma controls, they presented greater increase in abdominal volume and less pain evaluated by the FLACC scale, however without statistical significance. The study group had higher levels of pain, when compared to the WT controls (67% vs. 20%, $p = 0.0611$), and numbers very similar to the neuroblastoma controls (67% vs. 70%, $p = 0.889$). None of the symptoms, such as fever, vomiting, diarrhea, weight loss, constipation or enterorrhagia, were statistically significant. No patient in the study group presented with hematuria as in the WT controls or with lymphadenomegaly and tremors as in the neuroblastoma controls (**Table 1**).

Comparing the study group to WT and neuroblastoma controls showed similar results regarding hemoglobin, leukocytes, and platelets. The LDH cannot be compared to the WT group due to lack of data; only three patients had these data available. In the neuroblastoma controls, this value was slightly lower than that in the study group and without statistical significance (716 vs. 627, $p = 0.082$). With the exception of

TABLE 3 | Comparison between the study group, the Wilms group, and the neuroblastoma group in relation to US parameters.

US parameters		*p*-value
Heterogeneity		
Study group	3	
Wilms group	6	0.696
Neuroblastoma group	4	0.696
US parameters		*p*-value
Heterogeneity		
Study group	3	
Wilms group	6	0.696
Neuroblastoma group	4	0.696
Lobed outline		
Study group	1	
Wilms group	1	0.696
Neuroblastoma group	1	0.696
Size (cm)		
Study group	6.5	
Wilms group	12.7	0.155
Neuroblastoma group	7.1	0.447
Kidney restricted		
Study group	4	
Wilms group	6	0.789
Neuroblastoma group	10	0.109
Doppler flowmetry		
Study group	1	
Wilms group	1	0.696
Neuroblastoma group	1	0.696
Left side		
Study group	4	
Wilms group	7	0.889
Neuroblastoma group	5	0.515
Lobed outline		
Study group	1	
Wilms group	1	0.696
Neuroblastoma group	1	0.696
Size (cm)		
Study group	6.5	
Wilms group	12.7	0.155
Neuroblastoma group	7.1	0.447
Kidney restricted		
Study group	4	
Wilms group	6	0.789
Neuroblastoma group	10	0.109
Doppler flowmetry		
Study group	1	
Wilms group	1	0.696
Neuroblastoma group	1	0.696
Left side		
Study group	4	
Wilms group	7	0.889
Neuroblastoma group	5	0.515

TABLE 4 | Comparison between the study group, the Wilms group, and the neuroblastoma group in relation to CT parameters.

CT parameters		*p*-value
Heterogeneous		
Study group	4	
Wilms group	8	0.55
Neuroblastoma group	7	0.889
Calcification		
Study group	4	
Wilms group	0	**0.011***
Neuroblastoma group	8	0.55
Lymph node enlargement		
Study group	4	
Wilms group	4	0.301
Neuroblastoma group	3	0.152
Capsular involvement		
Study group	3	
Wilms group	3	0.423
Neuroblastoma group	1	0.073
Size (cm)		
Study group	11.3	
Wilms group	13.9	0.077
Neuroblastoma group	12.7	0.344
Necrosis		
Study group	3	
Wilms group	4	0.696
Neuroblastoma group	3	0.423
Crosses midline		
Study group	3	
Wilms group	2	0.21
Neuroblastoma group	0	0.385
Displaces larges vessels		
Study group	3	
Wilms group	3	0.423
Neuroblastoma group	2	0.21

**Bold values means statistical significance ($p < 0.05$).*

TABLE 5 | Comparison between our data with those published by Dickson et al. (18).

Authors	Carvalho et al. (2021)	Dickson et al. (18)
Patients (*n*)	6	9
Final diagnosis	Neuroblastoma (5) Xanthogranulomatous pyelonephritis (1)	Neuroblastoma (9)
Patient age (months)	31.3 (11–47)	N/A
CT parameters	Heterogeneous (4) Calcification (4) Lymph node enlargement (4) Capsular involvement (3) Necrosis (3) Crosses midline (3) Displaces larges vessels (3)	Calcification (6) Vascular encasement (4)
HVA (mg/24 h)	58.8 (29.4–91.6)	86.5 (47–126)
VMA (mg/24 h)	29.2 (4.8–71.5)	300.8 (58–806)

patient A, homovanillic acid (HVA) and vanillylmandelic acid (VMA) were measured in all others in the study group. Although HVA was increased in all patients in the study group (58.8 vs. 239, $p = 0.086$) and VMA was normal only in patient E (29.2 vs. 82.3, $p = 0.073$), the values were below those of the neuroblastoma group and did not show statistical significance (**Table 2**).

The ultrasound study analyzed heterogeneity, lobulated outline, size, tumor restricted to the kidney, and Doppler flowmetry. No data was statistically significant, but the mean size of the study group was half that of the WT group (6.5 vs. 12.7, $p = 0.155$) and similar to that of the neuroblastoma group (6.5 vs. 7.1, $p = 0.447$) (**Table 3**). The parameters analyzed at CT were heterogeneity, calcification, lymph node enlargement, capsular involvement, size, necrosis, crossing of the midline, and displacement of the great vessels. The only statistically significant result was calcification, which was present in 67% in the study group and absent in the WT group ($p = 0.011$). In the neuroblastoma group, 80% were found to have calcification ($p = 0.550$) (**Table 4**).

DISCUSSION

This is the first Brazilian study that focuses on the challenge of differential diagnosis between WT, neuroblastomas, and non-WT.

The study group had higher levels of pain, when compared to the WT controls (67% vs. 20%, $p = 0.0611$), and numbers very similar to the neuroblastoma controls, which can help to make differential diagnosis between WT and neuroblastoma, even though WTs can present with abdominal pain due to intratumoral bleeding or preoperative rupture.

Nowadays, there are various different image modalities to assist in the correct diagnosis of WT, but incorrect diagnosis still occurs in 5–12% of cases (14), which is a concern when the initial treatment is chemotherapy.

Despite having cases of misdiagnosis, diagnostic accuracy in the present series was 98.1%, which highlights the excellence of the multidisciplinary team and shows lower misdiagnosis rates than those described in the literature (15–18).

The diagnosis of WT tumor despite all the resources can be difficult in rare cases due to the intrarenal localization of neuroblastomas (4).

Even though complete resection is of essence in the treatment of solid tumors, accurate diagnosis is important due to the correct use of neoadjuvant chemotherapy (12).

While the abdominal mass or swelling is a sign almost always present in cases of WT, it is less common in neuroblastoma cases (19), which was also evidenced in our series.

Some CT findings, although not specific to a single type of tumor, may help the diagnosis. Displacement of large vessels, extension of the tumor beyond the midline, renal displacement, and calcifications are very suggestive of neuroblastomas but may be present in WT (20). In our series, tumor calcification was very characteristic of neuroblastoma, encompassing 80% of the cases in the study group and 80% of the cases in the neuroblastoma controls, whereas in the WT group, none of the cases had calcification. **Table 5** shows that calcification was an important diagnostic finding for neuroblastoma both in our series and Dickson et al. A greater numbers of image parameters were analyzed in the present series compared to Dickson et al.

In our study group, all patients had urinary catecholamines collected before surgery with the exception of the case of pyelonephritis. However, due to the delay in obtaining the results, it could not be used for treatment decision. Urinary catecholamine collection at presentation is very important and should be done with high priority to differentiate neuroblastoma cases.

Based on intraoperative findings, a trained surgeon sometimes suspects misdiagnosis and changes the surgical planning in order to achieve better prognosis without surgical morbidity. This can be illustrated in the two cases in which only biopsy was performed.

Further studies are needed to determine the occurrence of misdiagnosis in other Brazilian centers that use the SIOP protocol and evaluate the prognostic impact of preoperative treatment in tumors other than WT concerning survival.

CONCLUSION

Some pathologies can be misdiagnosed as WT, especially when they present unspecified symptoms and dubious images. Diagnostic accuracy was 98.1%, which highlights the quality of the multidisciplinary team. The increase in abdominal volume is highly suggestive of WT, especially if associated with the absence of intratumoral calcifications on CT. If possible, urinary catecholamines should be collected before surgery as they help in the differential diagnosis of neuroblastoma.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Federal University of São Paulo—CEP #0808/13. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

LC: writing, data collection and data analysis. SA: study design, data review, and article final review. MA: data review and article review. HL: images analysis. MC: data review and article review. TK: writing, data collection and data analysis. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Miniati D, Gay AN, Parks KV, Naik-Mathuria BJ, Hicks J, Nuchtern JG, et al. Imaging accuracy and incidence of Wilms' and non-Wilms' renal tumors in children. *J Pediatr Surg.* (2008) 43:1301–7. doi: 10.1016/j.jpedsurg.2008.02.077
2. Brodeur GM. Neuroblastoma: biological insights into a clinical enigma. *Nat Rev Cancer.* (2003) 3:203–16. doi: 10.1038/nrc1014
3. Gurney JG, Severson RK, Davis S, Robison LL. Incidence of cancer in children in the United States. Sex-, race-, and 1-year age-specific rates by histologic type. *Cancer.* (1995) 75:2186–95.
4. Muniz MTC, Araújo M, Pureza LMM, Morais A, Antunes C, Filho JR, et al. Neuroblastoma intrarenal mimetizando tumor de Wilms. *Revista Brasileira de Cancerologia.* (2005) 51:361–4. doi: 10.32635/2176-9745.RBC.2005v51n4.1942
5. Lonergan GJ, Schwab CM, Suarez ES, Carlson CL. Neuroblastoma, ganglioneuroblastoma, and ganglioneuroma: radiologic-pathologic correlation. *Radiographics.* (2002) 22:911–34. doi: 10.1148/radiographics.22.4.g02jl15911
6. Davidoff AM. Wilms' tumor. *Curr Opin Pediatr.* (2009) 21:357–64. doi: 10.1097/MOP.0b013e32832b323a
7. Kim S, Chung DH. Pediatric solid malignancies: neuroblastoma and Wilms' tumor. *Surg Clin North Am.* (2006) 86:469–87. doi: 10.1016/j.suc.2005.12.008
8. Wu Y, Song B, Xu J, Chen W, Zhao X, Jia R, et al. Retroperitoneal neoplasms within the perirenal space in infants and children: differentiation of renal and non-renal origin in enhanced CT images. *Eur J Radiol.* (2010) 75:279–86. doi: 10.1016/j.ejrad.2010.05.038
9. Papaioannou G, McHugh K. Neuroblastoma in childhood: review and radiological findings. *Cancer Imaging.* (2005) 5:116–27. doi: 10.1102/1470-7330.2005.0104
10. Zderic SA. Renal and adrenal tumors in children. *Urol Clin North Am.* (2004) 31(3):607–17, xi. doi: 10.1016/j.ucl.2004.04.014
11. Iumanne S, Shoo A, Akoko L, Scanlan P. Case report: Xanthogranulomutous pyelonephritis presenting as "Wilms' tumor." *BMC Urol.* (2016) 16:36. doi: 10.1186/s12894-016-0155-5
12. Kaste SC, Dome JS, Babyn PS, Graf NM, Grundy P, Godzinski J, et al. Wilms tumour: prognostic factors, staging, therapy and late effects. *Pediatr Radiol.* (2008) 38:2–17. doi: 10.1007/s00247-007-0687-7
13. Jackson TJ, Williams RD, Brok J, Chowdhury T, Ronghe M, Powis M, et al. The diagnostic accuracy and clinical utility of pediatric renal tumor biopsy: Report of the UK experience in the SIOP UK WT 2001 trial. *Pediatr Blood Cancer.* (2019) 66:e27627. doi: 10.1002/pbc.27627
14. Duarte RJ, Cristofani LM, Denes FT, Giron AM, Odone-Filho V, Srougi M. Misdiagnosis in Wilms tumor: concerns on the use of preoperative chemotherapy. *Eur Urol Suppl.* (2012) 11:E506–U99. Available online at: https://observatorio.fm.usp.br/handle/OPI/2949
15. Tournade MF, Com-Nougué C, Voûte PA, Lemerle J, de Kraker J, Delemarre JF, et al. Results of the sixth international society of pediatric oncology Wilms' tumor trial and study: a risk-adapted therapeutic approach in Wilms' tumor. *J Clin Oncol.* (1993) 11:1014–23. doi: 10.1200/JCO.1993.11.6.1014
16. Vujanić GM, Kelsey A, Mitchell C, Shannon RS, Gornall P. The role of biopsy in the diagnosis of renal tumors of childhood: Results of the UKCCSG Wilms tumor study 3. *Med Pediatr Oncol.* (2003) 40:18–22. doi: 10.1002/mpo.10216
17. Pritchard-Jones K. Controversies and advances in the management of Wilms' tumour. *Arch Dis Child.* (2002) 87:241–4. doi: 10.1136/adc.87.3.241
18. Dickson PV, Sims TL, Streck CJ, McCarville MB, Santana VM, McGregor LM, et al. Avoiding misdiagnosing neuroblastoma as Wilms tumor. *J Pediatr Surg.* (2008) 43:1159–63. doi: 10.1016/j.jpedsurg.2008.02.047
19. Aspectos clínicos, epidemiológicos e sobrevida de crianças com neuroblastoma: 21 anos de experiência do instituto de oncologia pediátrica, são paulo. *Rev. Paul. Pediatr.* (2021) 36: 254–60. Available from: https://pesquisa.bvsalud.org/portal/resource/pt/biblio-977062
20. Lowe RE, Cohen MD. Computed tomographic evaluation of Wilms tumor and neuroblastoma. *RadioGraphics.* (1984) 4:915–28. doi: 10.1148/radiographics.4.6.915

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

CASE REPORT
published: 26 October 2021
doi: 10.3389/fped.2021.765278

Case Report: Transoral Endoscopic Thyroidectomy *via* Vestibular Approach in Pediatric Thyroid Cancer

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Background: Transoral endoscopic thyroidectomy via vestibular approach (TOETVA) is a new technique that has become more popular worldwide because of its many advantages. However, this novel approach for thyroid cancer treatment in children is highly challenging, even for high-volume surgeons. In our study, we report our experiences with TOETVA for pediatric patients with thyroid cancer.

Patients and Methods: This study included four pediatric patients who underwent TOETVA performed by a single surgeon between June and December 2020. Patient demographics and surgical outcomes including operative time, incidence of complications, and length of hospital stay were evaluated.

Results: Four patients successfully underwent TOETVA with no complications. All patients were girls, aged from 13 to 18. Three patients underwent lobectomy and isthmusectomy, plus prophylactic unilateral central neck dissection. One patient had a total thyroidectomy, plus prophylactic bilateral central neck dissection. The mean operative time was 85 min for the lobectomy and 120 min for total thyroidectomy plus central neck dissection. The median hospital stay was 4.1 days. No drains were used. The histological examination showed four cases of malignant disease (papillary thyroid carcinoma). The mean number of harvested lymph nodes was 4.2 (ranged 3 to 8).

Conclusion: In the hands of a high-volume surgeon, TOETVA is a novel, feasible, and safe approach for treating selected pediatric patients with thyroid cancer.

Keywords: TOETVA, pediatric thyroid cancer, thyroid cancer in children, transoral approach, transoral thyroidectomy

OPEN ACCESS

Edited by:
Luca Pio,
Giannina Gaslini Institute (IRCCS), Italy

Reviewed by:
Alessandro Crocoli,
Bambino Gesù Children Hospital (IRCCS), Italy
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Specialty section:
This article was submitted to Pediatric Surgery, a section of the journal Frontiers in Pediatrics

Received: *26 August 2021*
Accepted: *27 September 2021*
Published: *26 October 2021*

Citation:
Ngo DQ, Le DT, Hoang GN, Ngo QX and Le QV (2021) Case Report: Transoral Endoscopic Thyroidectomy via Vestibular Approach in Pediatric Thyroid Cancer. Front. Pediatr. 9:765278. doi: 10.3389/fped.2021.765278

INTRODUCTION

Thyroid nodules are less common in children, but this age group has witnessed an increased rate of thyroid cancer (1). Surgery plays a major role in treatment. The prognosis for children with thyroid cancer is excellent, with a high survival rate after treatment (1, 2). Nevertheless, a cervical scar has been shown to have an impact on the confidence and the quality of life of children experiencing thyroid surgery, especially female patients (3). Thus, several new approaches to surgical methods have been introduced to reduce the risk of cervical scar (4, 5).

Trans-oral endoscopic thyroidectomy via vestibular approach (TOETVA) is a new technique, with the aesthetic result of truly scar-free healing and minimally invasive dissection. It also offers

an accessible approach to both thyroid lobes and facilitates the removal of neck lymph nodes (6–8). Thus, TOETVA is becoming more popular. However, while there is extensive research on TOETVA for adults, there is little on the pediatric population. In our study, we present the first case series of TOETVA used with children in Vietnam.

PATIENTS AND METHODS

Following the ATA guidelines, all patients included in this study were ≤18 yr old (1). Between June and December 2020, four patients were admitted to the Department of Head and Neck Surgery and selected for the transoral approach. All these patients had a preoperative assessment, including thyroid hormonal level tests, neck ultrasound examination, and fine-needle aspiration (in line with The Bethesda System for Reporting Thyroid Cytopathology—TBSRTC) (9).

Inclusion criteria for TOETVA were as follows: (1) thyroid nodule (TBSRTC II–IV) ≤6 cm in size; (2) thyroid nodule (TBSRTC V–VI) ≤2 cm in size with no lymph node involvement; (3) total size of thyroid gland ≤10 cm in diameter per lobe (10, 11).

Contraindications for TOETVA included (1) previous anterior neck surgery, (2) tracheal or esophageal invasion, (3) previous radiation to the head or neck, (4) recurrent laryngeal nerve palsy, (5) oral cavity infection, (6) uncontrolled hyperthyroidism (10, 11), and (7) patients under 10 years of age or less than 30 kg of weight.

SURGICAL TECHNIQUE

The surgical steps have been described in previous publications and are based on Anuwoong's technique (6). In brief, the patients were placed in a supine position. After intubation, three incisions were made in the oral vestibule for the insertion of endoscopic 5–10 mm trocars. To insert a 30-degree endoscope, a 5-mm central trocar is recommended, especially for pediatric patients. Working space was then created by using a hook cautery and/or an ultrasonic scalpel. Next, the strap muscles were retracted laterally with a transcutaneous suture. The pyramidal lobe was dissected and separated from the trachea. The isthmus was then divided, and the superior thyroid vessels were dissected and divided using an energy device. The upper parathyroid gland and then the recurrent laryngeal nerve (RLN) were identified. The

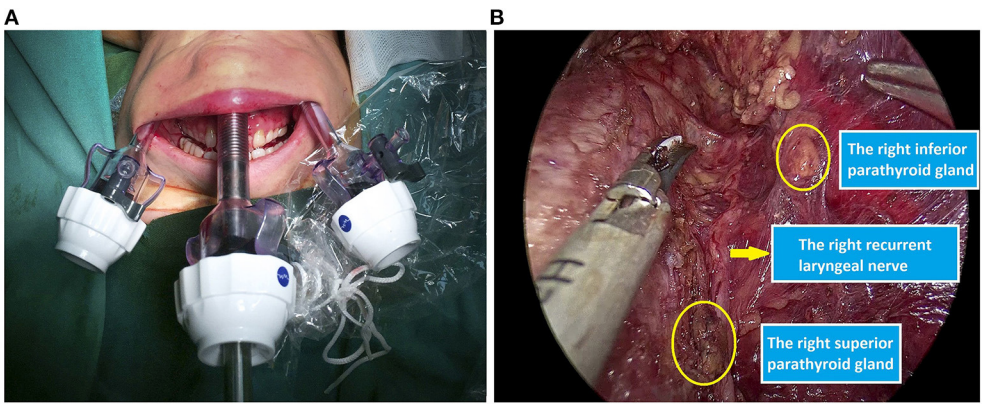

FIGURE 1 | Intraoperative images. **(A)** Trocar set-up. **(B)** Preservation of the recurrent laryngeal nerve and parathyroid glands.

TABLE 1 | Individual case characteristics and surgical outcomes.

Patient	Gender	Age (years)	Tumor location	Tumor size (mm)	FNA (Bethesda)	Surgery	Operative surgery (mins)	Number of haversted LN	Number of positive LN	pT	pN	Complication	Follow up (months)	Recurrence
Pt 1	Female	18	Left lobe	4 × 5	5	Hemithyroidectomy + uniteral CND	80	3	0	T1a	N0	None	14	No
Pt 2	Female	13	Left lobe	6 × 7	5	Hemithyroidectomy + uniteral CND	90	8	0	T1a	N0	None	15	No
Pt 3	Female	16	Right lobe	4 × 6	5	Hemithyroidectomy + uniteral CND	85	6	0	T1a	N0	None	15	No
Pt 4	Female	15	Right lobe	12 × 8	5	Bilateral thyroidectomy + bilateral CND	120	6	1	T1b	N1a	None	16	No

Pt, Patient; CND, central neck dissection; FNA, Fine needle aspiration; LN, Lymph nodes.

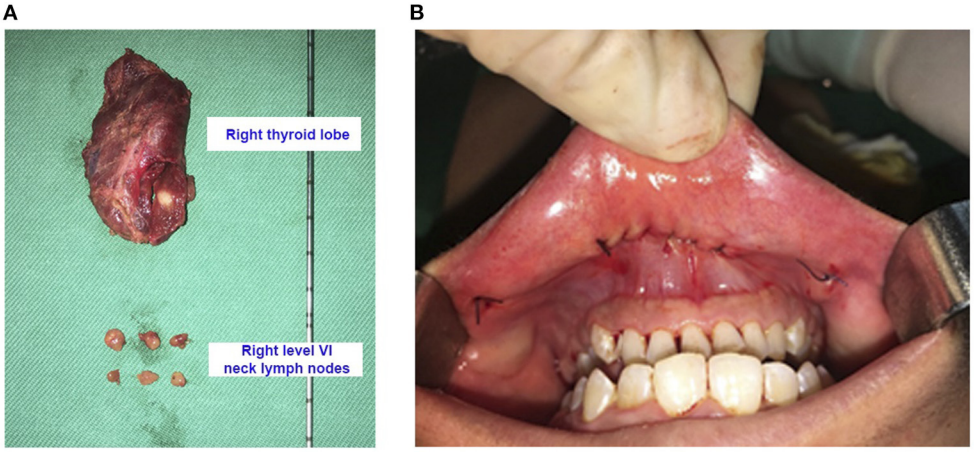

FIGURE 2 | (A) Surgical specimens after right hemithyroidectomy and right central neck dissection (16-year-old female patient with thyroid cancer of the right lobe pT1aN0M0). **(B)** The incisions were closed by two layers with absorbable interrupted sutures.

thyroid lobe was dissected from the trachea and the RLN while preserving the lower parathyroid. The specimen was put into an Endo Catch bag and removed via a central incision (**Figure 1**).

RESULTS

A summary of the preoperative data for the four patients is presented in **Table 1**. All the patients were girls, aged from 13 to18. Three patients underwent lobectomy and isthmusectomy plus prophylactic unilateral central neck dissection. One patient had a total thyroidectomy plus prophylactic bilateral central neck dissection. The median hospital stay was 4.2 days. All the TOETVA procedures were successfully carried out, with no need for conversion to the open approach. In each pediatric TOETVA, the RLNs and parathyroid glands were visualized while performing the dissection.

The mean operative time was 85 min (range 80–90 min) for lobectomy and isthmusectomy plus central neck dissection, while the time for total thyroidectomy plus central neck dissection was 120 min. No drains were used (**Figure 2**).

All patients were performed successfully via transoral approach without any surgical complication. No permanent or transient complications were documented, including RLN injury and hypocalcemia. No transient mental nerve injury or chin hypoesthesia was observed. There was no occurrence of skin injury, bleeding, seroma, or infection or need for reoperation in any of the patients. All pediatric patients were completely satisfied with the cosmetic results (**Figure 3**).

The histological examination showed four cases of malignant disease (papillary thyroid carcinoma). The mean number of harvested lymph nodes was 4.2 (range 3–8), while there was only one patient with positive lymph nodes (1/6 positive lymph nodes). All tumors were completely excised with negative margins.

After a median follow-up of 14.25 (range 10–16) months, none of these patients presented evidence of recurrent disease and any surgical complication related to transoral endoscopic thyroidectomy.

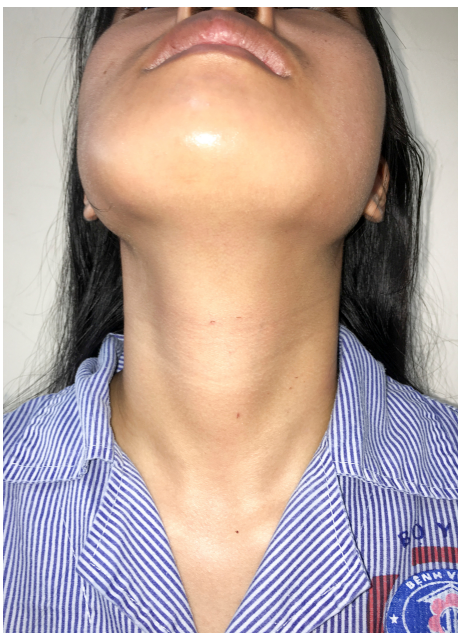

FIGURE 3 | Cosmetic results after surgery 5 days (16-year-old female patient with thyroid cancer of the right lobe pT1aN0M0).

DISCUSSION

Pediatric thyroid cancer is rare in children, accounting for approximately 1.5% of all cancers in the under-18 age group, with 4.8–5.9 cases per million children annually and an increasing trend (1, 2). The major pathology of pediatric patients is a well-differentiated thyroid carcinoma. However, the prognosis

of the group is excellent, with high survival rates (12). Thus, quality of life should be considered when choosing a surgical approach. With traditional open surgery, a neck scar following thyroidectomy leads to a decline in quality of life in children (3). A cervical scar is associated with an increased risk of depression in the pediatric population. Hence, different approaches have been developed to avoid the neck incision in children (4, 5). TOETVA is a new technique with many advantages, including an aesthetic result with scar-free healing. TOETVA has become popular worldwide (7, 10, 13). However, there has been only limited reported use in the pediatric population; thus, investigation of the feasibility and safety of TOETVA for use with this population is needed. To the best of our knowledge, this is the second case series for TOETVA for this group.

According to ATA guidelines, pediatric patients with thyroid cancer should be treated with a total thyroidectomy because of the risk of contralateral lobe cancer (1). However, a recent study by Tate Nice of the 15-yr follow-up of 4,000 pediatric patients showed that total thyroidectomy did not improve overall survival compared with a lobectomy in the low-risk group, which is similar to adults (14). However, despite the higher cervical lymph node metastatic rate in children compared with adults, prophylactic central neck dissection was found to increase the fatality risk in children due to the complications of hypoparathyroidism and RLN injury (1, 15, 16). Thus, indications for prophylactic central neck dissection should be considered carefully to balance the merits and risks. Thus, in cT1,2N0M0 patients with a unifocal disease, we performed the ipsilateral prophylactic central neck dissection and made a frozen section of level VI lymph nodes. If the result was positive, contralateral central neck dissection was carried out (15). In our case series, three patients with unifocal disease underwent lobectomy and unilateral prophylactic central neck dissection, and one patient underwent total thyroidectomy plus bilateral prophylactic central neck dissection because the results of the intraoperative frozen section of level VI lymph nodes were positive.

Thyroid surgery is uncommon in the pediatric population. Thus, there may be an increased percentage with surgical complications. A study of 464 pediatric patients who had undergone a thyroidectomy showed that the most common postoperative complication was temporary hypoparathyroidism, at 37% of patients, following by temporary RLN injury at 2.37% (17). The percentage of permanent hypoparathyroidism or RLN injury is less than 1%. Oden Cohen reported on 48 pediatric patients who had undergone thyroidectomy via a transoral approach; 33% experienced temporary hypoparathyroidism and 1.6% temporary RLN injury (18). No patient suffered permanent RLN injury or hypoparathyroidism.

Our study showed similar results, as no patient suffered temporary hypoparathyroidism or any other complications, such as RLN injury, mental nerve injury, hemorrhage, or seroma. Overall, we achieved good results in all four cases, with no long-term complications including recurrent laryngeal nerve injury or hypoparathyroidism. Although our report demonstrated good short-term outcomes, the pediatric patients needed to be followed to confirm longer-term oncological results. On the other hand, thyroid surgery in children is more difficult than in adults. Thus, we highly recommended that TOETVA in the pediatric population should only be performed by high-volume thyroid surgeons.

CONCLUSION

TOETVA is a new, feasible, and safe approach for pediatric patients with thyroid cancer. We recommend that only high-volume thyroid surgeons with experience of TOETVA could perform TOETVA on pediatric patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Local Institutional Review Board. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

DN and QL study conception and design. QN and DL data acquisition. DN, QN, and GH analysis and data interpretation. DN and GH drafting of the manuscript. QL critical revision. DL submit this form with the manuscript. All authors have read and agreed to the published version of the manuscript.

FUNDING

DN was funded by Vingroup Joint Stock Company and supported by the Domestic Master/PhD Scholarship Programme of Vingroup Innovation Foundation (VINIF), Vingroup Big Data Institute (VINBIGDATA), code [VINIF.2020.TS.11].

REFERENCES

1. Francis GL, Waguespack SG, Bauer AJ, Angelos P, Benvenga S, Cerutti JM, et al. Management guidelines for children with thyroid nodules and differentiated thyroid cancer. *Thyroid.* (2015) 25:716–59. doi: 10.1089/thy.2014.0460
2. Dermody S, Walls A, Harley EH. Pediatric thyroid cancer: an update from the SEER database 2007-2012. *Int J Pediatr Otorhinolaryngol.* (2016) 89:121–6. doi: 10.1016/j.ijporl.2016.08.005
3. Choi Y, Lee JH, Kim YH, Lee YS, Chang H-S, Park CS, et al. Impact of postthyroidectomy scar on the quality of life of thyroid cancer patients. *Ann Dermatol.* (2014) 26:693–9. doi: 10.5021/ad.2014.26.6.693

4. Lee F, Ha JF, Lannigan FJ. Minimal access thyroid surgery in children: a retrospective study and literature review. *Surg Tech Dev.* (2017) 7. doi: 10.4081/std.2017.6398
5. Lobe TE, Wright SK. The transaxillary, totally endoscopic approach for head and neck endocrine surgery in children. *J Laparoendosc Adv Surg Tech A.* (2011) 21:97–100. doi: 10.1089/lap.2010.0163
6. Anuwong A. Transoral endoscopic thyroidectomy vestibular approach: a series of the first 60 human cases. *World J Surg.* (2016) 40:491–7. doi: 10.1007/s00268-015-3320-1
7. Jongekkasit I, Jitpratoom P, Sasanakietkul T, Anuwong A. Transoral endoscopic thyroidectomy for thyroid cancer. *Endocrinol Metab Clin North Am.* (2019) 48:165–80. doi: 10.1016/j.ecl.2018.11.009
8. Ngo DQ, Tran TD, Le DT, Ngo QX, Van Le Q. Transoral endoscopic modified radical neck dissection for papillary thyroid carcinoma. *Ann Surg Oncol.* (2021) 28:2766. doi: 10.1245/s10434-020-09466-7
9. Cibas ES, Ali SZ. The 2017 bethesda system for reporting thyroid cytopathology. *Thyroid.* (2017) 27:1341–6. doi: 10.1089/thy.2017.0500
10. Russell JO, Anuwong A, Dionigi G, Inabnet WB, Kim HY, Randolph G, et al. Transoral thyroid and parathyroid surgery vestibular approach: a framework for assessment and safe exploration. *Thyroid.* (2018) 28:825–9. doi: 10.1089/thy.2017.0642
11. Razavi CR, Russell JO. Indications and contraindications to transoral thyroidectomy. *Ann Thyroid.* (2017) 2:12. doi: 10.21037/aot.2017.10.01
12. Paulson VA, Rudzinski ER, Hawkins DS. Thyroid cancer in the pediatric population. *Genes (Basel).* (2019) 10:E723. doi: 10.3390/genes10090723
13. Le QV, Ngo DQ, Tran TD, Ngo QX. Transoral endoscopic thyroidectomy vestibular approach: an initial experience in Vietnam. *Surg Laparosc Endosc Percutan Tech.* (2020) 30:209–13. doi: 10.1097/SLE.0000000000000764
14. Nice T, Pasara S, Goldfarb M, Doski J, Goldin A, Gow KW, et al. Pediatric papillary thyroid cancer >1 cm: is total thyroidectomy necessary? *J Pediatr Surg.* (2015) 50:1009–13. doi: 10.1016/j.jpedsurg.2015.03.031
15. Golpanian S, Perez EA, Tashiro J, Lew JI, Sola JE, Hogan AR. Pediatric papillary thyroid carcinoma: outcomes and survival predictors in 2504 surgical patients. *Pediatr Surg Int.* (2016) 32:201–8. doi: 10.1007/s00383-015-3855-0
16. Ngo DQ, Ngo QX, Van Le Q. Pediatric thyroid cancer: risk factors for central lymph node metastasis in patients with cN0 papillary carcinoma. *Int J Pediatr Otorhinolaryngol.* (2020) 133:110000. doi: 10.1016/j.ijporl.2020.110000
17. Baumgarten HD, Bauer AJ, Isaza A, Mostoufi-Moab S, Kazahaya K, Adzick NS. Surgical management of pediatric thyroid disease: Complication rates after thyroidectomy at the Children's Hospital of Philadelphia high-volume Pediatric Thyroid Center. *J Pediatr Surg.* (2019) 54:1969–75. doi: 10.1016/j.jpedsurg.2019.02.009
18. Cohen O, Tufano RP, Anuwong A, Russell JO, Assadi N, Dionigi G, et al. Trans-oral endoscopic thyroidectomy vestibular approach (TOETVA) for the pediatric population: a multicenter, large case series. *Surg Endosc.* (2021). doi: 10.1007/s00464-021-08537-4. [Epub ahead of print].

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frontiers in Pediatrics

ORIGINAL RESEARCH
published: 04 November 2021
doi: 10.3389/fped.2021.766610

Proliferation Pattern of Pediatric Tumor-Derived Mesenchymal Stromal Cells and Role in Cancer Dormancy: A Perspective of Study for Surgical Strategy

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The explanation for cancer recurrence still remains to be fully elucidated. Moreover, tumor dormancy, which is a process whereby cells enter reversible G_0 cell cycle arrest, appears to be a critical step in this phenomenon. We evaluated the cell cycle proliferation pattern in pediatric tumor-derived mesenchymal stromal cells (MSCs), in order to provide a better understanding of the complex mechanisms underlying cancer dormancy. Specimens were obtained from 14 pediatric patients diagnosed with solid tumors and submitted to surgery. Morphology, phenotype, differentiation, immunological capacity, and proliferative growth of tumor MSCs were studied. Flow cytometric analysis was performed to evaluate the cell percentage of each cell cycle phase. Healthy donor bone marrow-derived mesenchymal stromal cells (BM-MSCs) were employed as controls. It was noted that the DNA profile of proliferating BM-MSC was different from that of tumor MSCs. All BM-MSCs expressed the typical DNA profile of proliferating cells, while in all tumor MSC samples, ≥70% of the cells were detected in the G0/G1 phase. In particular, seven tumor MSC samples displayed intermediate cell cycle behavior, and the other seven tumor MSC samples exhibited a slow cell cycle. The increased number of tumor MSCs in the G0–G1 phase compared with BM-MSCs supports a role for quiescent MSCs in tumor dormancy regulation. Understanding the mechanisms that promote dormant cell cycle arrest is essential in identifying predictive markers of recurrence and to promote a dedicated surgical planning.

Keywords: mesenchymal stromal cells, proliferation pattern, cell cycle, tumor, children

OPEN ACCESS

Edited by:
Luca Pio,
Giannina Gaslini Institute (IRCCS), Italy

Reviewed by:
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Specialty section:
This article was submitted to Pediatric Surgery, a section of the journal Frontiers in Pediatrics

Received: *29 August 2021*
Accepted: *01 October 2021*
Published: *04 November 2021*

Citation:
Pelizzo G, Riva F, Croce S, Avanzini MA, Acquafredda G, de Silvestri A, Mazzon E, Bramanti P, Zuccotti G, Mazzini G and Calcaterra V (2021) Proliferation Pattern of Pediatric Tumor-Derived Mesenchymal Stromal Cells and Role in Cancer Dormancy: A Perspective of Study for Surgical Strategy. Front. Pediatr. 9:766610. doi: 10.3389/fped.2021.766610

INTRODUCTION

The overall incidence rates of childhood cancer vary between 50 and 200 per million children across the world (1). A thorough study on this issue revealed an incidence rate of 138.5 children in every 1 million children worldwide (2). However, cancer is the third leading cause of death among children ages 1–4 and the second leading cause of death among children age 5–14 (3), representing about 8% of all pediatric deaths (4–6). Indeed, after surgical and medical treatment, recurrence, or cancer relapse after an initial diagnosis has been frequently recorded (4–6).

A proposed mechanism underlying the persistence of covert cancer cells during and after treatment is that some cancer stem cells enter a reversible quiescent or dormant state in which they are relatively resistant to radiation and chemotherapy. Conventional chemotherapy regimens include DNA-damaging agents and spindle poisons, and their effect is, therefore, dependent on the active cycling of tumoral cells through the S and M cell cycle phases, respectively. However, both cell intrinsic characteristics and extrinsic influences from surrounding normal cells determine tumor cell dormancy (7). Extrinsic factors include mesenchymal stromal cells (MSCs), endothelial cells (ECs), and immune cells that form the niche of the tumor. As described in *in vitro* leukemia models, blasts communicate closely with MSCs (8), and contact with MSCs has been demonstrated to provide key survival signals to leukemic blasts, rendering them resistant against the non-genotoxic components of leukemia treatment protocols (8, 9). Mesenchymal stromal cells play different roles in modulating tumor progression, growth, and metastasis. They are recruited to the tumor site in large numbers and subsequently have an important microenvironmental role in modulating tumor progression and drug sensitivity. However, the effects of the tumor microenvironment (TME) on MSCs remain poorly understood. It has been reported that a paracrine effect of cancer cells slows cycling and chemoresistance, through the secretion of soluble factors promoting a more stem-like state of MSCs (10). Additionally, the contact between cancer cells and MSCs in regulating cancer dormancy should not be excluded (11).

Therefore, the aim of this study was to characterize the proliferation pattern of the cell cycle in pediatric tumor-derived MSCs, in order to enhance our understanding of the complex mechanisms, implicated in the cancer dormancy process, that may influence therapeutic response.

MATERIALS AND METHODS

Patients

Fourteen pediatric patients (eight females and six males; median age 5 years, range 9 months to 15 years), diagnosed with solid tumors (three neuroblastomas, three lymphomas, three nephroblastomas, and five others) and submitted to surgery were enrolled. Mesenchymal stromal cell isolation and expansion were performed starting from residual material for histological analysis. Samples were collected prior to chemotherapy. Stored bone marrow-derived mesenchymal stromal cells (BM-MSCs), obtained as previously described (12) from healthy donors (two females and two males; median age 5.5 years, range 4–7 years) enrolled for hematopoietic stem cell donation, were used as a control group.

The study was performed according to the Declaration of Helsinki and with the approval of the Institutional Review Board of the Children's Hospital "G. Di Cristina" (registry number 87 Civico 2017). Informed written consent was obtained from the parents and/or legal guardian after receiving information about the study.

Methods

Tumor Mesenchymal Stromal Cell Isolation and Expansion

Tumor tissue was mechanically dissociated and treated with collagenase type II as previously described (11). Tumor MSCs were expanded following the procedure normally used for BM-MSCs (12). Briefly, cells were plated in flasks or wells (Corning Costar, Corning, NY, USA) according to the cell number obtained, at a density of 160,000/cm^2 in complete medium [D-MEM + GlutaMAX (Gibco), supplemented with 10% FBS (Euroclone), 50 mg/ml of gentamicin, and 1% penicillin (Sigma Aldrich)] and cultured at 37°C, 5% CO_2.

Culture medium was changed twice a week until ≥80% confluence was reached; then tumor MSCs were trypsinized (Trypsin EDTA, Euroclone) and replated at a density of 4,000 cells/cm^2 for expansion (12). Cells were propagated to reach senescence.

Characterization of *ex vivo* Expanded Tumor Mesenchymal Stromal Cells

As defined by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT), MSCs must be plastic adherent and exhibit a spindle-shape morphology in standard culture conditions. Proliferative capacity was evaluated as cumulative population doubling (cPD) resulting from the sum of PD at each passage calculated with the following formula PD = $\log_{10}$ (no. of harvested cells/no. of seeded cells)/$\log_{10}2$.

Tumor MSCs were characterized by flow cytometry, using fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-labeled monoclonal antibodies specific for surface antigens: CD73, CD34, CD90, CD14, CD45, CD31, CD105, class I-HLA, and HLA-DR (Beckman Coulter, IL, Milan, Italy), as previously described (10). Analysis was performed by direct immunofluorescence with a FACS Navios flow cytometer (Beckman Coulter).

Tumor MSCs were cultured in osteogenic differentiation induction medium [αMEM, 10% FBS, 10^{-7} M dexamethasone, 50 mg/ml of L-ascorbic acid, and 5 mM β-glycerol phosphate (all from Sigma-Aldrich)], and in adipogenic differentiation medium [αMEM, 10% FBS, 10^{-7} M dexamethasone, 50 mg/ml of L-ascorbic acid, 5 mM β-glycerol phosphate, 100 mg/ml of insulin, 50 mM isobutyl methylxanthine (all from Sigma-Aldrich), and 0.5 mM indomethacin (MP Biomedica)]. The medium was replaced twice a week. After 21 days of culture, osteogenic differentiation was assessed by staining for alkaline phosphatase (AP) activity with Fast Blue and for calcium deposition, with Alizarin Red S stain (both from Sigma-Aldrich), while adipogenic

differentiation was demonstrated by staining of fat droplets with Oil Red O (Bio Optica, Milan, Italy).

Tumor MSC senescence was defined by the β-galactosidase (SA-β-gal) staining Kit (Cell Signaling Technology, Danvers, MA, USA), according to the instructions of the manufacturer. The evaluation of senescence was performed by bright-field microscopy.

DNA Staining for Cell Cycle Cytometric Analysis

Tumor MSCs at P3–P4, were collected after trypsinization. Cell suspensions were centrifuged at 1,200 rpm for 5 min, and then pellets were rinsed twice with phosphate-buffered solution (PBS). After the last centrifugation, 1×10^6 cells were resuspended in 2 ml of DNA staining solution (50 μg/ml of propidium iodide in PBS, 0.1% Igepal, 100 U/ml of RNase type 1A; all reagents from Sigma-Aldrich) and left for 2 h at room temperature before measurement. For the four MSC samples at P3–P4, from healthy hematopoietic stem cell donor (HD), bone marrow was used as a control group.

The cell percentage at each cell cycle phase was evaluated by flow cytometry, as previously described (13). Monoparametric conventional analysis was performed with a Partec PAS II flow cytometer (Sysmex, Milan) using a blue laser and with data recorded on a dedicated computer integrated in the system.

To ensure the best instrumental analytical performance, preliminary alignment, and control were always set up using standard calibration fluorescence beads (Sysmex Ref-4018 KW 160317). The best histogram resolution was achieved measuring a minimum of 50,000 cells. All measurements were performed blindly.

To excite and intercalate propidium iodide into the double-stranded nuclear DNA, the laser line was set at 488 nm, while a 610-nm-long pass filter permitted the selection and measurement of the red fluorescence emission. Data analyses were displayed as frequency histograms of red fluorescence intensities (equivalent to DNA content). Cell cycle analysis and estimation of the three G0/G1, S, and G2/M phases were analyzed with FlowMax software. Cell number in all phases was expressed as cell percentage frequency.

Statistical Analysis

Qualitative variables were described as count and percentage. Comparison between BM-MSCs and tumor MSCs was performed by Fisher exact test. Values of $p < 0.05$ were considered statistically significant. Analyses were performed using the SPSS statistical package (SPSS, Chicago, IL, USA) and Stata 8.0.

RESULTS

Features of the Tumor-Derived MSCs

Residual material from solid pediatric tumor biopsies taken for histological analysis was used as the starting material. In each case, MSC expansion was possible, and all MSC cultures met the minimal criteria defined by the ISCT (14).

As preliminarily reported for NB-derived MSCs (NB) (11), tumor MSCs exhibit spindle-shape morphology and are plastic adherent (**Figure 1A**). Our tumor MSCs expressed the following surface antigens: CD73, CD90, CD105, and HLA-class I ≥95% and CD34, CD14, CD45, CD31, and HLA-DR ≤5% (**Figure 1B**). They presented the capacity to differentiate into osteoblast and adipocytes (**Figure 1C**). Senescence was reached at a median passage of P11 (range P6–P23) (**Figure 1D**).

Tumor MSCs did not exhibit any differences in phenotypical or functional characteristics among the different kinds of tumors.

Cell Cycle Analysis by Flow Cytometry on Tumor Mesenchymal Stromal Cells

Blindly evaluated data related to MSC DNA content allowed us to define three different classes of cell cycle behavior. Proliferating samples were defined as cells at the following concentrations: 55–65, 20–25, and 20%, respectively, in G0/G1, S, G2/M. Samples were considered to be in an intermediate cell cycle condition when 70–75% of cells were observed in G1, 10–15% in S, and 10–15% in G2/M. A slow cell cycle had a high cell number in the G1 phase (80–90%) and lower cell percentage in S (5–10%) and G2/M (10–15%) phases, respectively (**Figure 2**; **Table 1**).

Different proliferating cell DNA profiles were noted when comparing BM-MSCs with tumor MSCs. All of the BM-MSCs had a typical DNA profile of proliferating cells, while all tumor-MSC samples had ≥70% of cells detected in the G0/G1 phase. In particular, seven tumor MSC samples (one neuroblastoma, two lymphomas, and four others) displayed intermediate behavior, and the other seven tumor MSC samples (two neuroblastomas, two nephroblastomas, one lymphoma, and one other) were in a slow cell cycle. The distribution of proliferating BM-MSCs and tumor MSCs resulted significantly different between the two groups ($p < 0.001$) (**Figure 3**).

DISCUSSION

Tumor relapse and metastasis in some cancers can arise years or decades after initial surgical and medical treatment and are responsible for the majority of cancer-related deaths (15). The identification of predictive markers for recurrence should be crucial to identify a correct surgical strategy. Cancer recurrence has not been fully elucidated. Moreover, tumor dormancy seems to be a critical condition in this phenomenon.

Cancer cell dormancy is defined as a process in which cells enter reversible G_0 cell cycle arrest (16), called quiescence. Quiescent cells may acquire additional mutations, survive in a new environment and initiate metastasis, become resistant to chemotherapeutic drugs, and evade immune destruction, thereby influencing cancer progression (16). Different factors have been suggested as contributors to cell dormancy, including complex interactions between metastatic cells and the microenvironment (17–19).

The TME includes endothelial cells, fibroblasts, MSCs, and various immune cells, which are together with cytokines and growth factors embedded in the tumor stroma endowed with specific physical and biomechanical cues (20). It is widely accepted that MSCs participate in each step of tumor

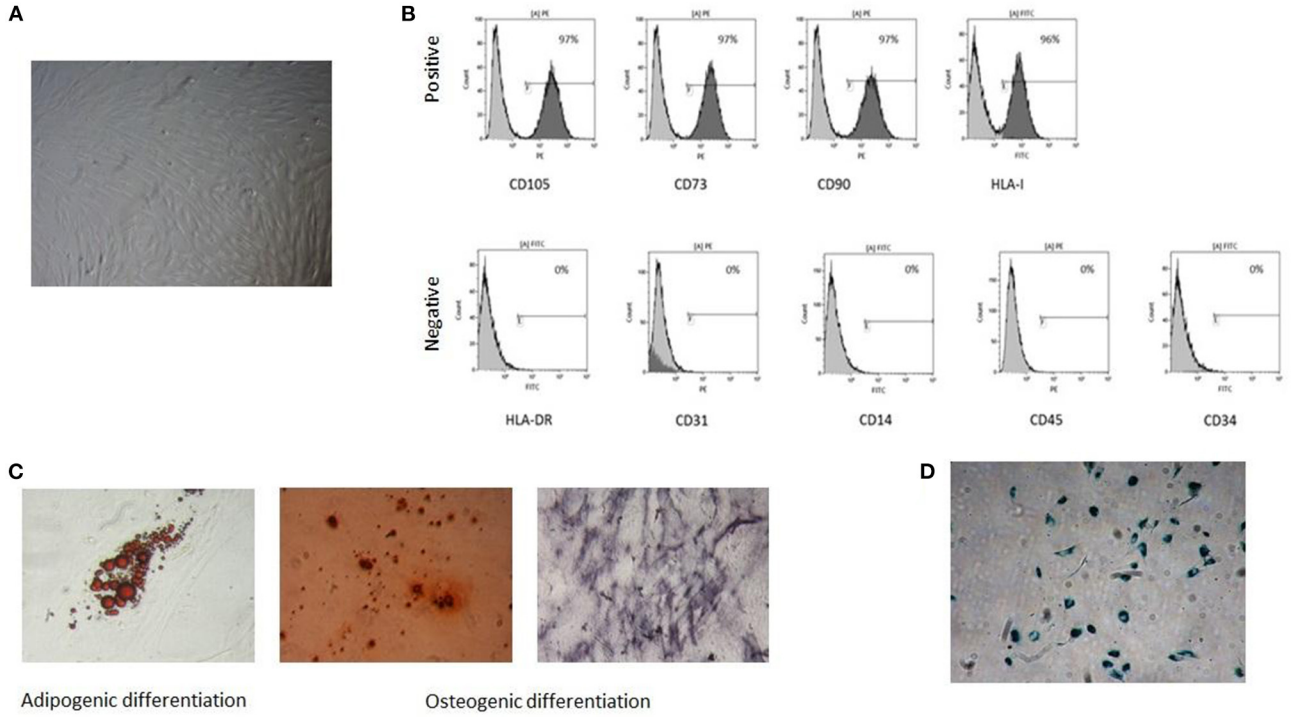

FIGURE 1 | Characterization of tumor mesenchymal stromal cells (MSCs). A representative sample is reported in the figure: **(A)** spindle-shape morphology, **(B)** immunophenotype with positive and negative surface antigens, **(C)** *in vitro* adipogenic and osteogenic differentiation capacity, **(D)** senescent tumor MSC at P13.

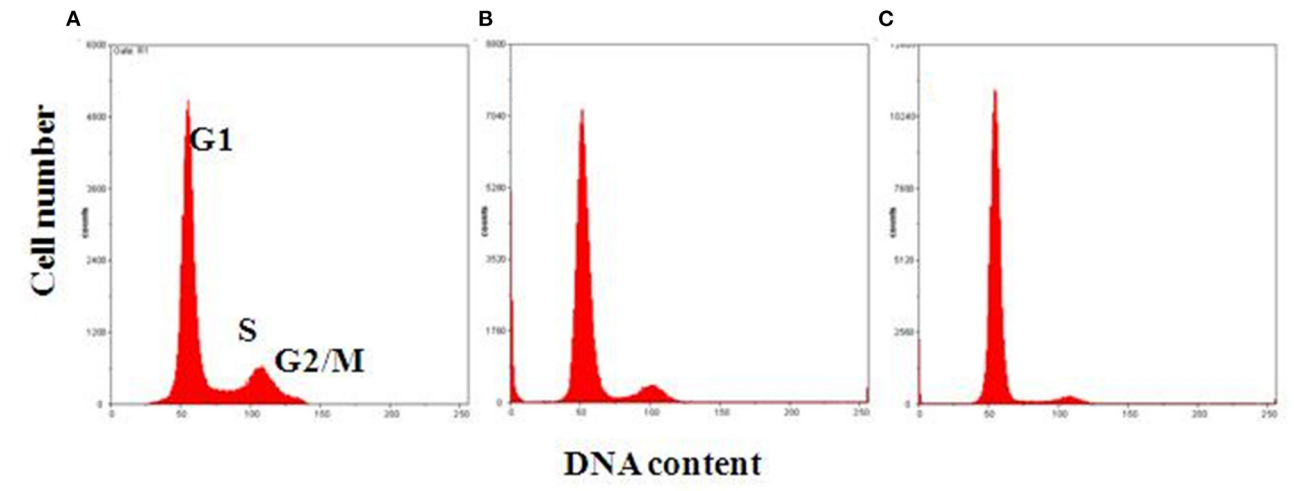

FIGURE 2 | Representative flow cytometric analysis of MSC DNA profiles. Histograms show cell cycle progression in G1, S, and G2/M phases for each representative condition. **(A)** Proliferating cells with 60% of cells in G1, 20% in S, and 20% in G2/M phase. **(B)** Intermediate condition of proliferation, with 70% of cells in G1, 15% in S, and 15% in G2/M phase. **(C)** Slow cell proliferation, with 85% of cells in G1, 5% in S, and 10% in G2/M phase on the x-, y-axes, respectively: DNA content vs. cell number.

development, including relapse and metastasis, due to tumor-homing ability, dynamic phenotype, and immunoregulatory activity (21). It has been reported that tumor MSCs may be driven by the tumor secretome determining their molecular and functional behavior. *In vitro* tumor MSC angiogenic capacity and tumor growth have been described to be supported by melanoma cells. On the contrary, glioblastoma cells reduced protumorigenic effect (22). Moreover, medullary thyroid carcinoma, human

TABLE 1 | Definition of the three classes of cell cycle behavior.

Proliferation pattern	Percentage of cells in G0/G1	Percentage of cells in S	Percentage of cells in G2/M
Proliferating	55–65	20–25	20
Intermediate	70–75	10–15	10–15
Slow	80–90	5–10	10–15

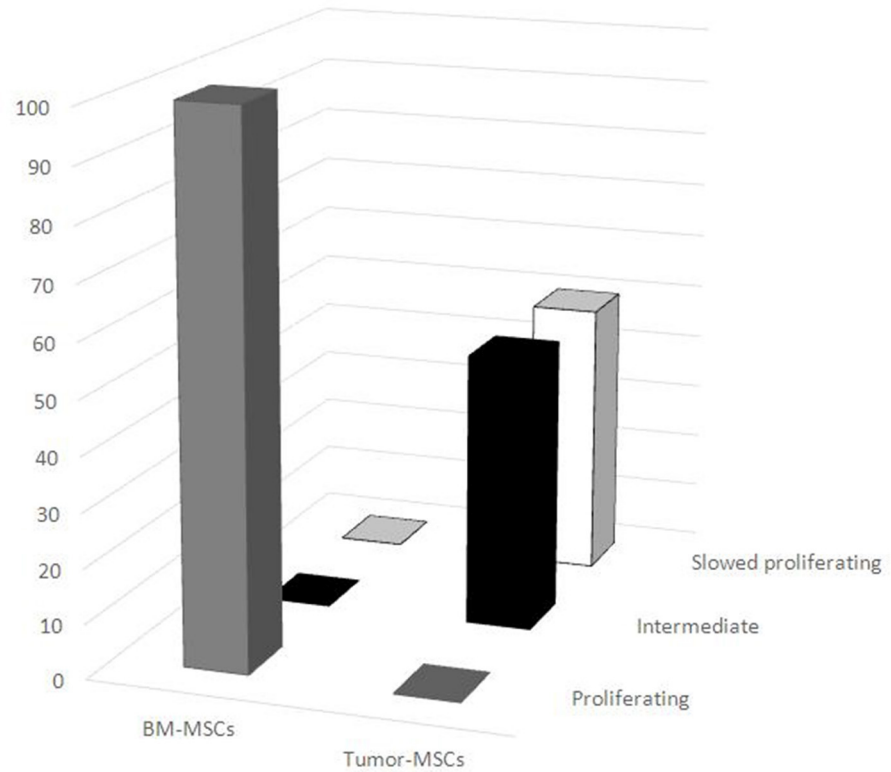

FIGURE 3 | Distribution of proliferating mesenchymal stromal cells (MSCs). Different DNA proliferating cell profiles were noted between BM-MSCs and tumor MSCs. All BM-MSCs expressed the typical DNA profile of proliferating cells, while all tumor MSC samples had a higher percentage of cells detected in the G0/G1 phase. BM-MSC, bone marrow-mesenchymal stromal cells; tumor MSCs, tumor-derived mesenchymal stromal cells.

breast carcinoma, and glioblastoma cells determined changes in MSCs, stimulating their inhibitory effect on cell proliferation and tumor growth (23–25).

Several cells, including MSCs, are recruited to the stroma of tumors where they acquire important microenvironmental roles in modulating tumor progression and drug sensitivity (26–28). Even though clear evidence has not been reported, it is known that the reaction of the host to tumors includes the release of proinflammatory cytokines and chemokines, which modulate the TME, support tumor growth, invasion, and metastasis (29–33). However, the crosstalk between tumor and tumor MSCs is sustained by a more complex pattern determined also by the tumor nature (34). It has been shown that tumor cells secreted several factors exerting different effects on the MSC activity and molecular changes bringing alterations in capacity to stimulate tumor growth (22). El-Badawy et al. (10) have recently reported that co-culture of tumor cell lines with BM-MSCs resulted in their phenotypic and functional alteration.

Additionally, regulation and modulation of the cytokine repertoire produced by various tissue-derived MSCs may affect the cancer cell cycle. Fathi et al. (35), reported that cell cycle progression of K562 co-cultured with BM-MSCs resulted in an accumulation of cells in the G0/G1 phase, with slowed entry into the S phase. Fonseka et al. (36) demonstrated that the arrest of K562 growth in the G0/G1 phase was due to the anti-proliferative effect of human umbilical cord blood-derived MSCs.

The role of cancer cells in modulating the cell cycle of MSCs derived from the TME has not been previously considered. A better knowledge of the mechanisms that promote dormant cell cycle arrest could allow to identify new perspectives of study in pediatric surgery. A dedicated surgical strategy for the prevention of cancer recurrence could be defined. In the present study, we

noted that tumor MSCs represent a population with phenotypical and functional characteristics of BM-MSCs, with a slow or intermediate cell cycle.

Considering the hypothesis that cytokines and growth factors might be highly involved in the anti-tumor effect mediated by MSCs, a high content of MSCs blocked in the G1 phase, as observed in our study, supports a scenario of "selective" cytokine secretion able to regulate tumor cell arrest in the G0/G1 phase, thereby inducing cancer dormancy (37). As documented by Li et al. (38), senescent MSCs may alter the tissue microenvironment and affect nearby malignant cells via cytokine secretion. In the same way, quiescent MSCs could have tumor-regulating effects.

Additionally, as reported by El-Badawy et al. (10), similar to cancer-induced stem cells, tumor-derived MSCs are slow cycling upon exposure to cancer cell-secreted factors. However, further studies are mandatory to support these hypotheses.

We recognize that this study has some limitations. The sample size was small, but the results were consistent with those of other studies investigating this specific population. The study was limited to characterizing pediatric tumor-derived MSCs, without investigation of the regulation mechanisms involved in cancer cellular processes. Additionally, our cases were not age-matched with controls; as reported (39), the same and identical results were found irrespective of whether matching or not matching was applied. Thus, it is possible that age and underlying condition may affect the outcomes studied. Finally, the follow-up of patients was not adequate to provide any correlation between the tumor MSC effect and tumor prognosis.

Despite these limitations, our data support a role for tumor MSCs in the cross talk between cancer cells and their microenvironment and promotion of cancer dormancy.

In conclusion, we characterized the proliferation pattern of pediatric tumor-derived MSCs. The increased number of tumor MSCs in the G0–G1 phase compared with BM-MSCs supports the role of quiescent MSCs in tumor dormancy regulation. Further studies focusing on the mechanisms, which enable a dormant cell cycle, are needed.

New predictive markers of risk recurrence should be evaluated as an innovative perspective of surgical strategy in children.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Istitutional Review Board of the Children's Hospital G. Di Cristina (registry number 87 Civico 2017). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

GP, FR, MA, GM, and VC conceptualized the study. GP, FR, SC, MA, GA, EM, AS, PB, GZ, GM, and VC developed the methodology. AS performed the formal analysis. SC, MA, and GA were in charge of the investigation. GP, FR, MA, SC, GA, AS, EM, PB, GM, and VC prepared and wrote the original draft. GP, FR, MA, EM, PB, GZ, GM, and VC wrote, reviewed, and edited the manuscript. GP, FR, MA, EM, PB, GZ, GM, and VC supervised the study. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGMENTS

The authors thank Dr. E. Lenta for technical assistance, Dr. L. Kelly for English revision, and the Fondazione Sicilia, Palermo, Italy, for supporting research in Pediatric Surgery.

REFERENCES

1. Gatta G, Capocaccia R, Coleman MP, Ries LA, Berrino F. Childhood cancer survival in Europe and the United States. *Cancer.* (2002) 95:1767–72. doi: 10.1002/cncr.10833
2. Kaatsch P. Epidemiology of childhood cancer. *Cancer Treat Rev.* (2010) 36:277–85. doi: 10.1016/j.ctrv.2010.02.003
3. Minino AM, Smith BL. Deaths: preliminary data for 2000. *Natl Vital Stat Rep.* (2001) 49:1–40.
4. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics 2010. *CA Cancer J Clin.* (2011) 61:133–4. doi: 10.3322/caac.20106
5. Edwards BK, Howe HL, Ries LA, Thun MJ, Rosenberg HM, Yancik R, et al. Annual report to the nation on the status of cancer, 1973-1999, featuring implications of age and aging on U.S. Cancer burden. *Cancer.* (2002) 94:2766–92. doi: 10.1002/cncr.10593
6. Bhakta N, Liu Q, Ness KK, Baassiri M, Eissa H, Yeo F, et al. The cumulative burden of surviving childhood cancer: an initial report from the St Jude Lifetime Cohort Study (SJLIFE). *Lancet.* (2017) 390:2569–82. doi: 10.1016/S0140-6736(17)31610-0
7. Meads MB, Gatenby RA, Dalton WS. Environment-mediated drug resistance: a major contributor to minimal residual disease. *Nat Rev Cancer.* (2010) 9:665–74. doi: 10.1038/nrc2714
8. Polak R, de Rooij B, Pieters R, den Boer ML. B-cell precursor acute lymphoblastic leukemia cells use tunneling nanotubes to orchestrate their microenvironment. *Blood.* (2015) 126:2404–14. doi: 10.1182/blood-2015-03-634238
9. Iwamoto S, Mihara K, Downing JR, Pui CH, Campana D. Mesenchymal cells regulate the response of acute lymphoblastic leukemia cells to asparaginase. *J Clin Invest.* (2007) 117:1049–57. doi: 10.1172/JCI30235
10. El-Badawy A, Ghoneim MA, Gabr MM, Salah RA, Mohamed IK, Amer M, et al. Cancer cell-soluble factors reprogram mesenchymal stromal cells to slow cycling, chemoresistant cells with a more stem-like state. *Stem Cell Res Ther.* (2017) 8:254. doi: 10.1186/s13287-017-0709-9
11. Pelizzo G, Veschi V, Mantelli M, Croce S, Di Benedetto V, D'Angelo P, et al. Microenvironment in neuroblastoma: isolation and characterization of tumor-derived mesenchymal stromal cells. *BMC Cancer.* (2018) 18:1176. doi: 10.1186/s12885-018-5082-2
12. Bernardo ME, Avanzini MA, Perotti C, Cometa AM, Moretta A, Lenta E, et al. Optimization of *in vitro* expansion of human multipotent mesenchymal stromal cells for cell-therapy approaches: further insights in the search for a fetal calf serum substitute. *J Cell Physiol.* (2007) 211:121–30. doi: 10.1002/jcp.20911

13. Danova M, Riccardi A, Ucci G, Luoni R, Giordano M, Mazzini G. Ras Oncogene expression and DNA content in plasma cell dyscrasias. A flow cytometric study. *Br J Cancer.* (1990) 62:781–5. doi: 10.1038/bjc.1990.379
14. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy.* (2006) 8:315–7. doi: 10.1080/14653240600855905
15. Gao XL, Zhang M, Tang YL, Liang XH. Cancer cell dormancy: mechanisms and implications of cancer recurrence and metastasis. *Onco Targets Ther.* (2017) 10:5219–28. doi: 10.2147/OTT.S140854
16. Recasens A, Munoz L. Targeting cancer cell dormancy. *Trends Pharmacol Sci.* (2019) 40:128–41. doi: 10.1016/j.tips.2018.12.004
17. Bahmad HF, Chamaa F, Assi S, Chalhoub RM, Abou-Antoun T, Abou-Kheir W. Cancer stem cells in neuroblastoma: expanding the therapeutic frontier. *Front Mol Neurosci.* (2019) 12:131. doi: 10.3389/fnmol.2019.00131
18. Lytle NK, Barber AG, Reya T. Stem cell fate in cancer growth, progression and therapy resistance. *Nat Rev Cancer.* (2018) 18:669–80. doi: 10.1038/s41568-018-0056-x
19. Ayob AZ, Ramasamy TS. Cancer stem cells as key drivers of tumour progression. *J Biomed Sci.* (2018) 25:20. doi: 10.1186/s12929-018-0426-4
20. Ye J, Wu D, Wu P, Chen Z. Huang J. The cancer stem cell niche: cross talk between cancer stem cells and their microenvironment. *Tumor Biol.* (2014) 35:3945–51. doi: 10.1007/s13277-013-1561-x
21. Han Z, Jing Y, Zhang S, Liu Y, Sh Y, Wei L. The role of immunosuppression of mesenchymal stem cells in tissue repair and tumor growth. *Cell Biosci.* (2012) 2:8. doi: 10.1186/2045-3701-2-8
22. Kucerova L, Zmajkovic J, Toro L, Skolekova S, Demkova L, Matuskova M. Tumor-driven molecular changes in human mesenchymal stromal cells. *Cancer Microenviron.* (2015) 8:1–14. doi: 10.1007/s12307-014-0151-9
23. Kucerova L, Matuskova M, Hlubinova K, Altanerova V, Altaner C. Tumor cell behaviour modulation by mesenchymal stromal cells. *Mol Cancer.* (2010) 9:129. doi: 10.1186/1476-4598-9-129
24. Kucerova L, Matuskova M, Hlubinova K, Bohovic R, Feketeova L, Janega P, et al. Bystander cytotoxicity in human medullary thyroid carcinoma cells mediated by fusion yeast cytosine deaminase and 5-fluorocytosine. *Cancer Lett.* (2011) 311:101–12. doi: 10.1016/j.canlet.2011.07.014
25. Kucerova L, Skolekova S, Matuskova M, Bohac M, Kozovska Z. Altered features and increased chemosensitivity of human breast cancer cells mediated by adipose tissue-derived mesenchymal stromal cells. *BMC Cancer.* (2013) 13:535. doi: 10.1186/1471-2407-13-535
26. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. *Nat Med.* (2013) 19:1423–37. doi: 10.1038/nm.3394
27. Pisco AO, Huang S. Non-genetic cancer cell plasticity and therapy-induced stemness in tumour relapse: 'what does not kill me strengthens me'. *Brit J Cancer.* (2015) 112:1725–32. doi: 10.1038/bjc.2015.146
28. Mao Y, Keller ET, Garfield DH, Shen K, Wang J. Stromal cells in tumor microenvironment and breast cancer. *Cancer Metastasis Rev.* (2013) 32:303–15. doi: 10.1007/s10555-012-9415-3
29. Shalapour S, Karin M. Immunity, inflammation, and cancer: an eternal fight between good and evil. *J Clin Invest.* (2015) 125:3347–55. doi: 10.1172/JCI80007
30. Hanahan D. Weinberg RA. The hallmarks of cancer. *Cell.* (2000) 100:57–70. doi: 10.1016/S0092-8674(00)81683-9
31. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell.* (2011) 144:646–74. doi: 10.1016/j.cell.2011.02.013
32. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis.* (2009) 30:1073–81. doi: 10.1093/carcin/bgp127
33. Brown J M. Wilson WR. Exploiting tumour hypoxia in cancer treatment. *Nat Rev. Cancer.* (2004) 4:437–47. doi: 10.1038/nrc1367
34. Spaeth EL, Dembinski JL, Sasser AK, Watson K, Klopp A, Hall B, et al. Mesenchymal stem cell transition to tumor-associated fibroblasts contributes to fibrovascular network expansion and tumor progression. *PLoS ONE.* (2009) 4:e4992. doi: 10.1371/journal.pone.0004992
35. Fathi E, Farahzadi R, Valipour B, Sanaat Z. Cytokines secreted from bone marrow derived mesenchymal stem cells promote apoptosis and change cell cycle distribution of K562 cell line as clinical agent in cell transplantation. *PLoS ONE.* (2019) 14:e0215678. doi: 10.1371/journal.pone.0215678
36. Fonseka M, Ramasamy R, Tan BC, Seow HF. Human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSC) inhibit the proliferation of K562 (human erythromyeloblastoid leukaemic cell line). *Cell Biol Int.* (2012) 36:793–801. doi: 10.1042/CBI20110595
37. Ingangi V, Minopoli M, Ragone C, Motti ML, Carriero MV. Role of microenvironment on the fate of disseminating cancer stem cells. *Front Oncol.* (2019) 9:82. doi: 10.3389/fonc.2019.00082
38. Li G, Zhang R, Zhang X, Shao S, Hu F, Feng Y. Human colorectal cancer derived-MSCs promote tumor cells escape from senescence via P53/P21 pathway. *Clin Transl Oncol.* (2019) 22:503–11. doi: 10.1007/s12094-019-02152-5
39. Faresjö T, Faresjö A. To match or not to match in epidemiological studiessame outcome but less power. *Int J Environ Res Public Health.* (2010) 7:325–32. doi: 10.3390/ijerph7010325

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frontiers in Pediatrics

ORIGINAL RESEARCH
published: 16 November 2021
doi: 10.3389/fped.2021.761896

Hemorrhage During Induction Chemotherapy in Neuroblastoma: Additional Risk Factors in High-Risk Patients

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Specialty section:
This article was submitted to Pediatric Surgery, a section of the journal Frontiers in Pediatrics

Received: 20 August 2021
Accepted: 11 October 2021
Published: 16 November 2021

Citation:
Voglino V, Persano G, Crocoli A, Castellano A, Serra A, Giordano U, Natali GL, Di Paolo PL, Martucci C, Stracuzzi A and Inserra A (2021) Hemorrhage During Induction Chemotherapy in Neuroblastoma: Additional Risk Factors in High-Risk Patients. Front. Pediatr. 9:761896. doi: 10.3389/fped.2021.761896

Background: Neuroblastoma is the most common solid extracranial tumor in children. Patients affected by neuroblastoma are stratified into low, intermediate, and high risk in terms of event-free and overall survival. Some high-risk patients have an additional risk of acute hemorrhagic complications during induction chemotherapy.

Aim: To find easily and rapidly assessed parameters that help clinicians identify those patients affected by high-risk neuroblastoma who have an additional risk of hemorrhagic complications.

Methods: The clinical notes of patients diagnosed with high-risk neuroblastoma from January 2013 until February 2021 were retrospectively reviewed. Clinical, demographic and laboratory data, biological characteristics of the tumor, and information about treatment and hospital stay were identified.

Results: In the examined period, 44 patients were diagnosed with high-risk neuroblastoma. Four of these patients had hemorrhagic complications within 2–7 days after the initiation of induction chemotherapy; two patients had hemothorax, one patient had hemoperitoneum and one patient had hemothorax and hemoperitoneum. The patient with isolated hemoperitoneum was treated with blood components transfusions, clotting factors and colloids infusions; the three patients with hemothorax underwent thoracostomy tube placement and respiratory support. At initial presentation, patients who suffered from hemorrhagic complications had a higher degree of hypertension (stage 2, $p = 0.0003$), higher levels of LDH (median 3,745 U/L, $p = 0.009$) and lower levels of hemoglobin (mean 7.6 gr/dl, $p = 0.0007$) compared to other high-risk patients.

Conclusions: A subgroup of "additional" high-risk patients can be identified within the high-risk neuroblastoma patients based on mean arterial pressure, LDH levels and hemoglobin levels at presentation. Further studies to define cut-off values and optimal management strategies for these patients are needed.

Keywords: high-risk neuroblastoma, hemorrhagic complications, hemothorax, hemoperitoneum, chemotherapy complications

INTRODUCTION

Neuroblastoma is the most common solid extracranial tumor in childhood worldwide, accounting for 8–10% of all cancer cases in children (1); it arises from the neural crest cells of the developing sympathetic system, typically resulting in adrenal or paravertebral tumors (2). Staging and pretreatment risk stratification of neuroblastoma are based on the International Neuroblastoma Risk Group (INRG) staging and classification system; patients are divided into low, intermediate and high risk in terms of Event-Free Survival (EFS) and Overall Survival (OS) (3, 4).

Clinical presentation of neuroblastoma varies widely, ranging from asymptomatic patients to symptoms related to local compression of adjacent structures and to catecholamines or vasoactive intestinal peptide (VIP) secretion, such as hypertension and intractable diarrhea, systemic non-specific symptoms, such as fever and weight loss, or cytopenia related to bone marrow metastases (5, 6).

On rare instances, children with neuroblastoma may present with acute hemorrhage such as hemothorax and hemoperitoneum (7–9). Such cases pose a great challenge for the caring clinicians.

The purpose of this study is to identify and describe a specific subset of high-risk patients who have additional risk of developing hemorrhagic complications, in order to find rapidly and easily assessed parameters that can help clinicians predict these complications and optimize their treatment.

PATIENTS AND METHODS

All the patients diagnosed with high-risk neuroblastoma at Bambino Gesù Children's Hospital from January 2013 until February 2021 were included in the study. Risk stratification was performed according to the criteria of the International Neuroblastoma Risk Group (INRG) Classification System (3); all the patients were evaluated and treated according to the High Risk Neuroblastoma Study 1.8 of SIOP-Europe (SIOPEN) (10). Patients who were referred from other institutions after the diagnosis had already been established were excluded from the present study.

At presentation, all the patients underwent full clinical assessment, serial measurement of arterial pressure, complete blood count, lactate dehydrogenase (LDH) and uric acid serum levels, urinary catecholamine metabolites, i.e., vanilmandelic acid (VMA) and homovanillic acid (HVA), coagulation tests, hepatic and renal function tests.

All the patients underwent total-body contrast-enhanced computed tomography (CT) and meta-iodobenzylguanidine (MIBG) scintigraphy.

Diagnosis was confirmed by histology performed on core needle biopsy and amplification of N-MYC on tumor specimens was determined for every patient. All the patients underwent bone marrow biopsy as part of initial work-up.

Patients were divided in two groups; patients who developed hemorrhagic complications during induction chemotherapy were categorized in group A, while patients who did not develop such complications were categorized in group B.

In order to differentiate anemia secondary to chemotherapy-induced bone marrow aplasia form anemia secondary to blood loss, hemorrhagic complications were defined by the concurrent presence of the following three criteria: (1) anemia (i.e., hemoglobin levels below 8.0 gr/dL) that persisted after the transfusion of 10 mL/kg of packed red cells, (2) the presence of respiratory distress or abdominal pain or distension, (3) radiological evidence of pleural effusion or free abdominal fluid.

The following variables were analyzed: clinical features, laboratory findings, radiologic assessment, histology/biology (see detailed description below).

Statistical analyses were performed using Prism 9.0.0.121 (GraphPad Software, Inc., San Diego, CA).

Categorical variables were analyzed using Fisher's test. Continuous variables were tested for normal distribution using D'Agostino-Pearson test: variables with normal distribution were analyzed using Student's *t*-test, while variables without normal distribution were analyzed with Mann-Whitney test.

Variables that resulted statistically significant on univariate analysis were subsequently tested on multivariate logistic regression; the outcome (dependent) variable was the occurrence of hemorrhage.

A value of $p < 0.05$ was considered statistically significant for each analysis.

Clinical Features

Age at diagnosis: median age at diagnosis in months was calculated separately in the two groups and data have been compared using Mann-Whitney test.

Time from onset of symptoms to diagnosis: time in weeks from onset of symptoms to diagnosis was recorded for each patient from the history reported in the clinical notes. Median time and range were calculated in each group and data were compared using Mann-Whitney test.

Systemic symptoms: the presence of fever >37.5°C, weight loss or asthenia was recorded for each patient in the two groups. Data were compared using Fisher's test.

Arterial pressure: as per institutional protocol, arterial pressure measurements were performed upon admission and every 8 h for each patient. Mean values for the first 4 days from admission were calculated for each patient. Patients were defined as having normal blood pressure, stage 1 hypertension or stage 2 hypertension according to the "Clinical Practice Guideline for Screening and Management of High Blood Pressure in Children and Adolescents" published in 2017 (11). Patients were grouped according to the presence of stage 2 hypertension vs. stage 1 or no hypertension. Data were compared using Fisher's test.

Laboratory Findings

Hemoglobin levels: full blood count was performed in every patient upon admission. Mean hemoglobin level and standard deviation (SD) have been calculated separately in the two groups and data have been compared using Student's *t*-test.

LDH levels: LDH serum levels were measured in every patient upon admission. Median levels and range have been calculated separately in the two groups and data have been compared using Mann-Whitney test.

Urinary VMA and HVA levels: VMA and HVA urinary levels were measured in every patient upon admission. Median levels and range for the two metabolites have been calculated separately in the two groups and data have been compared using Mann-Whitney test.

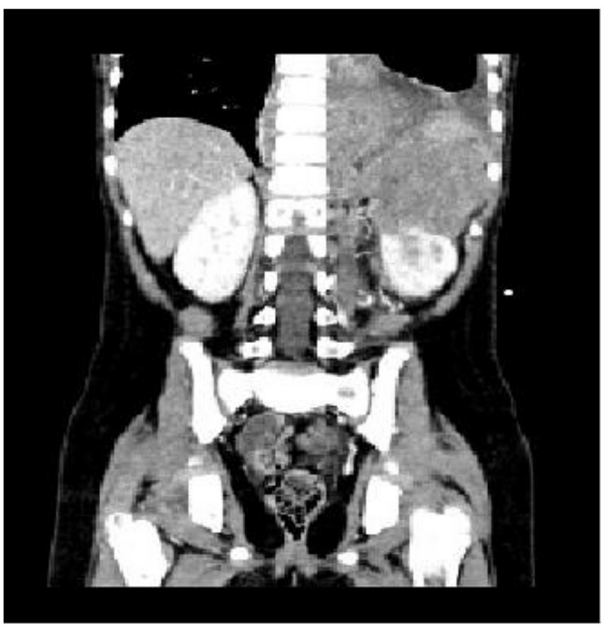

FIGURE 1 | Left retroperitoneal neuroblastoma with posterior mediastinum extension.

TABLE 1 | Patients' characteristics at initial presentation (group A).

Patient	Sex	Age (months)	Presentation					Laboratory				Radiology					Biology		Complications	
			Systemic symptoms	HR Mean	*Centile*	SBP Mean	*Centile*	HTN	LDH	Hb	VMA	HVA	Primary	Side	IDRF Vascular	Total	Max diam (cm)	Stage	n-MYC	Bone marrow	Hemo thorax	Hemoperitoneum
1	M	17	Yes	123	75th	121	>95th + 12 mmHg	2	4,561	7.6	16.8	20	Abd	Left	4	4	13.5	M	ampl	Neg	Yes	
2	M	19	Yes	145	95th	125	>95th + 12 mmHg	2	2,834	6.6	9	33	Abd	Left	4	4	14	L2	ampl	Neg	Yes	Yes
3	F	47	Yes	106	75th	123	>95th + 12 mmHg	2	2,928	8.7	24	197	Thor-Abd	Left	4	5	17	M	ampl	Pos	Yes	
4	M	15	Yes	123	75th	126	>95th + 12 mmHg	2	6,677	7.6	6.25	22	Abd	Left	4	4	14	L2	ampl	Neg		Yes

HR, Heart Rate; SBP, Systolic Blood Pressure (mmHg); HTN, Hypertension (stage); LDH, lactate dehydrogenase (U/L); Hb, hemoglobin (gr/dL); VMA, Vanilmandelic acid (mcg/mg creat); HVA, Homovanillic acid (mcg/mg creat); IDRF, Image-Defined Risk Factors.

TABLE 2 | Patients' characteristics at onset of hemorrhage (group A).

Patient	Onset of hemorrhage days from chemotherapy	Complication		Hemodynamics						Lysis marker*	
		Hemothorax	Hemoperitoneum	HR			SBP			LDH	Variation (x-fold)
				Mean	Centile	% Variation	Mean	Centile	% Variation		
1	2	Yes		153	99th	24	112	95th + 4 mmHg	−7	21,495	x 4.7
2	2	Yes	Yes	146	95th	0	114	95th + 6 mmHg	−9	11,163	x 3.9
3	7	Yes		132	95th	25	104	90th	−15	12,959	x 4.4
4	2		Yes	140	90th	14	107	95th	−15	20,771	x 3.1

HR, Heart Rate; SBP, Systolic Blood Pressure (mmHg); LDH, lactate dehydrogenase (U/L).

**Lysis marker value is measured as the highest level of LDH in the seven days following the first course of chemotherapy.*

Radiologic Assessment

Maximum diameter of the primary tumor: maximum diameter of the primary tumor was measured for each patient on initial CT images by the radiologist who performed the investigation and subsequently revised by GLN and PDP. Mean diameter and standard deviation (SD) have been calculated separately in the two groups and data have been compared using Student's *t*-test.

Vascular and total Image-Defined Risk Factors (IDRF): the presence and number of both vascular and total IDRF was assessed for each patient on initial CT images by the radiologist who performed the investigation and subsequently revised by GLN and PDP. IDRF were defined according to the International Neuroblastoma Risk Group (INRG) Staging System (4). Mean number and standard deviation (SD) have been calculated separately in the two groups and data have been compared using Student's *t*-test.

Stage: patients were staged by CT scan and MIBG scintigraphy according to the International Neuroblastoma Risk Group (INRG) Staging System (4). Stage distributions in the two groups were compared using Fisher's test.

Histology/Biology

Bone marrow infiltration: bone marrow biopsy was performed at initial presentation in every patient. Patients were grouped according to the presence vs. absence of neuroblastoma infiltrates in the bone marrow. Data were compared using Fisher's test.

N-MYC amplification: amplification of N-MYC on biopsy specimens was determined for every patient. Patients were grouped according to the presence vs. absence of N-MYC amplification. Data were compared using Fisher's test.

RESULTS

In the examined period, 44 patients were diagnosed with High-risk neuroblastoma at our institution and were included in the present study. None of the patient had pre-existing comorbidities and coagulation tests, hepatic and renal function tests did not reveal any abnormality in any patient.

All the patients had avid uptake on MIBG scan.

All the patients received induction chemotherapy according to the Rapid COJEC schedule of the High Risk Neuroblastoma Study 1.8 of SIOP-Europe (SIOPEN) (10).

Four patients (9%) developed hemorrhagic complications within 2–7 days (mean 3.25 days) after the administration of the first course of chemotherapy and were categorized in group A; two patients had hemothorax, one patient had hemoperitoneum and one patient had hemothorax and hemoperitoneum. All these patients had primary left retroperitoneal tumors, one patient also had extension of neoplastic tissue in the posterior mediastinum (**Figure 1**) and all of them presented with encasement of the aorta, the celiac tripod, the superior mesenteric artery and the left renal pedicle. Two patients had stage L2 disease, and two patients had stage M disease and no one of them had evidence of active bleeding on the initial staging CT scan. The clinical features of these patients at diagnosis and at the onset of hemorrhage are summarized in **Tables 1**, **2**, respectively.

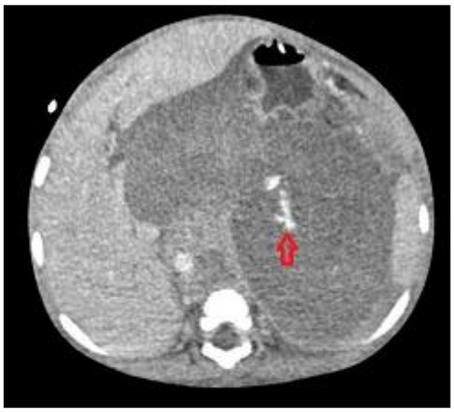

FIGURE 2 | Bleeding from left diaphragmatic artery (CT scan: circle).

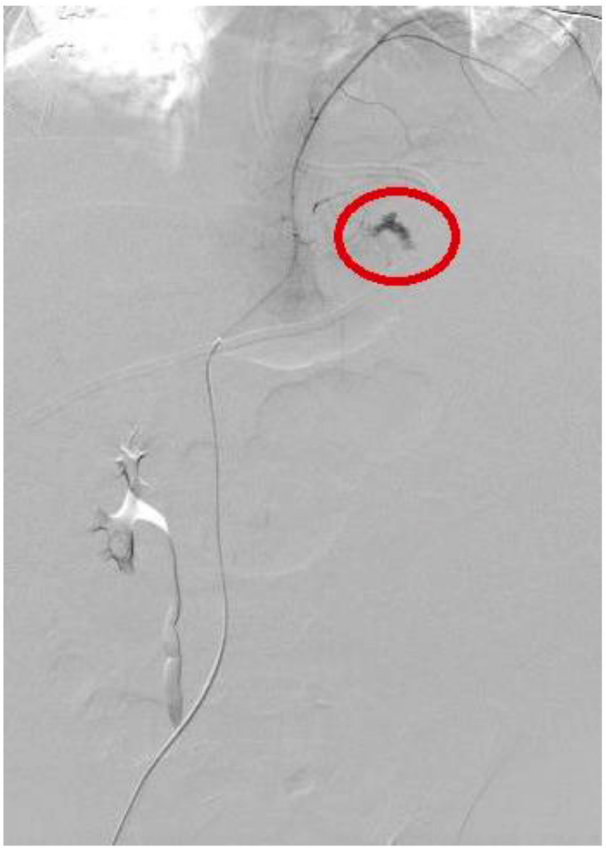

FIGURE 3 | Bleeding from left diaphragmatic artery (angiography: circle).

All these four patients received medical treatment consisting in packed red cells and platelets transfusions, plasma or purified vitamin K-dependent clotting factors infusions and albumin infusions. The two patients with isolated hemothorax underwent thoracostomy tube placement. The patient with associated hemothorax and hemoperitoneum had radiological evidence of bleeding from the left diaphragmatic artery (**Figures 2**, **3**); this patient underwent thoracostomy tube placement and angio-embolization of the bleeding vessel (**Figure 4**). The patient with isolated hemoperitoneum received medical treatment only.

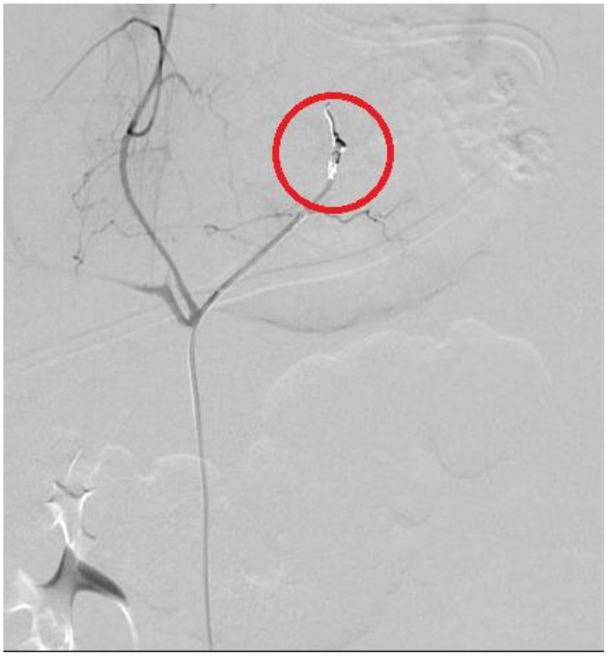

FIGURE 4 | Embolization of left diaphragmatic artery (angiography: circle).

All the patients successfully recovered after the hemorrhagic complications. **Table 3** summarizes the treatment for each patient. One patient died of progressive disease 4 months after diagnosis, two patients are currently on first line treatment and one patient is in complete remission with a follow up of 52 months.

Forty patients (91%) did not develop hemorrhagic complications and were therefore categorized in group B. Thirty-seven patients (92.5%) had primary retroperitoneal tumor, with mediastinal extension in two cases. Two patients (5%) had primary mediastinal tumors and one patient (2.5%) had primary cervical localization. Four patients (10%) had stage L2 disease and 36 (90%) had stage M disease. None of these patients developed hemorrhagic complications during subsequent courses of chemotherapy.

Clinical Features

Age at diagnosis: median age for patients in group A was 18 months (range 15–47 months) while mean age in group B was 42 months (range 11–199 months). Patients in group A were significantly younger than patients in group B ($p = 0.0343$; Mann-Whitney).

Time from onset of symptoms to diagnosis: median time was 3 weeks in group A (range 1–4 weeks) and 4 weeks in group B (range 1–22 weeks). The difference between the two groups was not statistically significant ($p = 0.2741$; Mann-Whitney).

Systemic symptoms: all the patients (100%) in group A presented with systemic symptoms while 26 patients (72.5%) in group B had systemic symptoms at diagnosis. There was no statistically significant difference between the two groups ($p = 0.2897$; Fisher's).

Arterial pressure: all the patients in group A (100%) presented with stage 2 hypertension while 3 patients (7.5%) in group B had stage 2 hypertension. Patients in group A had a statistically significant higher severity of hypertension ($p = 0.0003$; Fisher's).

Laboratory Findings

Hemoglobin levels: mean hemoglobin level was 7.6 gr/dL (SD 0.9 gr/dL; range 6.6–8.7 gr/dL) in group A and 9.9 gr/dL (SD 1.9 gr/dL; range 5.8–14.7 gr/dL) for group B. The difference between the two groups was statistically significant ($p = 0.0007$; Student's *t*-test).

LDH levels: median LDH serum level was 3,745 IU/L (range 2834–6677 IU/L) for group A and 1,089 IU/L (range 366–8,640 IU/L) for group B. Patients in group A had statistically significant higher levels of LDH compared to group B ($p = 0.009$; Mann-Whitney).

Urinary VMA and HVA levels: median VMA level was 12.9 mcg/mg creat (range 6.25–24.0 mcg/mg creat) for group A and 98.4 mcg/mg creat (range 5.0–2657.0 mcg/mg creat) for group B. The difference was statistically significant ($p = 0.0048$; Mann-Whitney).

Median HVA level was 27.5 mcg/mg creat (range 20.0–197.0 mcg/mg creat) for group A and 128.1 mcg/mg creat (range 9.5–2,191.0 mcg/mg creat) for group B. The difference was not statistically significant ($p = 0.0612$, Mann-Whitney).

Radiologic Assessment

Maximum diameter of the primary tumor: mean value for maximum diameter of the primary tumor was 14.6 cm (SD 1.6 cm; range 13.5–17.0 cm) for group A and 10.9 cm (SD 3.8 cm; range 4.1 – 18.0 cm). The difference between the two groups was not statistically significant ($p = 0.0625$, Student's *t*-test).

Vascular and total Image Defined Risk Factors (IDRF): all the patients in group A had 4 vascular IDRF, while mean number of vascular IDRF in group B was 2.2 (st. dev 1.1; range 0–4). The difference was statistically significant ($p = 0.0024$, Student's *t*-test).

The mean number of total IDRF was 4.2 (SD 0.5; range 4–5) in group A and 3.2 (SD 1.4; range 0–5) in group B. The difference between the two groups was not statistically significant ($p = 0.0752$, Student's *t*-test).

Stage: in group A 2 patients had stage L2 disease and 2 patients had stage M disease 50–50%). In group B 4 patients had stage L2 disease and 36 had stage M disease (10–90%). The difference was not statistically significant ($p = 0.0834$, Fisher's).

Histology/Biology

Bone marrow infiltration: one patient (25%) in group A had bone marrow infiltrates while 34 patients (85%) in group B had positive bone marrow biopsy. Patients with hemorrhagic complications had a significantly lower incidence of bone marrow metastases ($p = 0.0226$, Fisher's).

TABLE 3 | Patients' treatment (group A).

Patient	Complication		Medical treatment						Operative treatment	Respiratory support	ICU stay
	Hemothorax	Hemoperitoneum	Amlodipine	Carvedilol	Albumine	Blood prod red cells	Platelets	Clotting fact Plasma	Purified vit-k factors	Chest tube Embolization	
1	Yes		0.3 mg/Kg/day	0.5 mg/Kg/day	1.0 gr/kg x 6 dd	20 mL/kg		2 units	20 UI/Kg	Yes	HFNC (FiO2 30%; 20 l/min) x 3 days
2	Yes	Yes	0.3 mg/Kg/day	0.5 mg/Kg/day	1.5 gr/kg x 4 dd	120 mL/kg	10 units	1 unit	45 UI/Kg	Yes Yes	Mechanical ventilation x 5 days 6 days
3	Yes		0.3 mg/Kg/day	0.4 mg/Kg/day	0.5 gr/Kg x 10 dd	40 mL/kg	4 units			Yes	Mechanical ventilation x 3 days 9 days
4		Yes	0.4 mg/Kg/day	0.6 mg/Kg/day	0.5 gr/Kg x 10 dd	40 mL/kg	8 units		50 UI/Kg		

HFNC, High Flow Nasal Cannula.

TABLE 4 | Comparison and statistics.

	Group A		Group B		*p*-value	
Number of patients	4		40			
Clinical presentation						
Age (months: median + range)	18	15–47	42	11–199	0.0343	*Significant*
Time from onset to diagnosis (weeks: median + range)	3	1–4	4	1–22	0.2741	
Systemic symptoms (n. of pts + percentage)	4	100%	26	72.50%	0.2897	
Arterial pressure (n. of pts + percentage)						
Hypertension stage 2	4	100%	3	7.50%	0.0003	*Significant*
Hypertension stage 1	0	0%	7	17.50%		
Normal arterial pressure	0	0%	30	75%		
Laboratory findings						
Hb (gr/dL: mean + standard deviation)	7.6	0.9	9.9	1.9	0.0007	*Significant*
LDH (IU/L: median + range)	3,745	2,834–6,677	1,864	366–8,640	0.009	*Significant*
VMA (mcg/mg creat: median + range)	12.9	6.25–24.0	98.4	5.0–2,657.0	0.0048	*Significant*
HVA (mcg/mg creat: median + range)	27.5	20.0–197.0	128.1	9.5–2,191.0	0.0612	
Radiology						
Maximum diameter (cm: mean + standard deviation)	14.6	1.6	10.9	3.8	0.0625	
IDRF (number + standard deviation)						
Vascular	4	0	2.2	1.1	0.0024	*Significant*
Total	4.2	0.5	3.2	1.4	0.0752	
Stage (L2 vs. M)	2	2	4	36	0.0834	
Istology/biology						
Bone marrow infiltration (n. of pts + percentage)	1	25%	34	85%	0.0226	*Significant*
N-MYC amplification (n. of pts + percentage)	4	100%	23	57.50%	0.1468	

LDH, lactate dehydrogenase; Hb, hemoglobin; VMA, Vanilmandelic acid; HVA, Homovanillic acid; IDRF, Image-Defined Risk Factors.

N-MYC amplification: all the patients (100%) in group A had amplification of N-MYC while 23 patients (57.5%) in group B had N-MYC amplification. Such difference was not statistically significant ($p = 0.1468$, Fisher's).

The variables that were associated with bleeding on univariate analysis (i.e., age, stage 2 hypertension, hemoglobin levels, LDH levels, urinary VMA levels, vascular IDRF, bone marrow infiltration) were subsequently tested for multivariate logistic regression; the model resulted in a perfect separation.

Results are summarized in **Table 4**.

DISCUSSION

Hemorrhage is an uncommon, life-threatening event in patients affected by neuroblastoma (7, 12, 13). In a recent paper, Qin et al. reported 47 neuroblastoma patients with hemorrhage, either secondary to spontaneous tumor rupture or after chemotherapy or biopsy, on a total population of ∼1,800 patients, with an incidence of approximately 2.6% and poor outcome; treatment was withdrawn in 17 of these patients, while other 5 patients died as an immediate consequence of this complication (9).

The mechanism underlying spontaneous hemorrhage in neuroblastoma has been debated; in neonates, an adrenal mass could be crushed between the liver and the spine during delivery, causing tumor rupture and subsequent hemorrhage (14), while in older children the presence of neuroblastoma could predispose to adrenal hemorrhage following minor trauma (15).

Most authors report massive hemorrhage in patients younger than 18 months affected by high risk neuroblastoma with N-MYC amplification (7, 8, 13, 15–17). In their large case series, Qin et al. found two independent risk factors on multivariate analysis, i.e., the presence of N-MYC amplification and high tumor bulk, measured as maximum diameter of the primary mass. These authors also reported younger mean age (29 months vs. 43 months) and higher LDH values (3148.5 U/L vs. 723 U/L) in patients with hemorrhage secondary to tumor rupture compared to other neuroblastoma patients (9).

In the present case series, 4 out of 44 (9%) patients experienced hemorrhage, a proportion that is higher than previous reports (9). All the patients in group A and the majority of patients in group B had N-MYC amplification, without statistically significant difference between the two groups. Both observations are expected due to the selection of high risk patients only in the study.

The presence of systemic symptoms (i.e., fever, weight loss, asthenia) was similar in the two groups. Systemic symptoms are frequently associated with metastatic neuroblastoma (6, 18); a high prevalence of systemic symptoms is therefore anticipated in a population of high risk neuroblastoma patients.

Patients who experienced hemorrhage were significantly younger than other high risk patients; this observation is consistent with previously published data (9).

Patients in group A presented with a higher severity of hypertension compared to group B, i.e., stage 2 according to the clinical practice guidelines of the American Academy of

Pediatrics (11). Hypertension is classified as a life-threatening symptom that warrants chemotherapy in the European Low and Intermediate Risk Neuroblastoma Protocol (19), but the severity of hypertension is not part of the risk stratification algorithm and is not reported in previous literature as a risk factor for hemorrhage.

Hypertension in neuroblastoma patients has been associated with catecholamine release, although a linear correlation between the severity of hypertension and urinary catecholamine levels has not been demonstrated (20, 21). In the present case series, patients with hemorrhagic complications had lower levels of urinary VMA compared to patients without hemorrhage. Such observation is consistent with the study by Qin et al. who report lower urinary VMA and HVA levels in patients who experience neuroblastoma rupture. Streger et al. found low levels of urinary VMA in patients with N-MYC amplification and high levels of urinary dopamine in higher stage neuroblastoma (22).

Patients in group A presented with a high mean number of vascular IDRF compared to group B; vascular encasement, especially of the renal artery (20), may contribute to the development of hypertension and also predispose patients to vascular erosion and bleeding.

Patients who had hemorrhagic complications presented with lower hemoglobin levels at presentation compared to patients without hemorrhage despite a lower incidence of bone marrow metastases. Neuroblastoma can alter the microenvironment of bone marrow irrespective of neoplastic cell invasion, causing downregulation of genes involved in cell adhesion, and in erythrocyte, myeloid, and platelet differentiation pathways (23). Neuroblastoma can impair erythropoiesis by selectively disrupting the late stage of erythrocytes' maturation independently of the physical presence of neuroblastoma cells in the bone marrow, thus reducing hemoglobin levels in peripheral blood (24). All the patients in our study were affected by high risk neuroblastoma and therefore neuroblastoma-induced impaired erythropoiesis should have theoretically affected all the patients to a similar extent; patients who are prone to develop hemorrhagic complications may be more susceptible to the mechanism that inhibits erythropoiesis in neuroblastoma patients. Another explanation for lower hemoglobin levels could be related to slow, chronic intratumoral bleeding secondary to vascular erosion in patients who then develops frank hemorrhage; the extent of vascular encasement in these patients might support the second explanation. Large, multicentric series are necessary to clarify this issue.

Patients in group A had significantly higher LDH levels at diagnosis compared to group B; such data are consistent with the work by Qin et al. and other published case reports (8, 9, 17). High serum LDH levels at diagnosis are associated with poorer outcome in terms of event-free and overall survival in high-risk neuroblastoma (25) and can be interpreted as the serum marker of high tumor burden. Qin et al. have found a correlation between tumor burden, measured as the maximum diameter of the primary mass, and the risk of neuroblastoma rupture (9); in the present case series, a statistically significant correlation between tumor diameter and risk of hemorrhage could not be demonstrated.

In the present case series, all the patients who developed hemorrhagic complications presented with severe hypertension (i.e., stage 2), low hemoglobin levels and high serum LDH levels at diagnosis; such features are easy and immediate to detect and can be frequently reassessed with commonly available resources and minimal discomfort for the patient. These three criteria can therefore be used to differentiate patients who have an additional risk of hemorrhage from other high-risk neuroblastoma patients.

All the patients developed hemorrhage after the initiation of chemotherapy. We may speculate that chemotherapy-induced tumor lysis, evidenced by a sharp rise in LDH levels (see **Table 2**) might cause necrosis of the tissue encasing blood vessels; in such situation, vessel walls that have previously been eroded by neoplastic tissue might be more prone to bleeding. A similar clinical scenario has been demonstrated in patients with metastatic choriocarcinoma and is defined as "choriocarcinoma syndrome" (26). The postulation of a such mechanism in high-risk neuroblastoma patients, however, is speculative; further studies are needed to specifically investigate this issue.

All these patients had systolic pressure above 90th centile for age and height even during active bleeding and under anti-hypertensive treatment; the only abnormality in their hemodynamics was the development of tachycardia (see **Table 2**). Such observation is in contrast with several studies that report the development of frank hemorrhagic shock secondary to neuroblastoma rupture (7, 9, 13) and highlights the importance of a high index of suspicion and close monitoring of patients who present with the aforementioned risk factors.

Three patients had hemothorax ipsilateral to the primary retroperitoneal tumor. Pleural effusion sometimes can be associated with retroperitoneal neuroblastoma and is generally interpreted as reactive (27); in our cases, we believe that hemothorax can be secondary to the spreading of retroperitoneal hemorrhage through the diaphragmatic crura, as suggested by the angiography performed in patient 2 (**Figure 3**).

Neuroblastoma patients with hemorrhagic complications need multimodal treatment that should be focused at controlling and limiting the bleeding while supporting vital functions, consisting in blood products administration, crystalloid and colloid infusion, surgical drain of hemothorax and angiographic control of the bleeding source.

Some authors have reported cases of successful emergency surgery on the primary mass in patients with ruptured neuroblastoma (7, 13); in our case series, all the patients with hemorrhagic complications presented with encasement of the aorta and its major branches, that is a well-documented risk factor for major surgical complications and incomplete resection (28–30). In this scenario, emergency surgery on the primary mass should be reserved to patients who do not respond to other therapeutic measures.

The present study has an obvious limitation in its retrospective nature; another limitation is the single-institution design of the study, that reduces the number of patients. The main strong point of this study is the homogeneity of the patients in both groups, who are all affected by high risk neuroblastoma without pre-existing comorbidities, have a comparable prevalence of metastatic disease, a comparable size of the primary tumor and

are all treated according to the same protocol; such homogeneity reduces the presence of confounding factors in our analyses.

The result of a perfect separation on multivariate logistic regression is puzzling; it might be the expression of a substantial clinical difference between the two groups, or it might be simply related to the small sample size. Further studies with larger sample size might better clarify such result.

In conclusion, the present data suggest that within the population of patients affected by high-risk neuroblastoma there is a subgroup of children with some specific clinical features, i.e., stage 2 hypertension, anemia, elevated serum LDH levels and multiple vascular IDRF at diagnosis, who have an "additional" risk of developing hemorrhage during induction chemotherapy.

The small sample size of the present study does not allow to establish a clear causal relation; however, we suggest that patients who present with these features at diagnosis are carefully monitored so that hemorrhagic complications are promptly diagnosed and treated before hemorrhagic shock develops.

Further studies are needed to confirm the present observations, define cut-off values for these parameters and design optimal management strategies for these patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

VV, GP, ACr, ACa, and AI contributed to conception and design of the study. VV and GP organized the database. CM, ASe, and UG revised and analyzed the data. GN and PD revised radiology images. ASt revised the pathology specimens. All authors contributed to manuscript revision, read, and approved the submitted version.

ACKNOWLEDGMENTS

The authors thank Dr. Daniela Pacella, Ph.D., from the Department of Public Health and Medical Statistics of Federico II University in Naples, and Prof. Francesco Solombrino, Ph.D., from the Department of Mathematics of Federico II University in Naples, for critical revision of statistical analysis.

REFERENCES

1. Yan P, Qi F, Bian L, Xu Y, Zhou J, Hu J, et al. Comparison of incidence and outcomes of neuroblastoma in children, adolescents, and adults in the United States: a Surveillance, Epidemiology, and End Results (SEER) program population study. *Med Sci Monit.* (2020) 26:e927218. doi: 10.12659/MSM.927218
2. Irwin MS, Park JR. Neuroblastoma: paradigm for precision medicine. *Pediatr Clin North Am.* (2015) 62:225–562. doi: 10.1016/j.pcl.2014.09.015
3. Cohn SL, Pearson ADJ, London WB, Monclair T, Ambros PF, Brodeur GM, et al. The International Neuroblastoma Risk Group (INRG) classification system: an INRG task force report. *J Clin Oncol.* (2009) 27:289–97. doi: 10.1200/JCO.2008.16.6785
4. Monclair T, Brodeur GM, Ambros PF, Brisse HJ, Cecchetto G, Holmes K, et al. The International Neuroblastoma Risk Group (INRG) staging system: an INRG task force report. *J Clin Oncol.* (2009) 27:298–303. doi: 10.1200/JCO.2008.16.6876
5. Whittle SB, Smith V, Doherty E, Zhao S, McCarty S, Zage PE. Overview and recent advances in the treatment of neuroblastoma. *Expert Rev Anticancer Ther.* (2017) 17:369–86.. doi: 10.1080/14737140.2017.1285230
6. Tolbert VP, Matthay KK. Neuroblastoma: clinical and biological approach to risk stratification and treatment. *Cell Tissue Res.* (2018) 372:195–209. doi: 10.1007/s00441-018-2821-2
7. Meersman A, Wojciechowski M, Vaneerdeweg W, Jorens P, Michiels E, Ramet J. Acute retroperitoneal hemorrhage and shock as presenting signs of neuroblastoma in an infant. *Pediatr Emerg Care.* (2008) 24:37–8. doi: 10.1097/pec.0b013e31815f3c60
8. Shiokawa N, Okamoto Y, Kodama Y, Nishikawa T, Tanabe T, Mukai M, et al. Conservative treatment of massive hemothorax in a girl with neuroblastoma. *Pediatr Int.* (2016) 58:1090–2. doi: 10.1111/ped.13094
9. Qin H, Yang S, Cai S, Ren Q, Han W, Yang W, et al. Clinical characteristics and risk factors of 47 cases with ruptured neuroblastoma in children. *BMC Cancer.* (2020) 20:243. doi: 10.1186/s12885-020-06720-9
10. *High Risk Neuroblastoma Study 1.8 of SIOP-Europe (SIOPEN)*. Available online at: https://clinicaltrials.gov/ct2/show/NCT01704716 (accessed November 18, 2019).
11. Flynn JT, Kaelber DC, Baker-Smith CM, Blowey D, Carroll AE, Daniels SR, et al. Subcommittee on screening and management of high blood pressure in children. Clinical practice guideline for screening and management of high blood pressure in children and adolescents. *Pediatrics*. (2017) 140:e20171904. doi: 10.1542/peds.2017-3035
12. Vora D, Slovis TL, Boal DK. Hemoperitoneum and disseminated intravascular coagulation in two neonates with congenital bilateral neuroblastoma. *Pediatr Radiol.* (2000) 30:394–7. doi: 10.1007/s002470050769
13. Honda S, Miyagi H, Minato M, Kubota KC, Okada T. Hemorrhagic shock due to spontaneous rupture of adrenal neuroblastoma in an infant: a rare case and review of the literature. *J Pediatr Hematol Oncol.* (2012) 34:635–7. doi: 10.1097/MPH.0b013e3182678e1e
14. Brock CE, Ricketts RR. Hemoperitoneum from spontaneous rupture of neonatal neuroblastoma. *Am J Dis Child.* (1982) 136:370–1. doi: 10.1001/archpedi.1982.03970400088026
15. Normand C, Leblond P, Mazingue F, Nelken B, Defachelles AS, Bonnevalle M. A case of adrenal haemorrhage after minor trauma in a young child: think of neuroblastoma. *Eur J Pediatr Surg.* (2006) 16:365–8. doi: 10.1055/s-2006-924605
16. Tay M, Kapur J. Neuroblastoma with primary pleural involvement: an unusual presentation. *Pediatr Radiol.* (2011) 41:530–3. doi: 10.1007/s00247-010-1884-3
17. Lode HN, Henze G, Siebert N, Ehlert K, Barthlen W. Management of tumor rupture and abdominal compartment syndrome in an infant with bilateral high risk stage 4 neuroblastoma: a case report. *Medicine.* (2019) 98:e16752. doi: 10.1097/MD.0000000000016752
18. Chung C, Boterberg T, Lucas J, Panoff J, Valteau-Couanet D, Hero B. Neuroblastoma. *Pediatr Blood Cancer.* (2021) 68:e28473. doi: 10.1002/pbc.28473
19. *European Low and Intermediate Risk Neuroblastoma Protocol: a SIOPEN Study; Version 5.0; 1st October 2015*. Available online at: https://bspho.be/wp-content/uploads/2019/05/LINES-20170102-LINES_Protocol_version-5.0.pdf (accessed July 24, 2021).
20. Madre C, Orbach D, Baudouin V, Brisse H, Bessa F, Schleiermacher G, et al. Hypertension in childhood cancer: a frequent complication of certain tumor sites. *J Pediatr Hematol Oncol.* (2006) 28:659–64. doi: 10.1097/01.mph.0000212995.56812.bb
21. Kwok SY, Cheng FWT, Lo AFC, Leung WK, Yam MC, Li CK. Variants of cardiomyopathy and hypertension in neuroblastoma. *J Pediatr Hematol Oncol.* (2014) 36:e158-61. doi: 10.1097/MPH.0b013e318290c628

22. Strenger V, Kerbl R, Dornbusch H J, Ladenstein R, Ambros PF, Ambros IM, et al. Diagnostic and prognostic impact of urinary catecholamines in neuroblastoma patients. *Pediatr Blood Cancer.* (2007) 48:504–9. doi: 10.1002/pbc.20888
23. Scaruffi P, Morandi F, Gallo F, Stigliani S, Parodi S, Moretti S, et al. Bone marrow of neuroblastoma patients shows downregulation of CXCL12 expression and presence of IFN signature. *Pediatr Blood Cancer.* (2012) 59:44–51. doi: 10.1002/pbc.23339
24. Morandi F, Barco S, Stigliani S, Croce M, Persico L, Lagazio C, et al. Altered erythropoiesis and decreased number of erythrocytes in children with neuroblastoma. *Oncotarget.* (2017) 8:53194–209. doi: 10.18632/oncotarget.18285
25. Moreno L, Guo D, Irwin MS, Hogarty M, Kamijo T, Morgenstern D, et al. A nomogram of clinical and biologic factors to predict survival in children newly diagnosed with high-risk neuroblastoma: an International Neuroblastoma Risk Group project. *Pediatr Blood Cancer.* (2021) 68:e28794. doi: 10.1002/pbc.28794
26. Rejlekova K, Cursano MC, De Giorgi U, Mego M. Severe complications in testicular germ cell tumors: the choriocarcinoma syndrome. *Front Endocrinol.* (2019) 10:218. doi: 10.3389/fendo.2019.00218
27. Gupta H, Conrad J, Khoury JD, McGregor LM, Krasin MJ, Dome JS, et al. Significance of pleural effusion in neuroblastoma. *Pediatr Blood Cancer.* (2007) 49:906–8. doi: 10.1002/pbc.21199
28. Cecchetto G, Mosseri V, De Bernardi B, Helardot P, Monclair T, Costa E, et al. Surgical risk factors in primary surgery for localized neuroblastoma: the LNESG1 study of the European International Society of Pediatric Oncology Neuroblastoma Group. *J Clin Oncol.* (2005) 23:8483–9. doi: 10.1200/JCO.2005.02.4661
29. Irtan S, Brisse HJ, Minard-Colin V, Schleiermacher G, Galmiche-Rolland L, Le Cossec C, et al. Image-defined risk factor assessment of neurogenic tumors after neoadjuvant chemotherapy is useful for predicting intra-operative risk factors and the completeness of resection. *Pediatr Blood Cancer.* (2015) 62:1543–9. doi: 10.1002/pbc.25511
30. Temple WC, Vo KT, Matthay KK, Balliu B, Coleman C, Michlitsch J, et al. Association of image-defined risk factors with clinical features, histopathology, and outcomes in neuroblastoma. *Cancer Med.* (2021) 10:2232–41. doi: 10.1002/cam4.3663

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frontiers in Pediatrics

REVIEW
published: 20 December 2021
doi: 10.3389/fped.2021.777840

Above and Beyond Robotic Surgery and 3D Modelling in Paediatric Cancer Surgery

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Although the survival rates for children's cancers have more than doubled in the last few decades, the surgical practise has not significantly changed. Among the most recent innovations introduced in the clinic, robotic surgery and augmented reality are two of the most promising, even if they are not widespread. The increased flexibility of the motion, the magnification of the surgical field and the tremor reduction provided by robotic surgery have been beneficial to perform complex oncological procedures in children. Besides, augmented reality has been proven helpful in planning for tumour removal, facilitating early discrimination between cancer and healthy organs. Nowadays, research in the field of surgical oncology is moving fast, and new technologies and innovations wich will help to shape a new way to perform cancer surgery. Paediatric surgeons need to be ready to adopt these novel devices and intraoperative techniques to allow more radical tumour resections with fewer complications. This review aims to present the mechanism of action and indications of several novel technologies such as optical imaging surgery, high definition cameras, and intraoperative loco-regional treatments. We hope this will enhance early adoption and more research on how to employ technology for the benefit of children.

Keywords: paediatric surgery, oncology surgery, optical imaging, spectroscopy, cancer imaging, novel intraoperative technologies, fluorescence-guided surgery, children

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Specialty section:
This article was submitted to Pediatric Surgery, a section of the journal Frontiers in Pediatrics

Received: *15 September 2021*
Accepted: *17 November 2021*
Published: *20 December 2021*

Citation:
Privitera L, Paraboschi I, Cross K and Giuliani S (2021) Above and Beyond Robotic Surgery and 3D Modelling in Paediatric Cancer Surgery. Front. Pediatr. 9:777840. doi: 10.3389/fped.2021.777840

INTRODUCTION

With 367,000 new cases in the UK every year, paediatric and adult solid cancers are among the top causes of death worldwide. Although survival rates for children's cancers have more than doubled between the 1970s and 2000s, oncology surgery has not significantly changed in the last thirty years (1).

The surgical resection of tumours still represents one of the main treatments for nearly all the new solid cancer diagnosis (1). According to the literature, clear surgical margins and a maximal degree of tumour resection strongly impact patients' outcomes (2, 3). However, the delineation of cancer margins and the microscopical clearance of the disease remain significant challenges. One

of the techniques more recently adopted to enhance live surgical precision and accuracy during complex oncological procedures is robotic surgery (**Table 1**) (4, 6, 10, 16–20). The increased flexibility of the motion, the surgical field's magnification with enhanced 3D visualisation, and the tremor reduction have all been described as particularly useful to perform even challenging surgical procedures in children. Besides, the creation of 3D models in preparation for surgery or superimposed onto the surgical field have also been trialled to enhance the surgical practise (21–25). Furthermore, augmented reality acts as an effective adjunct by increasing peri-operative information, and it has been proven beneficial when removing tumours, facilitating discrimination between malignant tissue and adherent healthy organs (22, 26–28).

However, beyond the applications of robotic surgery and augmented reality, there has been a significant drive toward the clinical translation of other technologies, devices and intra-operative treatments to enable surgeons to perform safer and more radical resections. We envisage that optical imaging surgery, high definition cameras and intraoperative loco-regional therapies have the potential to revolutionise surgical oncology through more effective visualisation and removal of cancer. The aim of this article is, therefore, to review these novel promising technologies and devices to disseminate their application and facilitate a quicker adoption in the field of paediatric surgery.

OPTICAL IMAGING IN SURGERY

One of the crucial aspects of performing safe and effective cancer removal is visualising normal anatomical structures and differentiating them from the disease. Although human eyes can reconstruct the shape and architectural features, they cannot distinguish between spectra with slight separation in wavelengths, meaning that different tissues are very similar in colour (29). MRI, CT, ultrasonography, and nuclear imaging scans play an important role in the pre-operative assessment, allowing high-resolution whole-body imaging. However, their use in the operative field is limited (30, 31). There has been recently increased interest in the clinical application of optical imaging techniques in the operating theatre. Optical imaging offers a detailed picture of body anatomy, offering high-resolution images of entire organs down to molecules smaller than 10 μm, using non-ionising radiation, including visible, ultraviolet and infrared light (**Figure 1**) (29, 32).

Fluorescence-Guided Surgery and Dye-Loaded Targeted Probes in Cancer Surgery

One of the most commonly used optical imaging techniques is fluorescence-guided surgery (FGS). FGS principle is to generate a real-time fluorescence image of the surgical region to help the surgeon delineate their targets (33). Compared to other intra-operative devices, fluorescence imaging systems are relatively affordable, and they do not require specific training as they are generally intuitive to use. The key elements of FGS are a contrast agent (usually administered before the procedure), a light source, philtres for the excitation of the fluorescence agent and a camera to detect the signal designed for either laparoscopic, open or robotic procedures.

Near-infrared (NIR) fluorescence imaging provides a new and versatile platform for visualisation, resection and treatment. Imaging systems are often calibrated around the excitation and emission spectrum of indocyanine green (ICG), widely applied in surgery. In the paediatric field, ICG has been used safely and efficiently to delineate the biliary flow during laparoscopic cholecystectomies, assess bowel perfusion in neonates with necrotising enterocolitis, map arteries during urogenital surgeries and reconstruct the microvascularity in plastic surgery (34, 35). However, it is mainly in the oncological field that FGS has been emerging as a cutting-edge innovation. The live visualisation of the 3D tumour anatomy can facilitate the differentiation between the tumour and the normal tissue and lead to more radical tumour resections with improved surgical outcomes (34, 35). ICG-optical imaging has been adopted for the surgical treatment of several paediatric cancers, including hepatoblastomas, osteosarcomas, non-rhabdomyosarcomas, rhabdomyosarcomas, neuroblastomas, Ewing sarcoma, germ cells tumours, chondroblastoma, solid pseudopapillary neoplasms of the pancreas, lymphoma, and myoepithelial carcinoma (34, 35). Although ICG navigation imaging for paediatric hepatoblastomas is at its initial stages, early results seemed to be very promising (34–39). In particular, ICG-angiography has been very helpful to detect the primary tumour and its peritoneal and lung metastases (36, 36–39). Despite these encouraging results, some issues such as the background noise from adjacent organs, the tissue attenuation and the limitation in-depth penetration still must be addressed (35). Shortly, the record of more cases will lead to more standardised procedural protocols for establishing the optimal timing and dosage for ICG injection and a more accurate patients' selection. Further developments in optical imaging technology will soon overcome these limits, providing fluorescent dyes and detection cameras with greater tissue penetration.

Other than ICG, several reports, critical and systematic reviews have discussed the role of 5-Aminolevulinic acid (5-ALA)-guided surgery in performing a gross total resection of brain tumours in children, with the ultimate aim to improve patients' survival and reduce the risk of recurrences (40–43). The extent of surgical resection is a strong prognostic factor in paediatric brain tumours. However, performing a complete tumour excision can be a real challenge, as these tumours frequently infiltrate adjacent vulnerable tissue, making their clear intraoperative identification particularly demanding. In this regard, the literature seems to support the role of 5-ALA-guided resection to identify brain tumours more efficiently, especially in the case of glioblastoma, anaplastic ependymoma WHO grade III and anaplastic astrocytoma (43). Due to the very promising results achieved by these early studies, the significant impact of this new approach and its less clear role in infratentorial tumours, prospective randomised clinical trials have been advocated to increase the overall level of evidence concerning the usage of 5-ALA in the paediatric population (40–43).

TABLE 1 | Current literature focusing on robotic surgery used for paediatric oncology surgery.

References	Type of study	Number of patients	Mean age (years)	Pathologies included	Mean total operative time	Conversion rate	Mean days of hospitalisation	Robotic system
Blanc et al. (4)	Prospective observational study	89	8.2	Neuroblastomas ($n = 18$) Ganglioneuroblastomas ($n = 4$) Ganglioneuromas ($n = 9$) Wilms' tumours ($n = 20$) Neuroendocrine tumours ($n = 12$) Adrenal tumours ($n = 9$) Germ-cell tumours ($n = 7$) Pancreatic tumours ($n = 4$) Thymic tumours ($n = 4$) Inflammatory myofibroblastic ($n = 4$) Different rare tumours ($n = 5$)	215 min	8%	3	NA
Meehan et al. (5)	Retrospective study	14	NA	Neuroblastoma ($n = 3$) Ovarian teratoma ($n = 1$) Abdominal lymphangioma ($n = 1$) Retroperitoneal tumour ($n = 1$) Pancreatic tumour ($n = 1$) Mediastinal germ cell tumour ($n = 1$) Mediastinal teratoma ($n = 1$) Posterior mediastinal mass ($n = 2$) Not specified abdominal tumour ($n = 1$) Pheochromocytoma ($n = 1$) Mediastinal inflammatory mass ($n = 1$)	NA	29%	NA	Da Vinci system (Intuitive Surgical, Sunnyvale, CA)
Meignan et al. (6)	Multicenter retrospective study	11	7.65	Nephroblastoma ($n = 1$) Metanephric adenoma ($n = 1$) Neuroblastomas ($n = 3$) Pheochromocytomas ($n = 2$) Adrenocortical adenomas ($n = 2$) Cystic lymphangioma ($n = 1$) Paraganglioma ($n = 1$) Pancreatic cyst ($n = 1$)	145 min	8.3%	3	Da Vinci system (Intuitive Surgical, Sunnyvale, CA)
Blanc et al. (7)	Prospective study	10	5	Wilms tumour ($n = 8$) Renal sarcoma ($n = 1$) Renal tubulopapillary carcinoma ($n = 1$)	270 min	30%	5.6	Da Vinci system (Intuitive Surgical, Sunnyvale, CA)

(Continued)

TABLE 1 | Continued

References	Type of study	Number of patients	Mean age (years)	Pathologies included	Mean total operative time	Conversion rate	Mean days of hospitalisation	Robotic system
Varda et al. (8)	Retrospective study	8	12.5	Papillary renal cell carcinoma (n = 1) Segmental cystic dysplasia (n = 2) Benign heterologous tissue with nephrogenic rests (n = 1) Embryonal non-seminomatous germ cell tumour (n = 1) Rhabdomyosarcoma (n = 1) Ganglioneuroma (n = 1) Unclassified spindle cell sarcoma (n = 1)	277 min (PN) 540 min (RPLND)	0	3.7	NA
Meehan et al. (9)	Case reports	5	9.8	Ganglioneuroma (n = 1) Ganglioneuroblastoma (n = 1) Teratoma (n = 1) Germ cell tumour (n = 1) Large inflammatory mass (n = 1)	113 min	0	1.4	Da Vinci system (Intuitive Surgical, Sunnyvale, CA)
Navarrete Arellano and Garibay González (10)	Prospective, observational, longitudinal study	4	4.7	Mediastinal teratoma (n = 1) Retroperitoneal lipoma (n = 1) Pheochromocytoma (n = 1) Not specified (n = 1)	NA	NA	NA	Da Vinci system (Intuitive Surgical, Sunnyvale, CA)
Cost et al. (11)	Case report	1	14	Renal cell carcinoma	180 min	0	2	Da Vinci system (Intuitive Surgical, Sunnyvale, CA)
Hassan et al. (12)	Case report	1	16	Left ventricular myxoma	NA	0	3	Da Vinci system (Intuitive Surgical, Sunnyvale, CA)
Akar et al. (13)	Case report	1	15	Cystic adenomyoma	NA	0	NA	NA
Backes et al. (14)	Case report	1	18	Mullerian rhabdomyosarcoma	315 min	0	5	NA
Anderberg et al. (15)	Case report	1	1.8	Embryonal rhabdomyosarcoma	Min	0	NA	Da Vinci system (Intuitive Surgical, Sunnyvale, CA)

NA, not available; PN, partial nephrectomy; RPLND, retroperitoneal lymph node dissection.

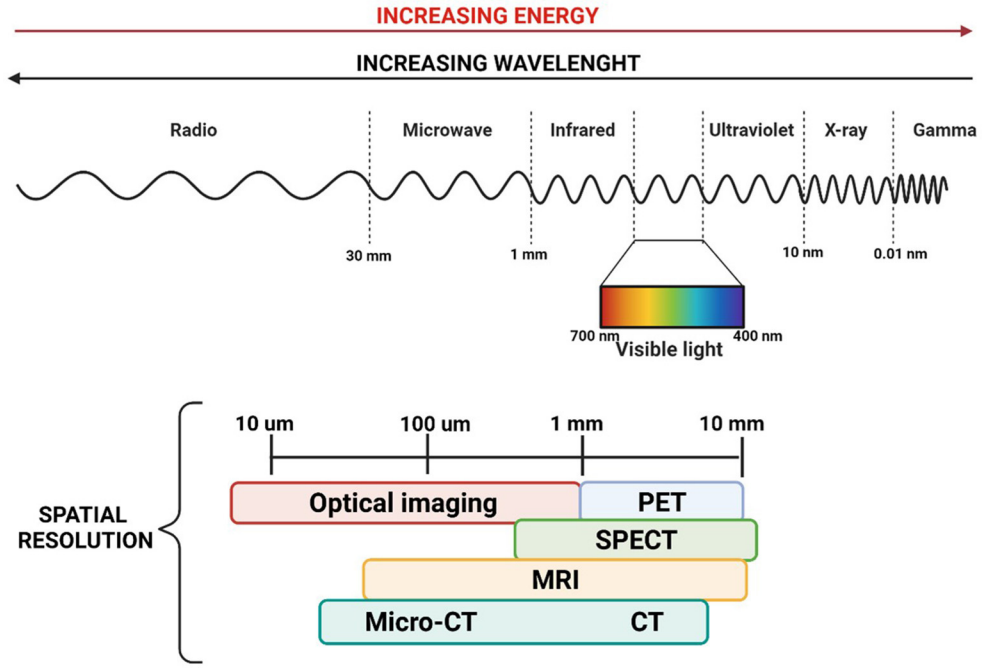

FIGURE 1 | Image of the electromagnetic spectrum and the associated wavelength and energies, with a focus on the spatial resolution of the dominant medical imaging modalities. TC, computer tomography; MRI, magnetic resonance imaging; SPECT, single-photon emission computed tomography; PET, positron emission tomography.

Although this decade has undoubtedly witnessed significant advances in the clinical application and technical development of ICG and 5-ALA optical imaging, there is still room for further developments. For example, tumour-specific targeted probes are currently under investigation to maximise the tumour signal and minimise background noise. This is of particular interest in the paediatric population, as there are tumour-specific monoclonal antibodies that have been already clinically approved (34, 44). The first preclinical study on a molecular-targeted fluorescent agent for FGS in paediatric oncology was provided by Wellens et al. (27) who developed and evaluated a GD2-specific tracer consisting of the immunotherapeutic antibody Dinutuximab-beta, conjugated to the NIR-I fluorescent dye (IRDye800CW). Their results showed the specific binding of anti-GD2-IRDye800CW to human neuroblastoma (NB) cells both *in vitro* and *in vivo* models. FGS using a tumour-specific tracer in paediatric oncology surgery remains experimental but is a promising modality for localising tumours and their metastases and protecting peritumoral organs and vital structures (34). The development of novel fluorescent probes and the identification of new tumour targets gives the surgeon the perfect combination to enhance cancer surgery, particularly for solid tumours. There is an excellent potential for this methodology to enter routinely in the surgical setting. As such, surgeons needs to familiarise themself with the basic pharmacokinetics of these novel molecules and the devices to detect them intraoperatively.

Spectroscopy-Guided Surgery

As previously stated, clear surgical margins and a maximal degree of tumour resection strongly impact patients' outcomes. Raman spectroscopy has emerged as a promising biochemical technique to perform a non-invasive, real-time, automated, and *in vivo* assessment in different types of cancer (45, 46). The principle behind Raman spectroscopy is the interaction of light with the chemical bonds within a material or tissue. The term "Raman spectrum" refers to a distinct chemical fingerprint of a tissue's current biological composition and activity that can be used to identify the tissue or distinguish it from others. Differences in the biochemical compositions (fatty-acid concentration, collagen content, DNA/RNA concentrations) of malignant tissues and healthy ones lead to the generation of different spectral signatures, with peaks associated with elevated concentrations of DNA, RNA, and peri-nuclear proteins in tumour sites (**Figure 2**) (47, 48).

Hale Wills et al. used Raman spectroscopy to analyse fresh NB specimens and other paediatric neural crest tumours. In their study, they collected 862 fresh and 252 frozen specimens from different samples (normal adrenal glands, NBs, ganglioneuromas, nerve sheath tumours, and pheochromocytomas) to compare the spectra of the frozen tissue against fresh tissue, and the results demonstrated a strong correlation between the two. They were able to distinguish between pathologic conditions and normal adrenal tissue with

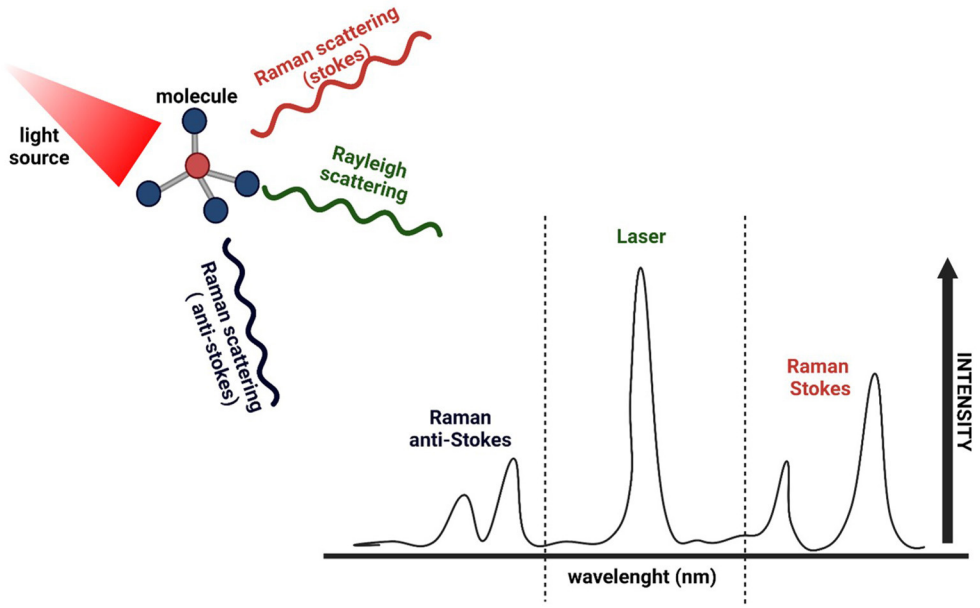

FIGURE 2 | Schematic representation of Raman spectroscopy. After the sample (molecule) is exposed to an intense beam of monochromatic light in the frequency range of visible, near-infrared or near-ultraviolet region, most of the scattered light is at the same wavelength as the laser source (Rayleigh scattering). In contrast, a small amount of light is scattered at different wavelengths depending on the chemical structure of the analyte (Raman scattering). A Raman spectrum is a vibrational spectrum, where each peak corresponds to a specific molecular bond vibration, showing the intensity and wavelength position of the Raman scattered light.

100% sensitivity and specificity (49). In 2012, Leslie et al. evaluated the use of Raman spectroscopy to diagnose paediatric brain tumours. They collected samples (fresh and frozen) of untreated paediatric medulloblastoma, gliomas, and normal brain samples and registered twelve Raman spectra per sample. The obtained spectra could accurately distinguish paediatric brain neoplasms from the normal brain tissue. Even within the same type of tumour, Raman spectroscopy differentiated high-grade ependymomas from low-grade ependymomas with 100% sensitivity and 96.0% specificity (50). More recently, Jabarkheel et al. presented their work on intraoperative detection of paediatric brain tumour margins. In detail, they investigated the potential for Raman spectroscopy to rapidly detect paediatric brain tumour margins with intraoperative images of fresh *ex vivo* paediatric brain tissue samples. All imaged samples underwent formal final histopathological analysis. They created a labelled Raman spectra dataset of paediatric brain tumours. Then they developed an end-to-end machine learning model to predict the final histopathology diagnosis from the spectral data, suggesting that machine learning approaches can be used to classify tumours and detect their margins (51).

As every tissue has distinct molecular fingerprint that can be used to differentiate it from other tissues, Raman spectroscopy has potentially endless applications. Creating a database for evaluating rare tumours and pathological conditions based on their spectrum might allow for a rapid, non-invasive, real-time diagnosis, with no need for tissue destruction, processing, or staining.

HIGH-DEFINITION CAMERAS

Short-Wave Infrared Camera

To date, biomedical fluorescence imaging has mainly relied on NIR-I (wavelength: 700–900 nm) dyes, which has been favoured over visible light (wavelength: 380–800 nm) due to less tissue autofluorescence and absorbance (34). However, the low tissue penetration, the high background noise and limited tissue contrast of NIR-I dyes have limited their surgical translations in children. In this respect, more recent studies are investigating short-wave infrared (SWIR, wavelength: 1,000–2,000 nm) fluorophores and cameras as promising tools for achieving higher contrast, greater sensitivity and improved penetration depths (52).

Compared to NIR-I molecules, SWIR technologies have negligible autofluorescence, reduced optical scatter, and lower or comparable absorption. These features improve spatial and contrast resolutions, particularly when imaging fluorescence below the tissue's surface (53). Although SWIR technologies are at a very early stage of development, they are proving to have a great potential that could promote their translation into the clinic.

Even if no SWIR dyes have been approved for clinical use in humans yet, the discovery that some NIR-I dyes (such as ICG) display bright emission tails over 1,000 nm offers exciting opportunities for enhanced surgical imaging, especially in the field of surgical oncology (34).

Hu et al. described using a multispectral imaging system able to cover a spectrum range from 400 to 1,700 nm, which allows the generation of SWIR, NIR-I, and visible light imaging. In

particular, the authors used this optical-imaging instrument for aiding the FGS resection of primary and metastatic liver tumours in adults. Their results showed that SWIR images provided a higher tumour-detection sensitivity, a higher tumour-to-liver signal ratio, and an enhanced tumour-detection rate than NIR-I images (54). In another study, Suo et al. reported the design and the construction of a SWIR fluorescence endoscopy imaging system compatible with most current clinic endoscopies, which was used to image animal models of colorectal cancer with ICG conjugated bevacizumab (Bev-ICG). SWIR *in vivo* images of a subcutaneous colorectal model showed a specific accumulation of Bev-ICG with high contrast, proving its potential as a promising contrast agent for non-invasive imaging of tumours overexpressing VEGF. Moreover, the use of the imaging system for imaging a rat orthotopic colorectal cancer model showed that the SWIR endoscope provided an accurate real-time delineation of colorectal tumour, highlighting the potential of SWIR imaging in the field of endoscopic (55).

As mentioned previously, SWIR fluorescence imaging is at a very early stage of development, but it shows great potential for *in vivo* detection of tumours *in vivo*. Although the literature found was based solely on adult series, the application of SWIR devices to paediatric instrumentation would easily translate into the use of these technologies in paediatric cancer surgery. Moreover, the use of specific targeting by conjugating SWIR molecules with tumours' markers is of particular interest as it would combine the advantages of SWIR imaging (reduced autofluorescence, deeper penetration and reduced scattering) with the selective targeting of tumour cells.

Radio-Guided Surgery Using Gamma Detection Probes

The concept of radio-guided surgery refers to the intraoperative detection of radionuclides using a radiation detection probe system. Intraoperative radiation probes can be divided into two main categories: gamma probes, which detect photon radiation (gamma rays or X-rays); and beta probes, which detect beta radiation (positrons or negatrons). There is a wide range of designs for small portable gamma cameras, which have been developed for intraoperative use (**Table 2**) (72). The use of radio-guided surgery in oncology provides real-time and specific visualisation of the extent of the disease, allowing the surgeon to assess surgical resection margins and to minimise the surgical invasivenes (72).

The use of gamma detection probes in the paediatric population is limited in the literature. Martinez et al. were the first to report the use of radio-guided surgery in 6 paediatric patients with stage II/IV NB. In their study, an intravenous injection of ^{125}I-Tyr3-octreotide was given between 1.8 and 7.5 h before surgery. Out of the 17 ^{125}I-Tyr3- octreotide binding sites found by the detection probe, 15 sites contained NB, which were not detected by standard intraoperative palpation and visualisation (73). The largest cohort of radio-guided procedures was published by Martinelli et al., who used either ^{123}I-MIBG (injected 24 48 h before surgery) or ^{125}I-MIBG (injected 3–5 days before surgery) for the intraoperative localisation of NB with a gamma detection probe. The probe was considered helpful in a vast majority of the cases. It permitted the detection of small, non-palpable, and difficult to access tumours and helped the surgeons define more accurately tumour margins (74).

Recently, there has been an increasing interest in developing hybrid gamma cameras (HGC) that provides the surgeon with fused optical and gamma images. Lees et al. described the first combined gamma and NIR fluorescence imaging producing dual-modality images in both laboratory simulations and the clinic. They reported the results of a patient's thyroid during the clinical investigation, showing that despite there was a reduction of the spatial resolution in the gamma image, due to the image processing performed before combining it with the optical image, there was an enhancement of the visual appearance when fused with its optical counterpart (75).

At the moment, there is no commercially available system offering hybrid imaging for clinical use. However, developing such systems has great potential both for cancer diagnosis and treatment, offering the surgeon the possibility of using a combination of probes to visualise different targets, improving the overall sensitivity and specificity of detection.

INTRA-OPERATIVE LOCO-REGIONAL TREATMENTS

The introduction of novel adjuvant treatment, and their potential to clear microscopic residual of the disease, can help consolidate the loco-regional control reducing the risk of recurrence and progression of the disease. Photodynamic therapy (PDT), for example, can be used as treatment, salvage therapy, and palliative care for different tumours. However, PDT is, in general, a non-selective treatment acting on both healthy and cancerous cells (76). This limit can be overcome by using near-infrared photoimmunotherapy (NIR-PIT) that selectively kill cancer cells due to the conjugation of a special fluorophore with a monoclonal antibody. This conjugate will spare healthy tissues and vital organs, not causing any local side effects. Another innovative treatment is hyperthermic intraperitoneal chemotherapy (HIPEC), consisting of the intraperitoneal administration of heated chemotherapy treatment after cytoreductive surgery (77, 78).

Photodynamic Therapy

From the wide range of therapies available in oncologic patients, PDT represents a novel adjuvant treatment capable of consolidating loco-regional control, aiming to reduce metastatic spread and progression of the tumour. The principle of PDT is a photochemical reaction between a photosensitive molecule (photosensitiser), light and molecular oxygen (76, 79). Administration of PDT starts with the intravenous, intraperitoneal or topical administration of a photosensitiser, followed by light exposure, which leads to the creation of radicals (**Figure 3**) (80).

The adult literature explored the role of PDT in tumours' treatment such as breast, brain, head, and neck gastrointestinal and genitourinary system tumours (81–85). Endoscopic

TABLE 2 | Summary of characteristics and performances of small gamma cameras.

Gamma camera	Detector	FOV	Size detector head	Energy (KeV)	Energy resolution (FWHM)	^{99m}Tc sensitivity
CarollReS (56–58)	Gd2SiO5 (Ce) PS-PMT	50 × 50 mm	78 × 78 × 275 mm		45%, ^{57}Co	1,000 cps/MBq (theoretical)
eZ-SCOPE® (59)	CdZnTe	32 × 32 mm	60 × 60 × 220 mm	71–364	8.6%, ^{99m}Tc	184 cps/MBq
GE camera (60, 61)	CdZnTe	40 × 40 mm	Height 150 mm	40–200	8%, ^{99m}Tc	100 cps/MBq
Imaging probe (62)	CsI (Tl) PS-PMT	49 × 49 mm			20%, ^{99m}Tc	210 cps/MBq
LumaGEM® (63)	CsI (Na) PS-PMT	20 × 20 mm		30–300	>20%, ^{99m}Tc	
MediProbe (64, 65)	CdTe	14.08 × 14.08 mm	200 × 70 × 30 mm			6.5–33 cps/MBq (5 cm source-to-aperture distance)
Minicam®	CdTe	49 × 49 mm	Φ 95 mm height 150 mm	20–200		
MinicamII®	CdTe	40 × 40 mm	70 × 170 × 250 mm	30–300	5–7%, ^{99m}Tc	
POCI (66)	YAP (Ce), IPSD	Φ 24 mm		Tc-99m, I-125, In-111	38%, ^{57}Co	200 cps/MBq
Second POCI (67)	CsI (Na) IPSD	Φ 40 mm	Φ 95 mm height 90 mm	105–175	32%, ^{99m}Tc	290 cps/MBq
Sentinella 102® (68)	CsI (Na) PS-PMT	40 × 40 mm	8 × 9 × 15 mm	50–200	15.9%, ^{99m}Tc	90–900 cps/MBq (1 cm source-to-aperture distance) 27–72 cps/MBq (10 cm source-to-aperture distance)
SSGC clinical-type (69)	CdTe	44.8 × 44.8 mm	82 × 86 × 205 mm	Max 550	6.9%, ^{99m}Tc	150 cps/MBq (high-resolution collimator) 1,600 cps/MBq (high-sensitivity collimator)
SSGC proto-type (70, 71)	CdTe	44.8 × 44.8 mm	152 × 166 × 65 mm	Max 550	7.8%, ^{99m}Tc	300 cps/MBq

FOV, field of view; FWHM, full-width half maximum of the ^{99m}Tc (140 KeV) or 57Co (122 KeV) photopeak; SSGC, Small CdTe Gamma Camera; Gd2SiO5 (Ce) PS-PMT, Gadolinium oxyorthsilicate doped with cerium coupled with position sensitive photomultiplier tube; CdZnTe, cadmium zinc telluride; CsI (Tl) PS-PMT, Thallium doped cesium iodide coupled with coupled with position sensitive photomultiplier tube; CsI (Na) PS-PMT, sodium-doped cesium iodide coupled with position sensitive photomultiplier tube; CdTe, cadmium telluride; YAP (Ce), IPSD, Cerium doped Yttrium Aluminium Perovskite coupled with intensified position-sensitive diode; cps, counts per second.

procedures with PDT in oesophageal cancer showed less morbidity and mortality than surgery, even though neoplastic recurrence may be higher (81, 86). The application of PDT also appears to be significantly correlated with more prolonged survival in advanced cholangiocarcinoma. A study by Gonzalez-Carmona et al. showed that the combination of PDT and chemotherapy resulted in significantly longer overall survival than either PDT or chemotherapy alone in patients with advanced unresectable cholangiocarcinoma (87). Moreover, although surgical resection remains the standard of care for non-small-cell lung cancer, PDT has been used intraoperatively after tumour debulking in a phase II clinical trial. This study showed an improvement of patients' survival with a 73.7% local control rate at 6 months and a median overall survival of 21.7 months, probably related to the eradication of microscopic residuals, which usually cause the recurrence of the disease (88).

Near-Infrared Photoimmunotherapy

The previous results on PDT encourage a potential use of this technique also in paediatric oncology. However, PDT exerts a non-selective action against cells relying on reactive oxygen species (ROS) generation, NIR-PIT allows a selective killing of target cells by conjugating NIR light-absorbing dye (IRDye700DX) to a cancer-targeting moiety (such as a monoclonal antibody) The conjugate is injected intravenously and binds to the specific cancer cells expressing the target antigen on the cell membrane. When the conjugate is exposed to the NIR light (∼690 nm), axial ligands of the IRDye700DX molecule dissociate from the main molecule causing the photoactivated chemical to change from a highly hydrophilic to a highly hydrophobic compound. This leads to the damage and rupture of the cellular membrane with micro-perforations, blebbing, and bursting, resulting in necrotic cell death (89). The rupture of the cell membrane leads to the release of tumour-specific antigens into the tumour microenvironment and promotes dendritic cell maturation, eliciting the host immune system against the dying tumour cells (90).

The literature on the use of NIR-PIT in the paediatric population is scant. Nouso et al. showed that the administration of anti-GD2-IRDye700DX followed by NIR-PIT significantly suppressed cell viability compared to an anti-GD2 monoclonal antibody. Thus, their results showed NIR-PIT as a promising anti-tumour strategy to enhance the therapeutic efficacy of anti-GD2 immunotherapy for high-risk NB (91). Maruoka et al. (92) hypothesised that the administration of IL-15 with cancer cell-targeted NIR-PIT could increase anti-tumour host

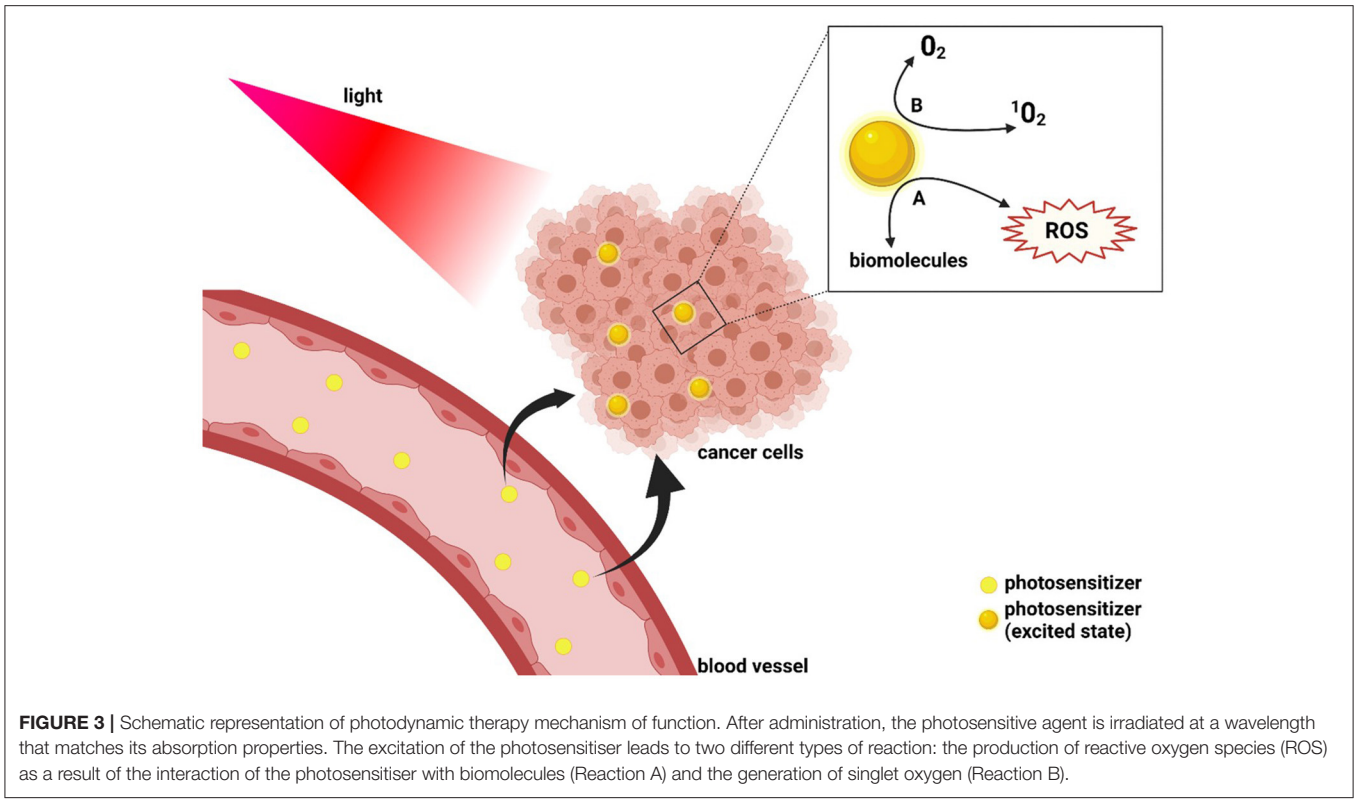

FIGURE 3 | Schematic representation of photodynamic therapy mechanism of function. After administration, the photosensitive agent is irradiated at a wavelength that matches its absorption properties. The excitation of the photosensitiser leads to two different types of reaction: the production of reactive oxygen species (ROS) as a result of the interaction of the photosensitiser with biomolecules (Reaction A) and the generation of singlet oxygen (Reaction B).

immunity. Most of the findings regarding the use of NIR-PIT in treating tumours can be found in the preclinical adult literature, where its potential has been explored in different types of tumours, going from head and neck tumours to gastrointestinal, lung, and lung and gynaecological tumours (93–95).

Overall, NIR-PIT could offer several benefits, like its tumour-cell specificity with virtually no damage to adjacent healthy tissues and vital organs. The same conjugate might also be used intra-operatively to guide the surgeon in localising the tumour. Lastly, NIR-PIT relies on a form of non-ionising radiation, with no limits to its total cumulative dose, meaning that multiple cycles could be safely employed. Further clinical studies are needed to prove the therapeutic potential of NIR-PIT in improving loco-regional control of tumours with reduced risk of recurrence and an overall benefit on survival.

Hyperthermic Intraperitoneal Chemotherapy

HIPEC is an innovative treatment that aims to treat cancers with deposits or involvement of the peritoneum. It consists of an infusion of a heated chemotherapeutic solution that circulates in the abdominal cavity for 60–120 min to maximise cancer cell killing. However, as the solution is not able to penetrate deep into tumours, the treatment must be performed after cytoreductive surgery to debulk the tumour from the abdominal cavity. When performed in specialised centres, HIPEC has a rate of side effects comparable to any other digestive surgery, including paralytic ileus, haemorrhages, infections, fistulas, abscesses, haematological toxicity, and kidney failure. While this technique is routinely used in adults, currently, there are no guidelines in the paediatric populations where its use remains to be determined (77).

In 2015 Hayes-Jordan et al. reported the first 50 cases treated in their institution in the USA with cytoreductive surgery and HIPEC. The age range of their cohort was from 3 to 21 years old and with a median follow-up of 21.9 months for the surviving patients. Patients diagnoses included desmoplastic small round cell tumours, rhabdomyosarcomas, mesotheliomas, and other carcinomas. The HIPEC was performed using a closed technique, and it was added to chemotherapy and radiotherapy treatment for all the patients who demonstrated a partial response to neoadjuvant chemotherapy. Patients with desmoplastic small round cell tumours also underwent postoperative total abdominal radiation and postoperative chemotherapy. The results of their study showed that patients who had a complete cytoreduction, who were then treated with HIPEC, had a reduced risk for recurrence than those who had an incomplete cytoreduction. Patients' outcome was also affected by the peritoneal cancer index, with patients with a significant tumour burden having a median overall survival lower than patients with a lower score (19.9 vs. 34 months). Overall, cytoreductive surgery and HIPEC proved to be a safe alternative for extensive or refractory abdominal tumours, with the best outcome experienced by patients with desmoplastic small round cell tumours and those with complete cytoreduction (78). The same group reported their experience with cytoreductive surgery and HIPEC on paediatric girls with ovarian carcinoma, diffuse peritoneal disease, and no disease outside the abdominal

cavity. Eight girls, previously treated with chemotherapy and surgery, were included in the study (age range 3–18). Three out of the eight patients recurred and died, while the remaining patients remained disease-free from 2 to 6 years post-HIPEC. The cohort showed an overall survival of 64% and relapse-free survival of 62%. Despite the small size of this cohort of patients, complete surgical resection, Cytoreductive surgery (CRS) and HIPEC proved to be a valid alternative that should be considered in paediatric patients with diffuse peritoneal disease from ovarian origin (96).

Most recently, Gesche et al. published their results on the use of CRS and HIPEC in a cohort of 6 patients below the age of 5 with intraperitoneal rhabdomyosarcoma. After surgery, HIPEC was performed using a closed technique with the administration of cisplatin and doxorubicin for 60 min at 42.5°C. Chemotherapy solution was eliminated by repetitive irrigation of the abdominal cavity with ringer solution. A peri- and postoperative hydration protocol was used to reduce the risk of HIPEC-associated renal failure. Their results demonstrate the safety and feasibility of CRS and HIPEC in this young age group with only low-grade side effects and no grade 3 or grade 4 toxicities (77). Sjoberg Bexelius et al. published the first case of CRS and HIPEC in a paediatric centre in the UK at the beginning of this year. This case report is about a 7-year-old girl diagnosed with an abdominal desmoplastic round cell tumour with peritoneal and liver metastases. The little girl, who is currently in complete remission 4 months after treatment, underwent CRS combined with HIPEC after six cycles of chemotherapy, followed by whole abdominopelvic radiotherapy and maintenance chemotherapy for 12 months. Although the long-term survival advantage of this technique is still uncertain and its use in children with this condition remains uncertain, this study proposed HIPEC as a potential alternative to selected children in the UK (97).

The use of HIPEC requires a multidisciplinary collaboration between adult peritoneal malignancy services, paediatric oncology, paediatric surgery, and intensive care services. To prove its clinical benefit, further data are needed, mainly concerning the paediatric population under 12 years of age. Furthermore, as there is no standardised way of administering HIPEC with variations in chemotherapy protocols, doses, lengths of treatment, a uniform guideline should be provided to have more consistent results.

CONCLUSIONS

Apart from robotic surgery and augmented reality, oncology surgery has not changed significantly in the last decades, especially for paediatric patients. However, the research in this field is rapidly evolving. The devices and technologies presented in this study have the potential to revolutionise surgical oncology through more effective visualisation and removal of cancer. Thus, the oncology surgeons of the future need to remain up to date with the vast range of bioengineering advances that will possibly reach clinical practise in the next few years. These technologies will improve the effectiveness of surgery, leading to significant benefits for the children we want to cure.

AUTHOR CONTRIBUTIONS

LP and SG contributed to the study conception and design. LP and IP took care of the data acquisition. Critical revision and final approval were provided by KC and SG. All the authors contributed to the drafting of the manuscript and approved the submitted version.

FUNDING

We thank the Medical Research Council (Grant No. MR/T005491/1) and the Wellcome/EPSRC Centre for Interventional and Surgical Sciences at University College London (WEISS, Grant No. 203145Z/16/Z), who supported the study.

REFERENCES

1. *Cancer Research UK*. Available online at: https://www.cancerresearchuk.org/health-professional/cancer-statistics/childrens-cancers#heading-Two (accessed June 5, 2021).
2. Ogrinc N, Saudemont P, Takats Z, Salzet M, Fournier I. Cancer surgery 2.0: guidance by real-time molecular technologies. *Trends Mol Med.* (2021) 27:602–15. doi: 10.1016/j.molmed.2021.04.001
3. Yang X, Chen J, Wang N, Liu Z, Li F, Zhou J, et al. Impact of extent of resection on survival in high-risk neuroblastoma: a systematic review and meta-analysis. *J Pediatr Surg.* (2019) 54:1487–94. doi: 10.1016/j.jpedsurg.2018.08.046
4. Blanc T, Meignan P, Vinit N, Ballouhey Q, Pio L, Capito C, et al. Robotic surgery in pediatric oncology: lessons learned from the first 100 tumors—a nationwide experience. *Ann Surg Oncol.* (2021). doi: 10.1245/s10434-021-10777-6. [Epub ahead of print].
5. Meehan JJ, Sandler A. Pediatric robotic surgery: A single-institutional review of the first 100 consecutive cases. *Surg Endosc.* (2008) 22:177–82. doi: 10.1007/s00464-007-9418-2
6. Meignan P, Ballouhey Q, Lejeune J, Braik K, Longis B, Cook AR, et al. Robotic-assisted laparoscopic surgery for pediatric tumors: a bicenter experience. *J Robot Surg.* (2018) 12:501–8. doi: 10.1007/s11701-017-0773-2
7. Blanc T, Pio L, Clermidi P, Muller C, Orbach D, Colin VM, et al. Robotic-assisted laparoscopic management of renal tumors in children: Preliminary results. *Pediatr Blood Cancer.* (2019) 66:53–9. doi: 10.1002/pbc.27867
8. Varda BK, Cho P, Wagner AA, Lee RS. Collaborating with our adult colleagues: A case series of robotic surgery for suspicious and cancerous lesions in children and young adults performed in a free-standing children's hospital. *J Pediatr Urol.* (2018) 14:182.e1–182.e8. doi: 10.1016/j.jpurol.2018.01.003
9. Meehan JJ, Sandler AD. Robotic Resection of Mediastinal Masses in Children. *J Laparoendosc Adv Surg Tech.* (2008) 18:114–9. doi: 10.1089/lap.2007.0092
10. Navarrete Arellano M, Garibay González F. Robot-Assisted laparoscopic and thoracoscopic surgery: prospective series of 186 pediatric surgeries. *Front Pediatr.* (2019) 7:200. doi: 10.3389/fped.2019.00200
11. Cost NG, Geller JI, DeFoor WR, Wagner LM, Noh PH. A robotic-assisted laparoscopic approach for pediatric renal cell carcinoma allows for both nephron-sparing surgery and extended lymph node dissection. *J Pediatr Surg.* (2020) 47:1946–50. doi: 10.1016/j.jpedsurg.2012.08.017

12. Hassan M, Smith JM. Robotic assisted excision of a left ventricular myxoma. *Interact Cardiovasc Thorac Surg.* (2012) 14:113–4. doi: 10.1093/icvts/ivr021
13. Akar ME, Leezer KH, Yalcinkaya TM. Robot-assisted laparoscopic management of a case with juvenile cystic adenomyoma. *Fertility and Sterility*. (2010) 94:e55–e56. doi: 10.1016/j.fertnstert.2010.06.001
14. Backes FJ, Seamon LG, Fowler JM. Robotic radical hysterectomy and pelvic lymphadenectomy for uterine rhabdomyosarcoma. *J Robotic Surg.* (2008) 2:197–200. doi: 10.1007/s11701-008-0095-5
15. Anderberg M, Backman T, Annerstedt M. Robot-assisted radical cystoprostatectomy in a small child with rhabdomyosarcoma: a case report. *J Robotic Surg.* (2008) 2:101–3. doi: 10.1007/s11701-008-0089-3
16. Cave J, Clarke S. Paediatric robotic surgery. *Ann R Coll Surg Engl.* (2018) 100(Supplement 7):18–21. doi: 10.1308/rcsann.supp2.18
17. Christison-Lagay ER, Thomas D. Minimally invasive approaches to pediatric solid tumors. *Surg Oncol Clin N Am.* (2019) 28:129–46. doi: 10.1016/j.soc.2018.07.005
18. Cundy TP, Marcus HJ, Clark J, Hughes-Hallett A, Mayer EK, Najmaldin AS, et al. Robot-assisted minimally invasive surgery for pediatric solid tumors: a systematic review of feasibility and current status. *Eur J Pediatr Surg.* (2014) 24:127–35. doi: 10.1055/s-0033-1347297
19. Cundy TP, Shetty K, Clark J, Pin Chang T, Sriskandarajah K, Gattas NE, et al. The first decade of robotic surgery in children. *J Pediatr Surg.* (2013) 48:858–65. doi: 10.1016/j.jpedsurg.2013.01.031
20. Meehan JJ. Robotic surgery for pediatric tumors. *Cancer J Sudbury Mass.* (2013) 19:183–8. doi: 10.1097/PPO.0b013e318289486c
21. Pennacchietti V, Stoelzel K, Tietze A, Lankes E, Schaumann A, Uecker FC, et al. First experience with augmented reality neuronavigation in endoscopic assisted midline skull base pathologies in children. *Childs Nerv Syst.* (2021) 37:1525–34. doi: 10.1007/s00381-021-05049-3
22. Souzaki R, Ieiri S, Uemura M, Ohuchida K, Tomikawa M, Kinoshita Y, et al. An augmented reality navigation system for pediatric oncologic surgery based on preoperative CT and MRI images. *J Pediatr Surg.* (2013) 48:2479–83. doi: 10.1016/j.jpedsurg.2013.08.025
23. Su L, Dong Q, Zhang H, Zhou X, Chen Y, Hao X, et al. Clinical application of a three-dimensional imaging technique in infants and young children with complex liver tumors. *Pediatr Surg Int.* (2016) 32:387–95. doi: 10.1007/s00383-016-3864-7
24. Zhang G, Zhou XJ, Zhu CZ, Dong Q, Su L. Usefulness of three-dimensional(3D) simulation software in hepatectomy for pediatric hepatoblastoma. *Surg Oncol.* (2016) 25:236–43. doi: 10.1016/j.suronc.2016.05.023
25. Zhao J, Zhou XJ, Zhu CZ, Wu Y, Wei B, Zhang G, et al. 3D simulation assisted resection of giant hepatic mesenchymal hamartoma in children. *Comput Assist Surg Abingdon Engl.* (2017) 22:54-59. doi: 10.1080/24699322.2017.1358401
26. Souzaki R, Kinoshita Y, Matsuura T, Tajiri T, Taguchi T, Ieiri S, et al. Successful resection of an undifferentiated sarcoma in a child using a real-time surgical navigation system in an open magnetic resonance imaging operation room. *J Pediatr Surg.* (2011) 46:608–11. doi: 10.1016/j.jpedsurg.2010.11.043
27. Wellens LM, Meulstee J, van de Ven CP, Terwisscha van Scheltinga CEJ, Littooij AS, van den Heuvel-Eibrink MM, et al. Comparison of 3-dimensional and augmented reality kidney models with conventional imaging data in the preoperative assessment of children with wilms tumors. *JAMA Netw Open.* (2019) 2:e192633. doi: 10.1001/jamanetworkopen.2019.2633
28. Maeda T, Hong J, Konishi K, Nakatsuji T, Yasunaga T, Yamashita Y, et al. Tumor ablation therapy of liver cancers with an open magnetic resonance imaging-based navigation system. *Surg Endosc.* (2009) 23:1048–53. doi: 10.1007/s00464-008-0123-6
29. De Boer E, Harlaar NJ, Taruttis A, Nagengast WB, Rosenthal EL, Ntziachristos V, et al. Optical innovations in surgery. *Br J Surg.* (2015) 102:e56–72. doi: 10.1002/bjs.9713
30. Han-bing S, Wei-guo Z, Wei-feng Z. Preoperative assessment using multimodal functional magnetic resonance imaging techniques in patients with brain gliomas. *Turk Neurosurg.* (2011) 22:558–65. doi: 10.5137/1019-5149.JTN.5332-11.1
31. Jiang Y, Sun J, Xia Y, Cheng Y, Xie L, Guo X, et al. Preoperative assessment for event-free survival with hepatoblastoma in pediatric patients by developing a ct-based radiomics model. *Front Oncol.* (2021) 11:644994. doi: 10.3389/fonc.2021.644994
32. Ma L, Fei B. Comprehensive review of surgical microscopes: technology development and medical applications. *J Biomed Opt.* (2021) 26:010901. doi: 10.1117/1.JBO.26.1.010901
33. Zheng Y, Yang H, Wang H, Kang K, Zhang W, Ma G, et al. Fluorescence-guided surgery in cancer treatment: current status and future perspectives. *Ann Transl Med.* (2019) 7:S6. doi: 10.21037/atm.2019.01.26
34. Paraboschi I, De Coppi P, Stoyanov D, Anderson J, Giuliani S. Fluorescence imaging in pediatric surgery: state-of-the-art and future perspectives. *J Pediatr Surg.* (2021) 56:655–62. doi: 10.1016/j.jpedsurg.2020.08.004
35. Abdelhafeez A, Talbot L, Murphy AJ, Davidoff AM. Indocyanine green–guided pediatric tumor resection: approach, utility, and challenges. *Front Pediatr.* (2021) 9:689612. doi: 10.3389/fped.2021.689612
36. Yamada Y, Ohno M, Fujino A, Kanamori Y, Irie R, Yoshioka T, et al. Fluorescence-Guided surgery for hepatoblastoma with indocyanine green. *Cancers.* (2019) 11:E1215. doi: 10.3390/cancers11081215
37. Chen-Yoshikawa TF, Hatano E, Yoshizawa A, Date H. Clinical application of projection mapping technology for surgical resection of lung metastasis. *Interact Cardiovasc Thorac Surg.* (2017) 25:1010–11. doi: 10.1093/icvts/ivx247
38. Takahashi N, Yamada Y, Hoshino K, Mori T, Abe K, Fujimura T, et al. Living donor liver re-transplantation for recurrent hepatoblastoma in the liver graft following complete eradication of peritoneal metastases under indocyanine green fluorescence imaging. *Cancers.* (2019) 11:E730. doi: 10.3390/cancers11050730
39. Souzaki R, Kawakubo N, Matsuura T, Yoshimaru K, Koga Y, Takemoto J, et al. Navigation surgery using indocyanine green fluorescent imaging for hepatoblastoma patients. *Pediatr Surg Int.* (2019) 35:551–7. doi: 10.1007/s00383-019-04458-5
40. Labuschagne JJ. The Use of 5-aminolevulinic acid to assist gross total resection of paediatric posterior fossa tumours. *Pediatr Neurosurg.* (2020) 55:268–79. doi: 10.1159/000511289
41. For the European ALA Pediatric Brain Tumor Study Group, Stummer W, Rodrigues F, Schucht P, Preuss M, Wiewrodt D, et al. Predicting the "usefulness" of 5-ALA-derived tumor fluorescence for fluorescence-guided resections in pediatric brain tumors: a European survey. *Acta Neurochir.* (2014) 156:2315–24. doi: 10.1007/s00701-014-2234-2
42. Zhang C, Boop FA, Ruge J. The use of 5-aminolevulinic acid in resection of pediatric brain tumors: a critical review. *J Neurooncol.* (2019) 141:567–73. doi: 10.1007/s11060-018-03004-y
43. Schwake M, Schipmann S, Müther M, Köchling M, Brentrup A, Stummer W. 5-ALA fluorescence–guided surgery in pediatric brain tumors—a systematic review. *Acta Neurochir.* (2019) 161:1099–108. doi: 10.1007/s00701-019-03898-1
44. Luo S, Zhang E, Su Y, Cheng T, Shi C. A review of NIR dyes in cancer targeting and imaging. *Biomaterials.* (2011) 32:7127–38. doi: 10.1016/j.biomaterials.2011.06.024
45. Pence I, Mahadevan-Jansen A. Clinical instrumentation and applications of raman spectroscopy. *Chem Soc Rev.* (2016) 45:1958–79. doi: 10.1039/C5CS00581G
46. Teh SK, Zheng W, Ho KY, Teh M, Yeoh KG, Huang Z. Diagnostic potential of near-infrared raman spectroscopy in the stomach: differentiating dysplasia from normal tissue. *Br J Cancer.* (2008) 98:457–65. doi: 10.1038/sj.bjc.6604176
47. Shafer-Peltier KE, Haka AS, Fitzmaurice M, Crowe J, Myles J, Dasari RR, et al. Raman microspectroscopic model of human breast tissue: implications for breast cancer diagnosis *in vivo*. *J Raman Spectrosc.* (2002) 33:552–63. doi: 10.1002/jrs.877
48. Zúñiga WC, Jones V, Anderson SM, Echevarria A, Miller NL, Stashko C, et al. Raman spectroscopy for rapid evaluation of surgical margins during breast cancer lumpectomy. *Sci Rep.* (2019) 9:14639. doi: 10.1038/s41598-019-51112-0
49. Wills H, Kast R, Stewart C, Rabah R, Pandya A, Poulik J, et al. Raman spectroscopy detects and distinguishes neuroblastoma and related tissues in fresh and (banked) frozen specimens. *J Pediatr Surg.* (2009) 44:386–91. doi: 10.1016/j.jpedsurg.2008.10.095
50. Leslie DG, Kast RE, Poulik JM, Rabah R, Sood S, Auner GW, et al. Identification of pediatric brain neoplasms using raman spectroscopy. *Pediatr Neurosurg.* (2012) 48:109–17. doi: 10.1159/000343285
51. Jabarkheel R, Parker JJ, Ho CS, Shaffer T, Gambhir S, Grant GA, et al. Intraoperative detection of pediatric brain tumor

margins using raman spectroscopy. *Neurosurgery*. (2019) 66:nyz310_132. doi: 10.1093/neuros/nyz310_132
52. Carr JA, Franke D, Caram JR, Perkinson CF, Saif M, Askoxylakis V, et al. Shortwave infrared fluorescence imaging with the clinically approved near-infrared dye indocyanine green. *Proc Natl Acad Sci USA*. (2018) 115:4465–70. doi: 10.1073/pnas.1718917115
53. Byrd BK, Marois M, Tichauer KM, Wirth DJ, Hong J, Leonor JP, et al. First experience imaging short-wave infrared fluorescence in a large animal: indocyanine green angiography of a pig brain. *J Biomed Opt*. (2019) 24:1. doi: 10.1117/1.JBO.24.8.080501
54. Hu Z, Fang C, Li B, Wirth D, Hong J, Leonor JP, et al. First-in-human liver-tumour surgery guided by multispectral fluorescence imaging in the visible and near-infrared-I/II windows. *Nat Biomed Eng*. (2020) 4:259–71. doi: 10.1038/s41551-019-0494-0
55. Suo Y, Wu F, Xu P, Shi H, Wang T, Liu H, et al. NIR-II fluorescence endoscopy for targeted imaging of colorectal cancer. *Adv Healthc Mater*. (2019) 8:1900974. doi: 10.1002/adhm.201900974
56. Mathelin C, Salvador S, Croce S, Andriamisandratsoa N, Huss D, Guyonnet JL. Optimisation of sentinel lymph node biopsy in breast cancer using an operative gamma camera. *World J Surg Oncol*. (2007) 5:132. doi: 10.1186/1477-7819-5-132
57. Mathelin C, Salvador S, Bekaert V, Croce S, Andriamisandratsoa N, Liégeois P, et al. A new intraoperative gamma camera for the sentinel lymph node procedure in breast cancer. *Anticancer Res*. (2008) 28:2859–64.
58. Mathelin C, Salvador S, Huss D, Guyonnet JL. Precise localisation of sentinel lymph nodes and estimation of their depth using a prototype intraoperative mini -camera in patients with breast cancer. *J Nucl Med*. (2007) 48:623–9. doi: 10.2967/jnumed.106.036574
59. Abe A, Takahashi N, Lee J, Oka T, Shizukuishi K, Kikuchi T, et al. Performance evaluation of a hand-held, semiconductor (CdZnTe)-based gamma camera. *Eur J Nucl Med Mol Imaging*. (2003) 30:805–11. doi: 10.1007/s00259-002-1067-z
60. Kopelman D, Blevis I, Iosilevsky G, Hatoum OA, Zaretzki A, Shofti R, et al. Sentinel node detection in an animal study: evaluation of a new portable gamma camera. *Int Surg*. (2007) 92:161–6.
61. Kopelman D, Blevis I, Iosilevsky G, Reznik A, Chaikov A, Weiner N, et al. A newly developed intra-operative gamma camera: performance characteristics in a laboratory phantom study. *Eur J Nucl Med Mol Imaging*. (2005) 32:1217–24. doi: 10.1007/s00259-005-1823-y
62. Bartolazzi A, D'Alessandria C, Parisella MG, Signore A, Del Prete F, Lavra L, et al. Thyroid cancer imaging in vivo by targeting the anti-apoptotic molecule galectin-3. *PLoS ONE*. (2008) 3:e3768. doi: 10.1371/journal.pone.0003768
63. MacDonald LR, Patt BE, Iwanczyk JS, Yamaguchi Y, McElroy DP, Hoffman EJ, et al. *High-Resolution Hand-Held Gamma Camera*. Doty FP, Barber HB, Roehrig H, Morton EJ, editors. San Diego, CA (2000). p. 242. doi: 10.1117/12.410569
64. Russo P, Curion AS, Mettivier G, Esposito M, Aurilio M, Caracò C, et al. Evaluation of a CdTe semiconductor based compact gamma camera for sentinel lymph node imaging: CdTe semiconductor based compact gamma camera. *Med Phys*. (2011) 38:1547–60. doi: 10.1118/1.3555034
65. Russo P, Mettivier G, Pani R, Pellegrini R, Cinti MN, Bennati P. Imaging performance comparison between a LaBr3:Ce scintillator based and a CdTe semiconductor based photon counting compact gamma camera: LaBr3:Ce and a CdTe compact gamma camera comparison. *Med Phys*. (2009) 36:1298–317. doi: 10.1118/1.3081412
66. Menard L, Charon Y, Solal M, Laniece P, Mastrippolito R, Pinot L, et al. POCI: a compact high resolution γ camera for intra-operative surgical use. *IEEE Trans Nucl Sci*. (1998) 45:1293–7. doi: 10.1109/23.682019
67. Pitre S, Ménard L, Ricard M, Solal M, Garbay JR, Charon Y. A hand-held imaging probe for radio-guided surgery: physical performance and preliminary clinical experience. *Eur J Nucl Med Mol Imaging*. (2003) 30:339–43. doi: 10.1007/s00259-002-1064-2
68. Sánchez F, Fernández MM, Giménez M, Benlloch JM, Rodríguez-Alvarez MJ, García de Quirós F, et al. Performance tests of two portable mini gamma cameras for medical applications: performance tests of two portable mini gamma cameras. *Med Phys*. (2006) 33:4210–20. doi: 10.1118/1.2358199
69. Oda T, Hayama K, Tsuchimochi M. [Evaluation of small semiconductor gamma camera–simulation of sentinel lymph node biopsy by using a trial product of clinical type gamma camera]. *Kaku Igaku*. (2009) 46:1–12.
70. Tsuchimochi M, Sakahara H, Hayama K, Funaki M, Ohno R, Shirahata T, et al. A prototype small CdTe gamma camera for radioguided surgery and other imaging applications. *Eur J Nucl Med Mol Imaging*. (2003) 30:1605–14. doi: 10.1007/s00259-003-1301-3
71. Tsuchimochi M, Hayama K, Oda T, Togashi M, Sakahara H. Evaluation of the efficacy of a small CdTe γ-camera for sentinel lymph node biopsy. *J Nucl Med*. (2008) 49:956–62. doi: 10.2967/jnumed.108.050740
72. Povoski SP, Neff RL, Mojzisik CM, O'Malley DM, Hinkle GH, Hall NC, et al. A comprehensive overview of radioguided surgery using gamma detection probe technology. *World J Surg Oncol*. (2009) 7:11. doi: 10.1186/1477-7819-7-11
73. Martinez DA, O'Dorisio MS, O'Dorisio TM, O'Malley DM, Hinkle GH, Hall NC, et al. Intraoperative detection and resection of occult neuroblastoma: a technique exploiting somatostatin receptor expression. *J Pediatr Surg*. (1995) 30:1580–9. doi: 10.1016/0022-3468(95)90161-2
74. Martelli H, Ricard M, Larroquet M, Wioland M, Paraf F, Fabre M, et al. Intraoperative localisation of neuroblastoma in children with 123I- or 125I-radiolabeled metaiodobenzylguanidine. *Surgery*. (1998) 123:51–7. doi: 10.1016/S0039-6060(98)70228-4
75. Lees J, Bugby S, Alqahtani M, Jambi L, Dawood N, McKnight W, et al. A multimodality hybrid gamma-optical camera for intraoperative imaging. *Sensors*. (2017) 17:554. doi: 10.3390/s17030554
76. Paraboschi I, Turnock S, Kramer-Marek G, Musleh L, Barisa M, Anderson J, et al. Near-InfraRed PhotoImmunoTherapy (NIR-PIT) for the local control of solid cancers: challenges and potentials for human applications. *Crit Rev Oncol Hematol*. (2021) 161:103325. doi: 10.1016/j.critrevonc.2021.103325
77. Gesche J, Beckert S, Neunhoeffer F, Kachanov D, Königsrainer A, Seitz G, et al. Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy: a safe treatment option for intraperitoneal rhabdomyosarcoma in children below 5 years of age. *Pediatr Blood Cancer*. (2019) 66:e27517. doi: 10.1002/pbc.27517
78. Hayes-Jordan A, Green H, Lin H, Owusu-Agyemang P, Mejia R, Okhuysen-Cawley R, et al. Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC) for children, adolescents, and young adults: the first 50 cases. *Ann Surg Oncol*. (2015) 22:1726–32. doi: 10.1245/s10434-014-4289-y
79. van Straten D, Mashayekhi V, de Bruijn H, Oliveira S, Robinson D. Oncologic photodynamic therapy: basic principles, current clinical status and future directions. *Cancers*. (2017) 9:19. doi: 10.3390/cancers9020019
80. Kwiatkowski S, Knap B, Przystupski D, Saczko J, Kedzierska E, Knap-Czop K, et al. Photodynamic therapy – mechanisms, photosensitizers and combinations. *Biomed Pharmacother*. (2018) 106:1098–107. doi: 10.1016/j.biopha.2018.07.049
81. Wolfsen HC. Carpe luz—seize the light: endoprevention of esophageal adenocarcinoma when using photodynamic therapy with porfimer sodium. *Gastrointest Endosc*. (2005) 62:499–503. doi: 10.1016/j.gie.2005.07.017
82. Zilidis G, Aziz F, Telara S, Eljamel MS. Fluorescence image-guided surgery and repetitive photodynamic therapy in brain metastatic malignant melanoma. *Photodiagnosis Photodyn Ther*. (2008) 5:264–6. doi: 10.1016/j.pdpdt.2009.01.001
83. Azzouzi AR, Vincendeau S, Barret E, Cicco A, Kleinclauss F, van der Poel HG, et al. Padeliporfin vascular-targeted photodynamic therapy versus active surveillance in men with low-risk prostate cancer (CLIN1001 PCM301): an open-label, phase 3, randomised controlled trial. *Lancet Oncol*. (2017) 18:181–91. doi: 10.1016/S1470-2045(16)30661-1
84. Meulemans J, Delaere P, Vander Poorten V. Photodynamic therapy in head and neck cancer: indications, outcomes, and future prospects. *Curr Opin Otolaryngol Head Neck Surg*. (2019) 27:136–41. doi: 10.1097/MOO.0000000000000521
85. Lamberti MJ. Breast cancer as photodynamic therapy target: enhanced therapeutic efficiency by overview of tumor complexity. *World J Clin Oncol*. (2014) 5:901. doi: 10.5306/wjco.v5.i5.901
86. Wu J, Pan Y min, Wang T tian, Gao D jian, Hu B. Endotherapy versus surgery for early neoplasia in barrett's esophagus: a meta-analysis. *Gastrointest Endosc*. (2014) 79:233–41.e2. doi: 10.1016/j.gie.2013.08.005

87. Gonzalez-Carmona MA, Bolch M, Jansen C, Vogt A, Sampels M, Mohr RU, et al. Combined photodynamic therapy with systemic chemotherapy for unresectable cholangiocarcinoma. *Aliment Pharmacol Ther.* (2019) 49:437–47. doi: 10.1111/apt.15050
88. Shafirstein G, Battoo A, Harris KE, Baumann H, Gollnick SO, Lindenmann J, et al. Photodynamic therapy of non-small cell lung cancer: narrative review and future directions. *Ann Am Thorac Soc.* 13:265–75. doi: 10.1513/AnnalsATS.201509-650FR
89. Wakiyama H, Kato T, Furusawa A, Choyke PL, Kobayashi H. Near infrared photoimmunotherapy of cancer; possible clinical applications. *Nanophotonics.* (2021) 0:20210119. doi: 10.1515/nanoph-2021-0119
90. Paraboschi I, Privitera L, Kramer-Marek G, Anderson J, Giuliani S. Novel treatments and technologies applied to the cure of neuroblastoma. *Children.* (2021) 8:482. doi: 10.3390/children8060482
91. Nouso H, Tazawa H, Tanimoto T, Tani M, Oyama T, Sato H, et al. Abstract 3831: development of near-infrared photoimmunotherapy targeting GD2-positive neuroblastoma. In: Dang CV, editor. *Immunology*. American Association for Cancer Research (2018). p. 3831. doi: 10.1158/1538-7445.AM2018-3831
92. Maruoka Y, Furusawa A, Okada R, Inagaki F, Wakiyama H, Kato T, et al. Interleukin-15 after near-infrared photoimmunotherapy (nir-pit) enhances t cell response against syngeneic mouse tumors. *Cancers*. (2020) 12:2575. doi: 10.3390/cancers12092575
93. Nagaya T, Sato K, Harada T, Nakamura Y, Choyke PL, Kobayashi H. Near infrared photoimmunotherapy targeting EGFR positive triple negative breast cancer: optimising the conjugate-light regimen. *PLoS ONE.* (2015) 10:e0136829. doi: 10.1371/journal.pone.0136829
94. Sato K, Nagaya T, Mitsunaga M, Choyke PL, Kobayashi H. Near infrared photoimmunotherapy for lung metastases. *Cancer Lett.* (2015) 365:112–21. doi: 10.1016/j.canlet.2015.05.018
95. Menke-van der Houven van Oordt CW, Gomez-Roca C, van Herpen C, Coveler AL, Mahalingam D, Verheul HMW, et al. First-in-human phase I clinical trial of RG7356, an anti-CD44 humanised antibody, in patients with advanced, CD44-expressing solid tumors. *Oncotarget.* (2016) 7:80046–58. doi: 10.18632/oncotarget.11098
96. Hayes-Jordan A, Lopez C, Green HL, Xiao LC, Huh W, Herzog CE. Cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) in pediatric ovarian tumors: a novel treatment approach. *Pediatr Surg Int.* (2016) 32:71–3. doi: 10.1007/s00383-015-3814-9
97. Sjoberg Bexelius T, Chisholm JC, Okoye B, Cecil T, Angelini P, Dayal S. Hyperthermic intraperitoneal chemotherapy (HIPEC) as another treatment modality for desmoplastic round cell tumour patients: first paediatric experience from UK. *BMJ Case Rep.* (2021) 14:e234876. doi: 10.1136/bcr-2020-234876

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frontiers in Pediatrics

ORIGINAL RESEARCH
published: 18 January 2022
doi: 10.3389/fped.2021.780830

Robotics-Assisted Pediatric Oncology Surgery—A Preliminary Single-Center Report and a Systematic Review of Published Studies

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Aim: The use of robotics-assisted surgery in oncology has been proved effective and safe in adults. Despite these results, the use of robotics has been rarely reported for pediatric oncology. Our review aims to evaluate the safety and feasibility of robotics-assisted surgery in this field, analyzing our experience and performing a systematic review of the most recent studies.

Methods: We reviewed all patients affected by an oncological disease who underwent a robotics-assisted procedure at our institute. We performed a systematic review of the literature from 2012 to 2021 on the subjects.

Findings: A total of 14 patients underwent robotics-assisted tumor resection. Eleven procedures (median age 13.2-years old) were carried out in children with adnexal lesions (seven tumor excision and four ovariectomies). Histological diagnosis was mature teratoma (six), serous papillary cystadenofibromas of the fallopian tube (two), ovarian serous cystadenoma (one), ovarian mucinous cystadenoma (one), and ovarian seromucinous cystadenoma. The median length of stay was 2 days. No recurrences or complications at a median follow-up of 2.1-years were observed. A 5-year-old girl underwent a complete posterior resection of a type 3 sacrococcygeal tumor with a robotics-assisted approach for the dissection of a possible intraabdominal residual component of the lesion. No intra- and postoperative complications were recorded. Complete excision of a recurrent differentiating neuroblastoma of the left para-renal region was performed on a 9-year-old girl. An idiopathic anaphylactic shock occurred 1 day after the procedure. At 9 months' follow-up, no local recurrences of the lesion were observed. Overall, we reported no conversion to open surgery. Lastly, a robotic excision of a growing left superior mediastinal intermixed ganglioneuroblastoma was performed on an 8-year-old girl with no postoperative complications. Follow-up was uneventful (7 months). In the literature, the rate of complications ranges from 0 to 28%, mainly related to difficult dissection and impaired anatomy. Conversion is reported in 5% of all oncological procedures, due to more invading tumors and altered anatomical features. No robotics-related complications were reported.

OPEN ACCESS

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Specialty section:
This article was submitted to Pediatric Surgery, a section of the journal Frontiers in Pediatrics

Received: *21 September 2021*
Accepted: *18 November 2021*
Published: *18 January 2022*

Citation:
Vatta F, Gazzaneo M, Bertozzi M, Raffaele A, Avolio L and Riccipetitoni G (2022) Robotics-Assisted Pediatric Oncology Surgery—A Preliminary Single-Center Report and a Systematic Review of Published Studies. Front. Pediatr. 9:780830. doi: 10.3389/fped.2021.780830

Conclusion: Robotics-assisted surgery in pediatric oncology has proven to be feasible. Nevertheless, its use should be limited to selected cases and performed by highly trained oncological surgeons. Preparation and patient positioning, alongside a correct port placement, are crucial to carrying out these procedures. Further innovations in robotics may allow a wider application of this technology in pediatric oncology.

Keywords: robotic-assisted surgery, oncology, pediatrics, children, mini-invasive surgery

INTRODUCTION

Robotics-assisted surgery (RAS) represents one of the most important advancements in minimally invasive surgery (MIS) in recent years and has progressively gained a predominant role in many fields of adult surgery. The da Vinci surgical system (Intuitive Surgical, Inc., Sunnyvale, CA, USA) is actually present in 67 countries, and more than 5,500 robots are used worldwide (1).

Well-known advantages are a stable magnified 3D view, tremor filtering, and motion scaling, which allow precise intracorporeal exposure and suturing (2).

RAS in pediatric surgery has initially struggled due to some limitations, notably port and arm dimensions, as well as high costs (3). Nevertheless, the growing number of case reports and series published every year reveals how RAS is increasingly applied for children (4).

Despite this spread, its use for pediatric oncology is still limited, and few studies have been conducted on the subject. The reasons are represented by the characteristics of pediatric tumors, as each type may be considered a rare disease. Moreover, most pediatric malignancies are embryonal tumors with rapid growth, which require frequently other therapies as neoadjuvant chemotherapy. All these distinctive features limit the creation of guidelines for the robotic approach. Nonetheless, accepted recommendations require an evaluation by a multidisciplinary tumor board and respecting oncological protocols for open surgery for each specific pathology (5).

We performed a retrospective study to critically review our experience in RAS.

In order to compare our results with those from the literature, we performed a systematic review, focusing on technical skills that could help pediatric surgeons to avoid intra- and postoperative complications.

MATERIALS AND METHODS

We performed a retrospective review of all pediatric oncological patients who underwent RAS at our institution from 2010 to 2021. From 2017 to 2020, the use of the robotic platform has been suspended due to technical reasons.

Patients over 18-years old were excluded, as well as all malignancies not treated with RAS.

We analyzed demographic data, including age at surgery, sex, pathology, possible comorbidities, operation time (OT), length of hospital stay (LHS), perioperative complications, and postoperative outcomes. Postoperative complications were classified according to the Clavien–Dindo classification and graded from I to V.

All procedures were carried out using the da Vinci Si Surgical System (Intuitive Surgical, Sunnyvale, CA, USA). All surgeries were performed with three robotic arms, placed accordingly depending on the lesion site and size. Some procedures required an accessory port (3 or 5 mm).

To compare our results with those of the literature, a systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria.

We selected articles reporting RAS in oncological pediatric patients between 2012 and September 2021 in MEDLINE and EMBASE using the following keywords: "(pediatric) or (children) and (robot) or (robotic) and (oncology) or (tumor)."

Inclusion criteria were as follows:

- Articles published between January 2012 and September 2021
- Articles written in English
- Median/mean age <18-years
- Case series with more than 3 patients
- Articles where data concerning demographics, surgical indications, complication, and conversion rates were clearly deductible.

All data were elaborated using the statistical software "R," version 3.4.1. Descriptive statistics were used to present findings, and quantitative variables were expressed as median (range) to express our data. Data elaborated from the literature review were expressed as median (range) or mean ± SDs depending on the reference found in the original articles.

RESULTS

A total of 14 pediatric patients underwent RAS for oncological pathologies from 2010 to 2021 at our institute. All data are displayed in **Table 1**. No patients required a conversion to open surgery.

Among our cohort, 11 gynecological procedures were performed (7 mass excisions and 4 ovariectomies) for the following tumors: 6 ovarian mature cystic teratomas (**Figure 1**), 2 serous papillary cystadenofibroma of the fallopian tube, 1 ovarian mucinous cystadenoma, 1 ovarian serous cystadenoma, and 1 ovarian seromucinous cystadenoma. The median age at surgery was 13.2 [8.0–16.9], with median operative time including docking time 120 [65–260]. Most of the procedures were carried out using an 8-mm optic port and two 5-mm operative ports.

TABLE 1 | Summarized data of all oncological patients undergoing RAS (in chronological order).

Year	Patient	Sex	Age	Region/specialty	Surgical intervention	Side	Diagnosis	Robotic port (optic–operative–operative)	Accessory port	OT	Conversion	Perioperative complication	Postoperative complication	LHS (days)	Follow-up (years)
2011	NA	F	8.6	Gynecology	Ovariectomy	L	Ovarian mature cystic teratoma	8–5–5	No	130	No	No	No	1	0.63
2011	PLC	F	13.2	Gynecology	Ovariectomy	L	Ovarian mature cystic teratoma	8–5–5	No	260	No	No	No	2	6.11
2012	BG	F	5.4	Abdomen	Robotics-assisted explorative laparoscopy (mass debulking via posterior approach)	NA	Mature sacrococcygeal teratoma	8–5–5	No	NA	No	No	No	4	LAF
2015	AS	F	8.7	Gynecology	Ovariectomy	L	Ovarian mature cystic teratoma	8–8–8	1 (3 mm)	215	No	No	No	2	6.29
2015	KA	F	14.8	Gynecology	Mass excision	R	Ovarian seromucinous cystadenoma	8–5–5	No	120	No	Spillage	No	2	5.58
2016	SS	F	12.9	Gynecology	Mass excision	L	Serous papillary cystadenofibroma of the fallopian tube	8–5–5	No	105	No	No	No	1	0.88
2017	BN	F	16.9	Gynecology	Ovariectomy	R	Ovarian mature cystic teratoma	12–8–8	No	195	No	No	No	2	3.28
2017	GC	F	8.0	Gynecology	Mass excision	L	Ovarian mucinous cystadenoma	8–5–5	No	65	No	No	No	1	0.15
2017	CSE	F	13.6	Gynecology	Mass excision (concomitant urachal remnant excision)	L	Ovarian serous cystadenoma	8–5–5	No	155	No	No	No	4	3.32
2017	CV	F	16.4	Gynecology	Mass excision	R	Serous papillary cystadenofibroma of the fallopian tube	8–5–5	No	90	No	No	No	2	0.92
2020	GA	F	9.4	Abdomen	Mass excision	L	Differentiating neuroblastoma	12–8–8	1 (5 mm)	320	No	No	Anaphylactic shock (1 day postop)—Cl. Dindo IV	8	1.22*
2020	FE	F	7.6	Thoracic	Mass excision	L	Intermixed ganglioneuroblastoma	8–8–8	1 (5 mm)	290	No	No	No	7	0.78
2020	SM	F	13.5	Gynecology	Mass excision	R	Ovarian mature cystic teratoma	8–5–5	1 (5 mm)	110	No	No	No	2	LAF
2021	SG	F	13.1	Gynecology	Mass excision	L	Ovarian mature cystic teratoma	12–8–8	1 (5 mm)	105	No	Spillage	No	2	0.63

RAS, robotics-assisted surgery; OT, operation time; LHS, length of hospital stay.

**Neuroblastoma localization in a supraclavicular lymph node at 6 months postop treated surgically.*

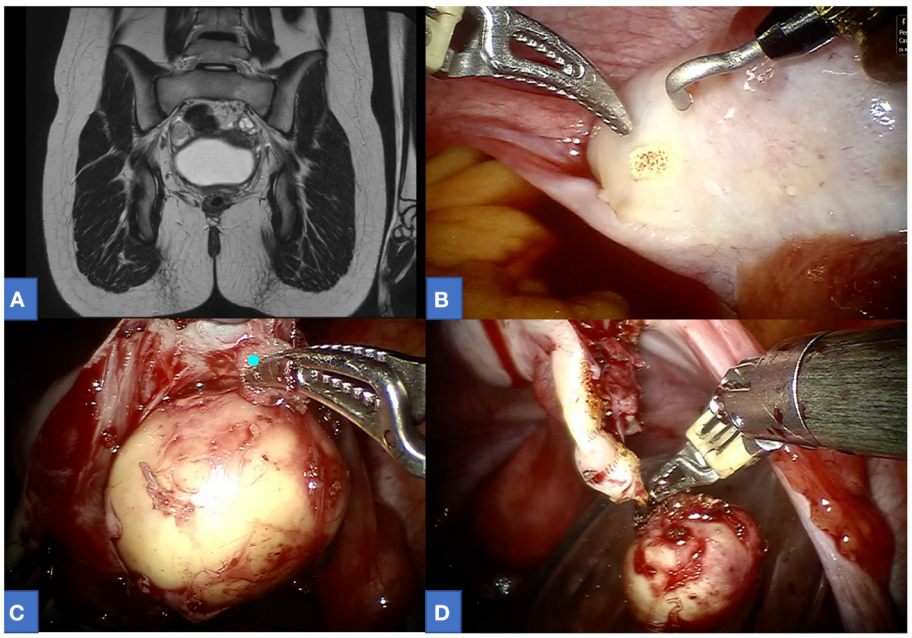

FIGURE 1 | A 13-year-old affected by right ovarian mature teratoma. **(A)** Preoperative MRI. **(B–D)** Intraoperative view.

In only three patients, an additional accessory port (either 5 or 3 mm) was positioned. In two cases (14.2%), intraoperative spillage was reported. No other perioperative complications nor conversion were reported. Follow-up was uneventful for all patients (median follow-up 2.1-years [0.2–6.3]). The median length of hospitalization was 2 days (1–4).

We performed one thoracic procedure on a 7-year-old girl for a growing intermixed ganglioneuroblastoma located on the supero-posterior mediastinum encasing the subclavian vessels. An 8-mm optic port was positioned in the sixth intercostal space on the midaxillary line. Two 8-mm operative ports were positioned 8 cm away from the optic port, in the fifth intercostal space on the anterior axillary line and in the seventh intercostal space on the paravertebral line. Finally, a 5-mm auxiliary port was placed in the fourth intercostal space on the anterior axillary line. No postoperative complications were reported.

We report one robotics-assisted explorative laparoscopy on a 5-year-old girl who previously underwent posterior excision of a type 3 mature sacrococcygeal teratoma, as the preoperative imaging showed suspicion of tumor extension in the pelvis. The robotics-assisted exploration result was negative.

We completed an excision of a left perirenal recurrence of neuroblastoma in a 9-year-old girl. A 12-mm optic port was placed trans-umbilically, whereas two 8-mm operative ports were placed in the left hypochondrium and in the left iliac region. An accessory port was then positioned in the epigastric region. No intraoperative complication occurred. The patient suffered from an anaphylactic shock on the first postoperative day that required adrenaline and corticosteroid administration. Further postoperative course was uneventful.

A systematic review was performed according to the PRISMA guidelines (**Figure 2**). Eight studies met the eligibility criteria, for a total of 137 procedures in 134 patients. Data are summarized in **Table 2**. The male-to-female (M:F) ratio was ∼1:2, the median age was 9-years [0.9–19.0], and the median weight was 35 kg (when reported). Treated conditions were represented by a broad group of tumors, and the most common were adrenal. The malignancy rate was on average 65%. The median conversion to open surgery rate was 5%.

The intraoperative complication rate ranged from 0 to 28%, and the main reported causes were difficult dissection and intraoperative discovery of more invading tumors than expected. Moreover, two conversions were performed due to a lack of confidence in the anatomy. No robotics-related complication was reported (e.g., injury to the patients due to robotic arms).

The median operative time, including docking, was 184 min. The postoperative complication rate accounted for 4% (most reported complications were pneumothorax, unexpected drug reaction, and adhesions). The median hospital stay was 4.6 days.

Follow-up, when stated, was carried out for a median of 14.4 months. The recurrence rate was 1.4%.

A comparison between our experience and the literature is reported in **Table 3**.

DISCUSSION

The role of RAS is becoming progressively more important in every field, including pediatric oncology. In the last decade, several papers have been published on the subjects, even if the sample of the cohort is often small since most of the studies are case reports (5–12).

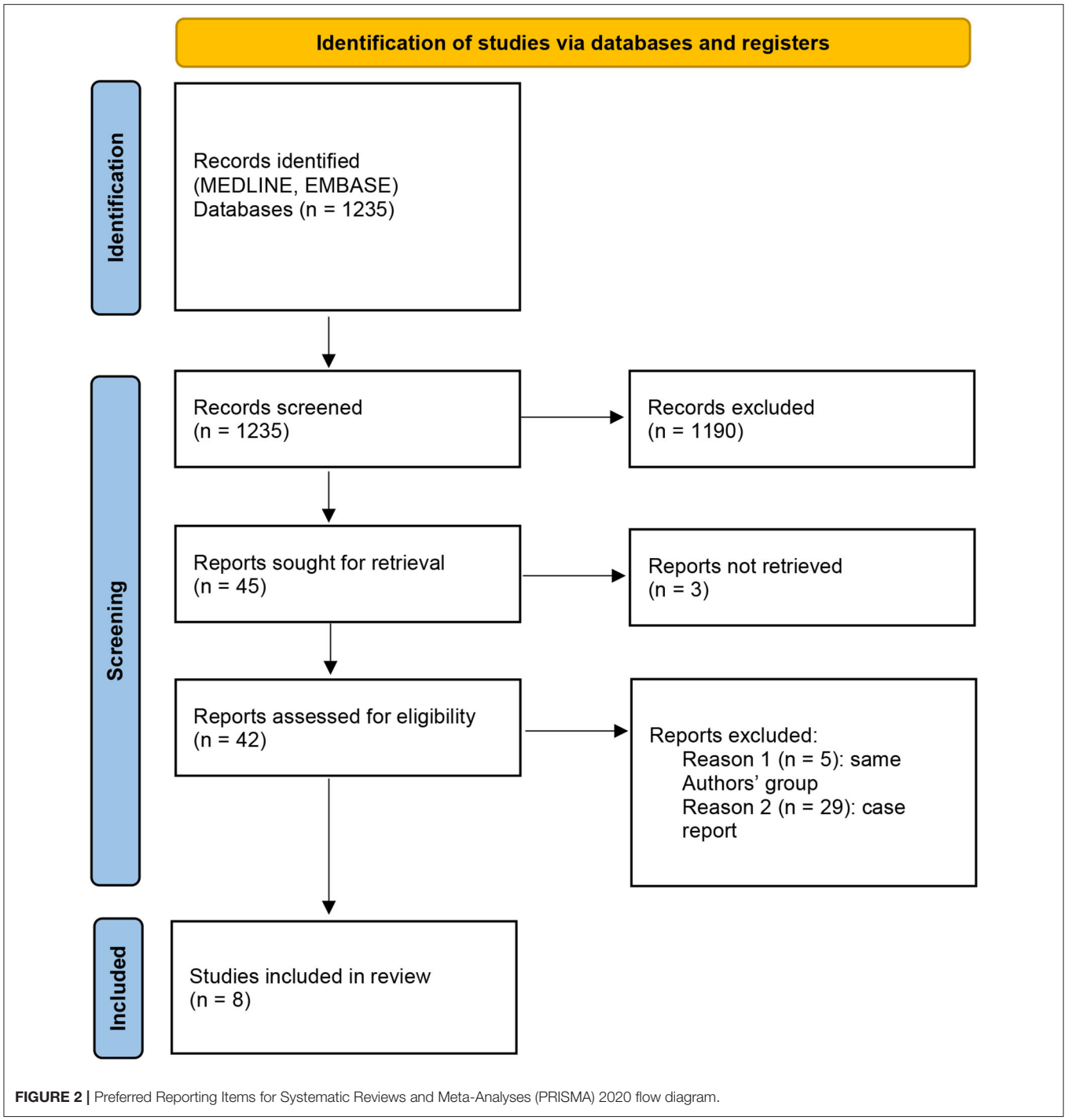

FIGURE 2 | Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 flow diagram.

The still limited spread of this technique is due to both the concerns of the use of MIS for tumors and the well-known limitations of RAS in children (13, 14). Nevertheless, since the first cases reported by Meehan and Sandler (15), results have been encouraging.

To date, the published paper underlines the necessity to perform a strict selection of all children undergoing RAS, in order to adhere to oncological guidelines. The application of this technique requires an in-depth knowledge of pediatric oncology and the revision of each case by an ongoing multidisciplinary team, composed of medical oncologists, radiologists, anesthesiologists, and surgeons experienced in MIS and oncologic surgery (16).

Comparing our experience with the literature (**Table 3**), we found a different M:F ratio (0:1 vs. 1:2), probably due to our initial selection to perform surgery in adnexal lesions. In our practice, the first oncological procedures that were performed concerned adnexal lesions, as we believed that RAS is a perfect

TABLE 2 | Results of the systematic review.

Author	Publication year	Patients (n)	Tumor type	Diagnosis	M:F ratio	Procedures (n)	Median age (years)	Median weight (kg)	Mean total operative time (minutes)	Malignancy rate (%)	Conversion rate (%)	Intraoperative complications (%)	Type of intraoperative complication/ cause of conversion	Postoperative complication rate (%)	Clavien–Dindo complications	Postoperative complications	LHS (Days)	Follow-up (months)	Recurrence rate (%)
Meehan (5)	2013	14	Mediastinal ($n = 4$), retroperitoneal ($n = 4$), adrenal ($n = 3$), ovarian ($n = 1$), colonic ($n = 1$), pancreatic ($n = 1$)	Germ cell mediastinal tumor ($n = 1$), mature mediastinal teratoma ($n = 1$), ganglioneuroma ($n = 2$), ganglioneuroblastoma ($n = 1$), neuroblastoma ($n = 3$), pheochromocytoma ($n = 1$), adrenal carcinoma ($n = 1$), begin adrenal mass ($n = 1$), colon cancer ($n = 1$), pancreatic tumor ($n = 1$)	NA	14	NA	NA	NA	NA	28 ($n = 4$)	28 ($n = 4$)	Not confident with the anatomy ($n = 2$: retroperitoneal ganglioneuroma, pancreatic mass), acute hypertensive crisis during adrenal pheochromocytoma resection ($n = 1$), unexpected discovery of a large colon tumor invading the anterior abdominal wall ($n = 1$)	0	/		NA	NA	0
Varda et al. (6)	2018	7	Renal ($n = 4$), retroperitoneal ($n = 2$), adrenal ($n = 1$)	Ganglioneuroma ($n = 1$), papillary renal cell carcinoma ($n = 1$), non-seminomatous germ cell tumors ($n = 1$), renal tumor ns ($n = 1$), rhabdomyosarcoma ($n = 1$), cystic renal dysplasia ($n = 2$)	NA	7	12.5 (3–19)	45 (14–79)	277 (172–508)	42 ($n = 3$)	0	0		0	/		NA	7	0
Xie et al. (7)	2019	4	Ovarian ($n = 4$)	Ovarian mature cystic teratoma ($n = 2$), mucinous tumor ($n = 1$), ovarian teratoma ($n = 1$)	0:04	4	7.5 (1–13)	36.8 (8.5 –69.5)	120	NA	0	0		0	/		3	6	0
Navarrete Arellano et al. (8)	2019	4	Mediastinal teratoma (n =), renal ($n = 1$), retroperitoneal ($n = 1$), adrenal ($n = 1$)	Mediastinal teratoma ($n = 1$), retroperitoneal lipoma ($n = 1$), pheochromocytoma ($n = 1$), renal tumor ns ($n = 1$)	NA	4	NA	NA	NA	NA	NA	NA		0	/		2.6	NA	NA
Esposito et al. (9)	2020	5	Ovarian ($n = 5$)	Mature teratoma ($n = 3$), seromucinous cystadenoma ($n = 2$)	0:05	5	13.5 (11–16)	NA	78 (66–90)	0	0	0		0	/		NA	NA	NA
Mitra et al. (10)	2020	3	Adrenal ($n = 3$)	Ganglioneuroblastoma ($n = 2$), pheochromocytoma ($n = 1$)	2:01	3	6.3 (2–13)	NA	244 (244–265)	NA	0	0		33 ($n = 1$)	II ($n = 1$)	Unexpected drug reaction ($n = 1$)	2	19 (12–30)	0

(Continued)

TABLE 2 | Continued

Author	Publication year	Patients (n)	Tumor type	Diagnosis	M:F ratio	Procedures (n)	Median age (years)	Median weight (kg)	Mean total operative time (minutes)	Malignancy rate (%)	Conversion rate (%)	Intraoperative complications (%)	Type of intraoperative complication/ cause of conversion	Postoperative complication rate (%)	Clavien–Dindo complications	Postoperative complications	LHS (Days)	Follow-up (months)	Recurrence rate (%)
Blanc et al. (11)	2021	89	Neuroblastic ($n = 31$), renal ($n = 24$), neuroendocrine ($n = 12$), adrenal ($n = 9$), germ-cell ($n = 7$), pancreatic ($n = 4$), thymic ($n = 4$), IMT ($n = 4$), soft tissue ($n = 5$)	Pheochromocytoma ($n = 6$), paraganglioma ($n = 6$), adrenocortical adenoma ($n = 1$), bilateral carney complex ($n = 2$), bilateral McCune–Albright ($n = 2$), mature teratoma ($n = 2$), malignant seminoma ($n = 1$), non-seminomatous ($n = 4$), neuroendocrine tumor ($n = 1$), focal hyperinsulinism ($n = 3$), thymoma ($n = 4$), myasthenia ($n = 1$), MEN1 ($n = 1$), IMTs ($n = 4$), embryonal rhabdomyosarcoma ($n = 1$), neurofibroma ($n = 1$), bronchial carcinoid tumor ($n = 1$), leiomyoma ($n = 1$), lipoma ($n = 1$)	3:5.6	92	8.2 (3.6–13)	26 (15–47)	215 (156–282)	57 ($n = 51$)	8 ($n = 7$)	8 ($n = 7$)	Renal vein injury in Wilms' tumor ($n = 1$), misdiagnosed renal vein tumor thrombus and spillage ($n = 1$), poor respiratory tolerance after diaphragmatic resection and spillage due to tumor rupture after the conversion in WT infiltrating the liver ($n = 1$), Sliding Hem-O-Lock clip-on renal vein ($n = 1$), difficult renal hilum dissection in renal sarcoma ($n = 1$), difficult dissection in a neuroblastoma and ganglioneuroma for narrow space and vascular involvement ($n = 2$)	5.7 ($n = 5$)	III ($n = 4$), II ($n = 1$)	Pneumothorax ($n = 2$), anastomotic stenosis ($n = 1$), adhesions ($n = 1$), retroperitoneal collection ($n = 1$)	3	27 (18–29)	2% ($n = 2$)
Li et al. (12)	2021	8	Bladder/prostate tumor ($n = 8$)	Bladder rhabdomyosarcoma ($n = 8$)	5:03	8	6 (0.9–11)	NA	172 (104–316)	100 ($n = 8$)	0	0		0	/		12.5	13.3	0

LHS, length of hospital stay.

TABLE 3 | Comparison between our experience and literature.

	Our cohort	Literature
Patients (*n*)	14	134
M:F	0:1	1:2
Age at surgery (years)	11.5 (5.4–16.9)	9 (0.9–19)
Malignancy rate	14%	65%
Operative time (min)	166	184
Intraoperative complication rate	14.2% (2 spillage)	4.5% (0–28%)
Conversion rate	0%	5%
Recurrence rate	0%	3.20%

fit for these indications. Adult gynecology has already proven the feasibility of robotic procedures for both benign and malign pathologies (7, 17–20), and experience in pediatrics is growing (7, 9, 21). A robot allows a superb visualization of the pelvis, and in the majority of cases, port placement may be carried out easily, as most girls undergoing this kind of surgery are adolescents. Alongside, MIS offers good cosmetic results, which is an important factor, especially in this group of patients (22). Nevertheless, the relative simplicity of the surgical procedure must not let the surgeons underestimate the risk of spillage and/or rupture of ovarian lesions.

Although extremely rare, malignant ovarian neoplasm in children and adolescents may occur (23–27). If preoperative examinations point out the risk of malignancy, oophorectomy should be strongly considered, and, when performed, no salpingectomy is required, which is preferable in this age group (24, 25, 28, 29). Nevertheless, in pediatrics, there is an interest in preserving as much ovarian tissue as possible, to assure the development of normal puberty and future fertility (30). As many articles describe how laparoscopy may be safely applied to perform ovarian-sparing surgery in pediatrics, this topic is sometimes debated (31). In our opinion, the already cited technological advantages of the da Vinci system may further allow a surgeon to perform a safe excision minimizing the risk of spillage, as long as all oncological principles are followed (e.g., preoperative tumor markers, adequate imaging, and extraction of the mass using an Endobag).

Risks of tumor rupture and/or spillage, risk of incomplete resection, and risk of port-site recurrences count as the most cited problems for MIS/RAS.

In our experience, the complication rate was higher than in other series (14.2%, two spillages, vs. 4.5%). Spillage during RAS is reported in only one case by Blanc et al. (11), due to the leakage of a renal vein thrombus of a Wilms tumor, discovered after renal vein control. Overall, the spillage rate was 0.7%. Despite that the risk should not be underestimated, the use of MIS in malignancies where spillage or rupture is particularly dangerous has been accepted in selected cases. For example, in 2014, the Renal Tumor Strategy Group of the International Society of Paediatric Oncology (SIOP) published the largest cohort of laparoscopic excision of Wilms tumor (32). Moreover, in the same year, the SIOP Umbrella protocol (33, 34) proposed inclusion criteria to safely perform laparoscopic nephrectomy in Wilms tumors. Finally, in 2018, Bouty et al. showed, by performing a systematic review, that in highly selected cases, MIS in Wilms tumor did not worsen prognosis (35).

Although detractors of RAS are skeptical about its use due to the absence of haptic feedback, the technological advantages of the da Vinci system (3D vision, seven degrees of freedom, tremor filtration, and precise camera control) have expanded the possibilities of performing and reproducing difficult operations, especially when there is a deep and narrow field and when fine dissection is required for delicate tissue manipulation, as is the case in pediatric oncology surgery (16, 36).

Regarding the suspected incidence of port-site recurrences, a recent publication in adults shows equivalent outcomes between laparoscopic/robotic and open approaches (17, 37). In pediatrics, no recurrences have been cited so far.

In literature, the overall conversion rate to open surgery was about 5%, and difficult dissection or surgeon diffidence in continuing RAS were the most reported causes.

Conversion is required every time there is the possibility to upstage the tumor. Nevertheless, as the experience of the surgeon grows, a reduction in the rate of conversion is reported (11).

This is certainly due to improved confidence in RAS, associated with a better selection of patients addressed to this technique. Blanc et al. suggest beginning the experience with RAS with smaller tumors and converting in cases of difficult dissection, stressing that the main objective is to respect the oncological surgical principles (11).

For several authors, surgeons with or without previous laparoscopic or robotic experience could perform independently and properly robotic procedures (38, 39). In surgical oncology, the passage from open to laparoscopy or RAS is far from being easy. The approach to pediatric tumors needs an important surgical background that comes from open surgery. To apply RAS in tumor resection, it is not only necessary to improve personal learning curve, training, and exercising on virtual and animal models mastering basic and advanced robotic skills. For any surgeon, it is necessary to perform at least 250 procedures to consider himself/herself independent and a mentor in surgery.

The availability of senior surgeons with experience in both oncologic surgery and MIS provides valid support to the steep learning curve.

In our experience, the availability of a simulating station for the da Vinci system allowed us to perform specific personal training, both virtual and *in vivo*. The approach to pediatric tumors came after a consistent experience in other RAS and specific training of the whole surgical team. Thanks to the presence of 2 consoles, it was possible for younger surgeons to approach tumors, with senior surgeons mentoring live, even those with less experience in RAS.

An oncological procedure carried out with RAS, especially at the beginning, may require a long operative time. Installation of the patient requires meticulous attention. Comfortable positioning as well as the use of adequate padding and skin protection must always be verified (4, 40). It is important to avoid hyperextension or flexion in small children, as they are more pliable compared with adolescents and adults (4). Once the patient is correctly installed, the docking procedure needs

to be correctly planned, especially in infants and toddlers, as the working space is limited. Particular attention is needed to avoid conflict between the robotic arms and, more importantly, between the robot and the patient. The key role in assuring and controlling potential harm to the child during surgery is played by the scrubbed nurse and the scrubbed assistant who need to control and verify patient safety throughout the intervention, alongside assisting the lead surgeon by passing needles, bandages, or other instruments through the assistant port (41).

In our experience, OT was comparable with that in the literature (166 vs. 184 min).

Anesthesiologists involved in RAS procedures must be familiar with the robot and its installation, as well as the degree of movements of the arms. All vascular accesses must be positioned before docking and arranged to minimize any possible conflicts with the robot. At the same time, robot installation must not prevent the work of the anesthesiology team during surgery.

In literature, concerning pediatric oncology, no case of robotics-related complication has been reported, in terms of injury to the patient due to a robotic arm, nor cases of robot malfunction. When operating with the da Vinci robot, especially in the case of delicate surgeries such as oncological procedures, all members of the surgical team have to keep in mind the possibility of malfunction and must be able to respond and properly provide assistance if necessary. In fact, during any robotics-assisted procedure, the role of the technical assistance team is crucial. Technical support should always be available and consists of in-person and phone support provided (42). Their help can solve most cases of dysfunction of the robot or any of its components. In our experience, we were assisted by a da Vinci specialist during the most complicated surgeries.

LIMITATIONS

The limitations of this study are represented by the retrospective nature of the analysis and the small cohort of patients (14) with a high prevalence of adnexal lesions. Since the application of RAS to pediatric oncology represents a new experience, even the systematic review is limited by a low number of papers with a small series.

CONCLUSIONS

RAS in pediatric oncology has proven to be feasible for different pathologies. Although optimistic reports have been published in the literature, the use of RAS should be limited to selected cases and performed by highly trained oncological surgeons. So far, the literature strongly recommends the presence of a multidisciplinary board of experts (surgeon, anesthesiologist, radiologist, and oncologist) to evaluate candidates to RAS. All procedures must be carried out while respecting oncological protocols. Preparation and patient positioning, alongside a correct port placement, are crucial to safely perform these surgeries.

Further studies are needed to assess the role of RAS in pediatric oncology, as well as to implement specific technical standards for each pediatric tumor.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

GR contributed to conception and design of the study. FV and MG organized the database and performed the statistical analysis. FV, MB, and AR wrote the first draft of the manuscript. FV, MB, and GR wrote sections of the manuscript. LA and RV reviewed the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

REFERENCES

1. Koukourikis P, Rha KH. Robotic surgical systems in urology: what is currently available? *Investig Clin Urol.* (2021) 62:14–22. doi: 10.4111/icu.20200387
2. Mizuno K, Kojima Y, Nishio H, Hoshi S, Sato Y, Hayashi Y. Robotic surgery in pediatric urology: current status. *Asian J Endosc Surg.* (2018) 11:308–17. doi: 10.1111/ases.12653
3. Bruns NE, Soldes OS, Ponsky TA. Robotic surgery may not "make the cut" in pediatrics. *Front Pediatr.* (2015) 3:10. doi: 10.3389/fped.2015.00010
4. Denning NL, Kallis MP, Prince JM. Pediatric robotic surgery. *Surg Clin North Am.* (2020) 100:431–43. doi: 10.1016/j.suc.2019.12.004
5. Meehan JJ. Robotic surgery for pediatric tumors. *Cancer J.* (2013) 19:183–8. doi: 10.1097/PPO.0b013e318289486c
6. Varda BK, Cho P, Wagner AA, Lee RS. Collaborating with our adult colleagues: a case series of robotic surgery for suspicious and cancerous lesions in children and young adults performed in a free-standing children's hospital. *J Pediatr Urol.* (2018) 14:182.e1-8. doi: 10.1016/j.jpurol.2018.01.003
7. Xie XX, Wang N, Wang ZH, Zhu YY, Wang JR, Wang XQ. Robotic-assisted resection of ovarian tumors in children: a case report and review of literature. *World J Clin Cases.* (2019) 7:2542–8. doi: 10.12998/wjcc.v7.i17.2542
8. Navarrete Arellano M, Garibay González F. Robot-assisted laparoscopic and thoracoscopic surgery: prospective series of 186 pediatric surgeries. *Front Pediatr.* (2019) 7:200. doi: 10.3389/fped.2019.00200
9. Esposito C, Settimi A, Del Conte F, Cerulo M, Coppola V, Farina A, et al. Image-guided pediatric surgery using Indocyanine Green (ICG) fluorescence in laparoscopic and robotic surgery. *Front Pediatr.* (2020) 8:314. doi: 10.3389/fped.2020.00314
10. Mitra AP, Vasquez E, Kokorowski P, Chang AY. Robotic adrenalectomy in the pediatric population: initial experience case series from a tertiary center. *BMC Urol.* (2020) 20:155. doi: 10.1186/s12894-020-00727-x
11. Blanc T, Meignan P, Vinit N, Ballouhey Q, Pio L, Capito C, et al. Robotic surgery in pediatric oncology: lessons learned from the first 100 tumors-a nationwide experience. *Ann Surg Oncol.* (2021) 28(Suppl 3):901. doi: 10.1245/s10434-021-10861-x
12. Li P, Zhou H, Cao H, Xu B, Guo T, Zhu W. Robotic-assisted Laparoscopic Management of Bladder/Prostate Rhabdomyosarcoma in Children: initial series and 1-yr outcomes. *J Endourol.* (2021) 35:1520–5. doi: 10.1089/end.2020.1238
13. Motterle G, Ahmed ME, Andrews JR, Moschini M, Kwon ED, Karnes J. Tumor seeding after robot-assisted radical prostatectomy: literature

review and experience from a single institution. *J Urol.* (2020) 203:1141–6. doi: 10.1097/JU.0000000000000701
14. Cundy TP, Shetty K, Clark J, Chang TP, Sriskandarajah K, Gattas NE, et al. The first decade of robotic surgery in children. *J Pediatr Surg.* (2013) 48:858–65. doi: 10.1016/j.jpedsurg.2013.01.031
15. Meehan JJ, Sandler A. Pediatric robotic surgery: a single-institutional review of the first 100 consecutive cases. *Surg Endosc.* (2008) 22:177–82. doi: 10.1007/s00464-007-9418-2
16. Blanc T, Pio L, Clermidi P, Muller C, Orbach D, Minard-Colin V, et al. Robotic-assisted laparoscopic management of renal tumors in children: preliminary results. *Pediatr Blood Cancer.* (2019) 3:e27867. doi: 10.1002/pbc.27867
17. Qadan M, Kingham TP. Technical aspects of gallbladder cancer surgery. *Surg Clin North Am.* (2016) 96:229–45. doi: 10.1016/j.suc.2015.12.007
18. Zanagnolo V, Garbi A, Achilarre MT, Minig L. Robot-assisted surgery in gynecologic cancers. *J Minim Invasive Gynecol.* (2017) 24:379–96. doi: 10.1016/j.jmig.2017.01.006
19. Conrad LB, Ramirez PT, Burke W, Naumann RW, Ring KL, Munsell MF, et al. Role of minimally invasive surgery in gynecologic oncology: an updated survey of members of the society of gynecologic oncology. *Int J Gynecol Cancer.* (2015) 25:1121–7. doi: 10.1097/IGC.0000000000000450
20. Gala RB, Margulies R, Steinberg A, Murphy M, Lukban J, Jeppson P, et al. Systematic review of robotic surgery in gynecology: robotic techniques compared with laparoscopy and laparotomy. *J Minim Invasive Gynecol.* (2014) 21:353–61. doi: 10.1016/j.jmig.2013.11.010
21. Pelizzo G, Nakib G, Calcaterra V. Pediatric and adolescent gynecology: treatment perspectives in minimally invasive surgery. *Pediatr Rep.* (2019) 11:8029. doi: 10.4081/pr.2019.8029
22. Pelizzo G, Nakib G, Romano P, Avolio L, Mencherini S, Zambaiti E, et al. Five millimetre-instruments in paediatric robotic surgery: advantages and shortcomings. *Minim Invasive Ther Allied Technol.* (2015) 24:148–53. doi: 10.3109/13645706.2014.975135
23. Helmrath M, Shin C, Warner B. Ovarian cysts in the pediatric population. *Semin Pediatr Surg.* (1998) 7:19–28. doi: 10.1016/S1055-8586(98)70002-2
24. Cass D, Hawkins E, Brandt M, Chintagumpala M, Bloss RS, Milewicz AL, et al. Surgery for ovarian masses in infants, children, and adolescents: 102 consecutive patients treated in a 15-year period. *J Pediatr Surg.* (2001) 36:693–9. doi: 10.1053/jpsu.2001.22939
25. Hermans AJ, Kluivers KB, Wijen MH, Bulten J, Massuger LF, Coppus SF. Diagnosis and treatment of adnexal masses in children and adolescents. *Obstet Gynecol.* (2015) 125:611–15. doi: 10.1097/AOG.0000000000000665
26. Kelleher CM, Goldstein AM. Adnexal masses in children and adolescents. *Clin Obstet Gynecol.* (2015) 58:76–92. doi: 10.1097/GRF.0000000000000084
27. Strickland J. Ovarian cysts in neonates, children and adolescents. *Curr Opin Obstet Gynecol.* (2002) 14:459–65. doi: 10.1097/00001703-200210000-00004
28. Amies Oelschlager AM, Gow KW, Morse CB, Lara-Torre E. Management of large ovarian neoplasms in pediatric and adolescent females. *J Pediatr Adolesc Gynecol.* (2016) 29:88–94. doi: 10.1016/j.jpag.2014.07.018
29. ACOG Committee. Adnexal torsion in adolescents: ACOG Committee Opinion No, 783 Summary. *Obst Gynecol.* (2019) 134:435–6. doi: 10.1097/AOG.0000000000003376
30. Templeman CL, Fallat ME, Lam AM, Perlman SE, Hertweck SP, O'Connor DM. Managing mature cystic teratomas of the ovary. *Obstet Gynecol Surv.* (2000) 55:738–45. doi: 10.1097/00006254-200012000-00004
31. Guillén G, Martín-Giménez MP, López-Fernández S, Molino JA, López M. Results of ovarian sparing surgery in pediatric patients: is there a place for laparoscopy? *J Laparoendosc Adv Surg Tech.* (2020) 30:458–63. doi: 10.1089/lap.2019.0515
32. Warmann SW, Godzinski J, van Tinteren H, Heij H, Powis M, Sandstedt B, et al. Minimally invasive nephrectomy for Wilms tumors in children – data from SIOP 2001. *J Pediatr Surg.* (2014) 49:1544–8. doi: 10.1016/j.jpedsurg.2014.06.005
33. Burnand K, Roberts A, Bouty A, Nightingale M, Campbell M, Heloury Y. Laparoscopic nephrectomy for Wilms. *Pediatr Urol.* (2018) 14: 253.e1-8. doi: 10.1016/j.jpurol.2018.01.005
34. Van den Heuvel-Eibrink MM, Hol JA, Pritchard-Jones K, van Tinteren H, Furtwängler R, Verschuur AC, et al. Position paper: rationale for the treatment of Wilms tumor in the UMBRELLA SIOP-RTSG 2016 protocol. *Nat Rev Urol.* (2017) 14: 743–52. doi: 10.1038/nrurol.2017.163
35. Bouty A, Burnand K, Nightingale M, Roberts A, Campbell M, O'Brien M, et al. What is the risk of local recurrence after laparoscopic transperitoneal radical nephrectomy in children with Wilms tumors? Analysis of a local series and review of the literature. *J Pediatr Urol.* (2018) 14: 327. e1-7. doi: 10.1016/j.jpurol.2018.03.016
36. Anderberg M, Backman T, Annerstedt M. Robot-assisted radical cystoprostatectomy in a small child with rhabdomyosarcoma: a case report. *J Robotic Surg.* (2008) 2:101–3. doi: 10.1007/s11701-008-0089-3
37. Mantica G, Smelzo S, Ambrosini F, Tappero S, Parodi S, Pacchetti A, et al. Port-site metastasis and atypical recurrences after robotic-assisted radical cystectomy (RARC): an updated comprehensive and systematic review of current evidences. *J Robot Surg.* (2020) 14:805–12. doi: 10.1007/s11701-020-01062-x
38. Pio L, Musleh L, Paraboschi I, Pistorio A, Mantica G, Clermidi P, et al. Learning curve for robotic surgery in children: a systematic review of outcomes and fellowship programs. *J Robot Surg.* (2020) 14:531–41. doi: 10.1007/s11701-019-01026-w
39. O'Brien ST, Shukla AR. Transition from open to robotic-assisted pediatric pyeloplasty: a feasibility and outcome study. *J Pediatr Urol.* (2012) 8:276–81. doi: 10.1016/j.jpurol.2011.04.005
40. Villanueva J, Killian M, Chaudhryn R. Robotic urologic surgery in the infant: a review. *Curr Urol Rep.* (2019) 20:35. doi: 10.1007/s11934-019-0902-8
41. Nayyar R, Yadav S, Singh P, Dogra PN. Impact of assistant surgeon on outcomes in robotic surgery. *Indian J Urol.* (2016) 32:204–9. doi: 10.4103/0970-1591.185095
42. *Product Services DaVinci Surgical System.* Intuitive (2021). Available online at: https://www.intuitive.com/en-us/products-and-services/da-vinci/services (accessed September 21, 2021).

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frontiers in Pediatrics

ORIGINAL RESEARCH
published: 04 February 2022
doi: 10.3389/fped.2021.783754

Locoregional Control and Survival in Children, Adolescents, and Young Adults With Localized Head and Neck Alveolar Rhabdomyosarcoma—The French Experience

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Introduction: The head and neck (HN) are the most frequent sites of pediatric rhabdomyosarcoma (RMS). Alveolar RMS (ARMS) represents ∼20% of all RMS cases and frequently spread to lymph nodes (LNs). The aim was to report locoregional control, event-free survival (EFS), and overall survival (OS), according to clinical and pathological features, LN staging, and treatment modalities.

Methods: The study included all patients prospectively enrolled in EpSSG RMS 2005 study under 21 years of age with localized HN ARMS and diagnosed between 2005 and 2016 in France. Medical data including imaging, surgical report, and radiation therapy planes were analyzed.

Results: Forty-eight patients (median age 6 years; range 4 months−21 years), corresponding to 30 parameningeal and 18 non-parameningeal ARMS, were included. There were 33 boys (69%). Tumor locations included the following: orbit ($n = 7$) among which four cases had bone erosion, paranasal sinuses and nasal cavity ($n = 16$), deep facial spaces ($n = 10$), nasolabial fold ($n = 8$), and other non-parameningeal HN sites ($n = 7$). A fusion transcript of PAX3-FOXO1 or PAX7-FOXO1 was expressed in 33 of the

OPEN ACCESS

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Reviewed by:
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Specialty section:
This article was submitted to Pediatric Surgery, a section of the journal Frontiers in Pediatrics

Received: *26 September 2021*
Accepted: *23 November 2021*
Published: *04 February 2022*

Citation:
Machavoine R, Helfre S, Bernier V, Bolle S, Leseur J, Corradini N, Rome A, Defachelles A-S, Deneuve S, Bernard S, Fayoux P, Nicollas R, Mondain M, Luscan R, Denoyelle F, Simon F, Kadlub N, Kolb F, Honart J-F, Orbach D, Minard-Colin V, Moya-Plana A and Couloigner V (2022) Locoregional Control and Survival in Children, Adolescents, and Young Adults With Localized Head and Neck Alveolar Rhabdomyosarcoma—The French Experience. Front. Pediatr. 9:783754. doi: 10.3389/fped.2021.783754

45 cases (73%) with molecular analysis. At diagnosis, 10 patients had primary resection of the primary tumor (PRPT) (none with microscopic complete resection) and 9 had LN staging. After induction chemotherapy, 26 patients (54%) had secondary resection of the primary tumor (SRPT) and 13 patients (27%) had cervical LN dissection. A total of 43 patients (90%) were treated with radiation therapy.

With a median follow-up of 7 years (range 2–13 years), 5-year OS and EFS were 78% (95% CI, 63–88%) and 66% (95% CI, 51–78%), respectively. We observed 16 events (10 deaths): 4 local, 4 regional, 1 local and regional, and 7 metastatic. In univariate analysis, OS was only superior for patients under 10 years of age ($p = 0.002$), while *FOXO1*-negative ARMS, SRPT for parameningeal ARMS, and LN surgery were associated with significantly better EFS.

Conclusion: Our study confirms a better outcome for fusion-negative ARMS and ARMS in children under 10 years. Moreover, LN surgery and SRPT of parameningeal tumor may improve EFS of ARMS. Larger studies are needed to confirm our findings.

Keywords: alveolar rhabdomyosarcoma (ARMS), head and neck neoplasm, children, neck dissection, survival

INTRODUCTION

Rhabdomyosarcoma (RMS) represents about 2–5% of childhood and adolescent cancers (1, 2), with ∼40% arising in the head and the neck (3–5). Several RMS histologic subtypes can be distinguished. The two most prevalent ones are embryonal rhabdomyosarcoma (ERMS) that has an intermediate prognosis and alveolar rhabdomyosarcoma (ARMS) that represents about 20–25% of RMS and has a poorer prognosis (6–8). ARMS is proportionately more common than ERMS in children over 10 years old (9). More than 80% of ARMS express fusion transcripts (FTs) between the *FOXO1* and *PAX3* or *PAX7* genes (10, 11). These genetic *FOXO1* anomalies are associated with a poorer prognosis (12). The treatment of these high-risk tumors includes systemic chemotherapy associated with local treatment that may rely on surgery, radiotherapy, or a combination of both. ARMS spreads rapidly locally but also by lymphatic and hematogenous routes. The most frequent extension sites are lymph nodes (LNs), lungs, and bone marrow (13). About half of the patients treated for localized ARMS undergo a relapse (14). The 5-year overall survival (OS) of patients with head and neck ARMS (HN-ARMS) ranges from 35% (15) to 80% (16). To evaluate the prognosis value of clinical and pathological features and the impact on outcome of LN staging and locoregional therapies, we reviewed all patients <21 years with localized ARMS treated in France in the prospective EpSSG RMS 2005 study.

PATIENTS AND METHODS

Population

This multicenter study included all French patients prospectively enrolled in the EpSSG RMS 2005 protocol under 21 years of age with localized HN-ARMS diagnosed between 2005 and 2016 (17). Patient's consent or his/her legal representative's was collected. Analyses were performed on the data derived from EpSSG RMS 2005 study. Additional data, particularly those concerning modalities of LN staging, surgery, and radiotherapy, were retrieved from medical center files. Based on initial RMS2005 criteria, pathologists should consider ARMS diagnosis when tumor showed any focal alveolar pattern histology. However, since RMS2005 trial ran from 2005 to 2016, it was next recommended in the pathologist community to consider ARMS if alveolar pattern was predominant. The location of the primary tumor was determined by imaging at the time of diagnosis and classified into three sites: orbit, non-parameningeal (non-PM), and PM sites. For PM tumors, a cranial nerve palsy, a skull base erosion, an intracranial extension, and the presence of tumor cells in the cerebrospinal fluid (CSF) were systematically looked for at diagnosis.

Staging

Initial staging was established according to the TNM (18) and Intergroup Rhabdomyosarcoma Study Group (IRSG) (both surgical-pathologic grouping and staging systems) (19) classifications. LN involvement was assessed by initial computed tomography (CT), magnetic resonance imaging (MRI), or positron emission tomography–computed tomography (PET-CT). For ARMS, systematic LN evaluation was further recommended by cytological or pathological analysis of nodal samples. Any distant metastasis at the time of diagnosis was researched by technetium bone scan or PET CT, bone marrow biopsy, and aspiration.

Treatment

Treatment protocol EpSSG RMS 2005 has been previously reported (20). All localized ARMS were considered as high-risk RMS. For N0 ARMS, patients were randomized to receive either standard IVA (ifosfamide, vincristine, dactinomycin) or IVADo (ifosfamide, vincristine, dactinomycin, and doxorubicin)/IVA for a total of 9 courses. Patients with tumor in remission after 9 courses, surgery, and/or radiation therapy (RT), were randomly assigned to stop treatment or receive maintenance

chemotherapy [six 28-day cycles of intravenous (i.v.) vinorelbine and oral cyclophosphamide]. For N1 ARMS, patients received intensified induction chemotherapy (IVADo/IVA) and additional maintenance chemotherapy with systematic local treatment to primary and nodal sites. The total duration of chemotherapy was 50 weeks.

Surgical Strategy

In case of primary resection of the primary tumor (PRPT) (resection of the primary site of the tumor prior to any other treatment), the status of surgical margins was categorized from R0 to R2 (R0: macroscopically and microscopically complete resection; R1: microscopically incomplete resection; R2: macroscopically incomplete resection), and the quality of tumor resection was defined using the IRSG surgical-pathologic grouping system (20). When secondary surgery was performed after induction chemotherapy, 3 types of procedure were distinguished: extensive resection of the initial tumor extensions ("ghost surgery"), resection of the residual [both considered as secondary resection of the primary tumor (SRPM)], or exploration. As in the case of PRPT, the status of surgical margins was ranked from R0 to R2. The necessity of a reconstruction and its type (pedicled flap, free flap) and the transient or definitive need for a tracheostomy or gastrostomy were noted. Mutilating surgery was defined by the presence of a permanent postoperative cranial nerve paralysis and by the necessity of infratemporal fossa, maxillary or mandibular resection. Concerning LN surgery, 4 strategies were distinguished: sentinel LN biopsy (SLNB), suspicious node excision, LN sampling, and LN dissection. After pathological analysis, resected LNs were divided into healthy ones (pN0) and pathological ones (pN1).

Radiation Therapy

Different types of RT, brachytherapy or external radiation therapy [proton beam therapy (PBT) or intensity-modulated radiation therapy (IMRT)], were performed. RT target could concern the initial or residual tumor volume, LN chains, and the transit pathway from the tumor primary site to the nearest LN chain. In the RMS 2005 protocol, reduction of radiation dose for patients who underwent secondary surgery was not planned.

Statistics

Follow-up was defined as the time between diagnosis and the patient's last visit or death date. Relapse was defined by cancer recurrence after a period of complete remission: it could be local, regional (nodal), or metastatic, regardless of the initial T and N status. Progression was defined by tumor volume increase or the occurrence of new lesions during treatment. OS was defined as the time between diagnosis and date of last visit or death. Event-free survival (EFS) was defined as the time between diagnosis and occurrence of an event such as relapse, progression, or death from any cause. In case of RT, local or nodal relapses were defined as in-field, marginal, or out-of-field. Univariate analysis and correlation between two qualitative variables were estimated with chi-square test and Fisher's exact test. Survival curves were calculated by the Kaplan–Meier method. The 5-year OS and the 5-year event-free percent survival were expressed with their confidence interval. The log-rank test was used for univariate analysis of survival data. For all statistical tests used, results were considered significant for a $p \leq 0.05$. Data were analyzed using GraphPad Prism software (version 8.00 GraphPad Software, La Jolla, CA, USA; www.graphpad.com).

RESULTS

Patient Characteristics

Forty-eight patients diagnosed between 2005 and 2016 in France were included in the analysis. Median age was 6.2 years (range 4 months−20.3 years) (**Table 1**). Fifteen patients had a non-PM and 30 a PM HN-ARMS of which locations are detailed

TABLE 1 | Patient characteristics.

		n	%
Age			
	0–12 months	3	6.25%
	13 months−10 years	30	62.5%
	>10 years	15	31.25%
Sex			
	Female	15	31.25%
	Male	33	68.75%
Histology			
	ARMS	46	95.8%
	Solid ARMS	2	4.2%
PAX3/PAX7-FOXO1 FT expression			
	Yes	33	68.75%
	No	12	25%
	Investigation not done	3	6.25%
Tumor stage at diagnosis			
	T1	21	43.75%
	T2	27	56.25%
Tumor size at diagnosis			
	a <5 cm	25	52.08%
	b >5 cm	21	43.75%
	x: unavailable	2	4.17%
Nodal stage at diagnosis			
	N0	31	64.6%
	N1	17	35.4%
IRS group			
	I	0	0%
	IIa	3	6.2%
	IIb	0	0%
	IIc	0	0%
	IIIa	38	79.2%
	IIIb	7	14.6%
	IV	0	0%
Tumor location			
	Orbit	3	6.25%
	Non-parameningeal	15	31.25%
	Parameningeal	30	62.5%

ARMS, alveolar rhabdomyosarcoma.

in **Figure 1**. Three patients had an orbital tumor without bone erosion. Of the 30 patients with PM tumor, 21 (70%) had a skull base erosion, 6 (20%) had an intracranial tumor extension, and 10 (33.3%) had a cranial nerve palsy at the time of diagnosis. The tumor expressed a *PAX3/PAX7-FOXO1* FT in 33 patients over 45 tested (73%). Thirty-one patients were N0 (65%) and 17 N1 ARMS. There was no significant difference in LN status (N0 or N1) at diagnosis depending on initial tumor extension (T status) ($p = 0.544$).

Lymph Node Staging

PET-CT was performed for 34/48 patients (71%). Twenty-eight patients had negative LN on PET-CT. Among them, three had abnormal LN on CT and/or MRI. Among the 6 patients with abnormal LN fixation on PET-CT, 2 patients were considered N0 (1 pN0 after biopsy and 1 for whom LNs were not visualized on MRI and were not considered for LN staging). None of these 2 patients had LN relapse. The 4 other patients received a nodal treatment (radiotherapy only $n = 2$ or combination of LN dissection and RT $n = 2$). Among these last 4 patients, 3 were alive on first complete remission and 1 deceased after displaying a nodal relapse. Nine patients underwent initial cytological or pathological assessment of LN areas (3 because of abnormal LN on conventional imaging, 2 because of abnormal LN fixation on PET-CT, 1 because of anomalies on both conventional imaging and PET-CT; in the last 3 cases, LN cytological or pathological assessment was performed despite normal CT/MRI/PET-CT results): 4 underwent LN fine-needle aspiration (of which 3 were

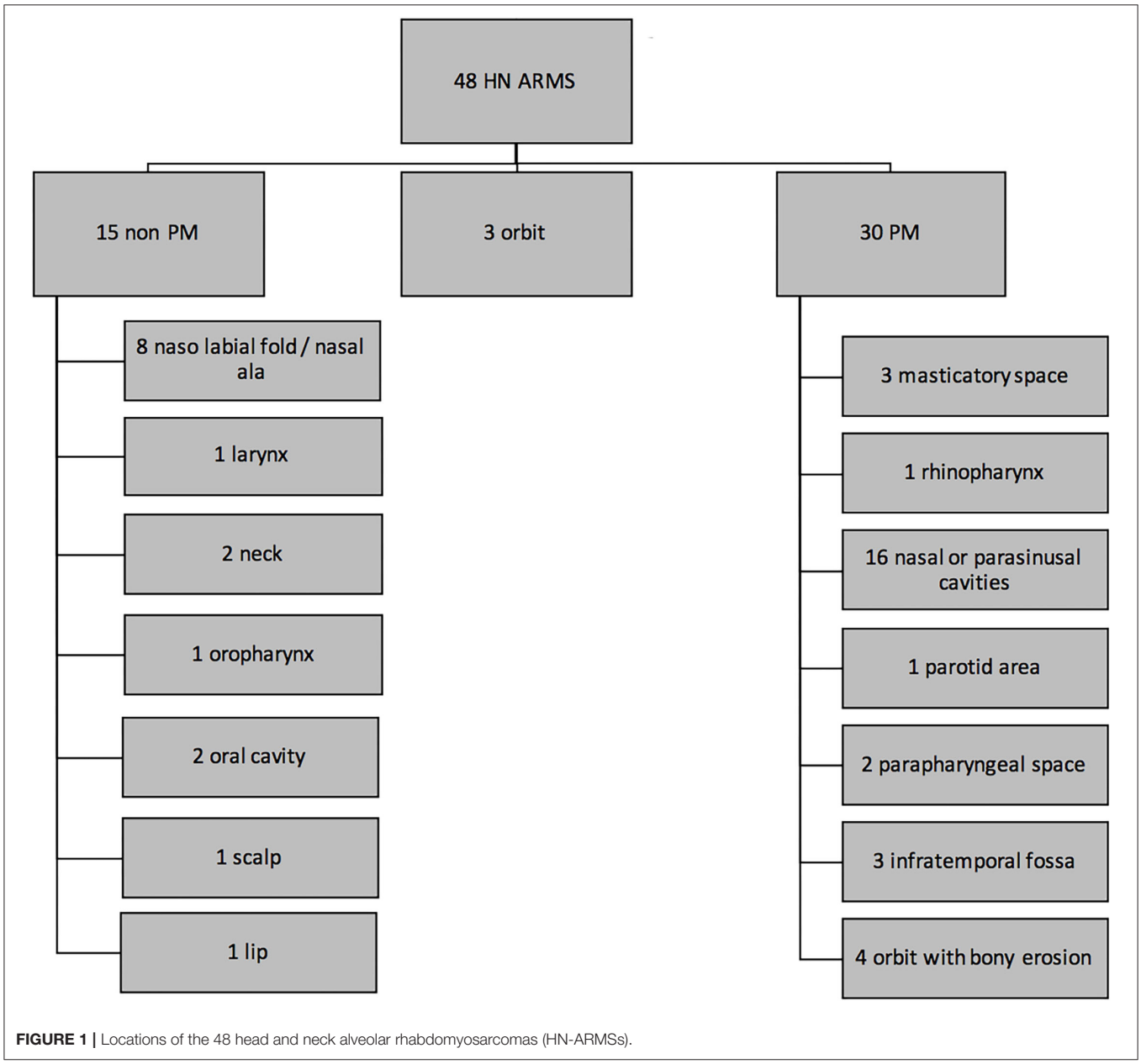


FIGURE 1 | Locations of the 48 head and neck alveolar rhabdomyosarcomas (HN-ARMSs).

pN1), 3 had LN biopsy (1 pN1), 1 had sentinel node biopsy (pN0), and 1 had LN dissection (pN1). Among the 17 patients classified as N1: 10 were on clinical evaluation and conventional imaging (CT and/or MRI), 2 were on PET/CT only, and 5 were confirmed by pathological examination of LN. There was no correlation between the initial N status and the performance of PET-CT at diagnosis ($p = 0.201$). The PET-CT positive predictive and negative predictive values for proven pathological nodal disease (pN1) or nodal relapse were 75 and 50%, respectively.

Response to Chemotherapy

After induction chemotherapy, out of the 42 patients with evaluable disease, 6 (14.3%) had a complete response, 10 (23.8%) had a very good partial response, 16 (38.1%) had a partial response, 6 (14.3%) had a minor partial response, 3 (7.1%) had tumor stability, and 1 (2.4%) had tumor progression.

Surgical Strategy

For 32 patients (67%), diagnosis of ARMS was made by surgical biopsy. For 4 patients, the diagnosis was made by ultrasound (US)-guided "tru-cut®" biopsy. PRPT was performed in 10 patients: 7 resections were macroscopically incomplete (R2) (70%) and 3 were microscopically incomplete (R1) (30%). For 1 patient, diagnosis was made on pathological analysis of an LN dissection. One diagnosis of ARMS was made on fine-needle aspiration.

Twenty-eight (58.3%) patients underwent secondary surgery after chemotherapy (**Figure 2**). Twenty-six were SRPT (10 non-PM, 16 PM): 24 had extensive surgery with the aim of removing initial tumor volume ("ghost surgery"), and 2 were limited to the residual mass. In 1 case of nasal sinus tumor, secondary surgery only consisted of surgical exploration with tumor mapping. In case of nasal ala ARMS, no tumor remnant was identified during surgery: in **Figure 2**, this case was also categorized as a surgical exploration. None of the 3 patients with localized orbital tumor underwent secondary surgery. The 26 SRPT were 2 total parotidectomies, 1 with sacrifice of the facial nerve and the other extended to the masseter and the zygomatic and malar region; 5 resections of the nasolabial fold extended to the nasal ala, cheek, upper lip, lower turbinate, and nasal bones, and in 4 cases extended to the maxillary bone at the level of the piriform aperture; 1 revision surgery in the anterior neck region with resection of the hyoid bone; 1 partial glossectomy; 1 revision of scalp resection; 1 labiectomy; 5 total maxillectomies; 2 external temporal fossa resections; 2 spheno-ethmoidal surgeries, one of which was limited to the excision of the residual tumor; 5 infratemporal fossa resections, two of which were extended to the parotid with 1 postoperative facial paralysis; 1 arytenoidectomy. Thirteen SRPTs were classified as mutilating according to the criteria as defined in the *Patients and Methods* section: 3 facial paralysis, one after parotidectomy with sacrifice of the facial nerve and two after laterally extended resection

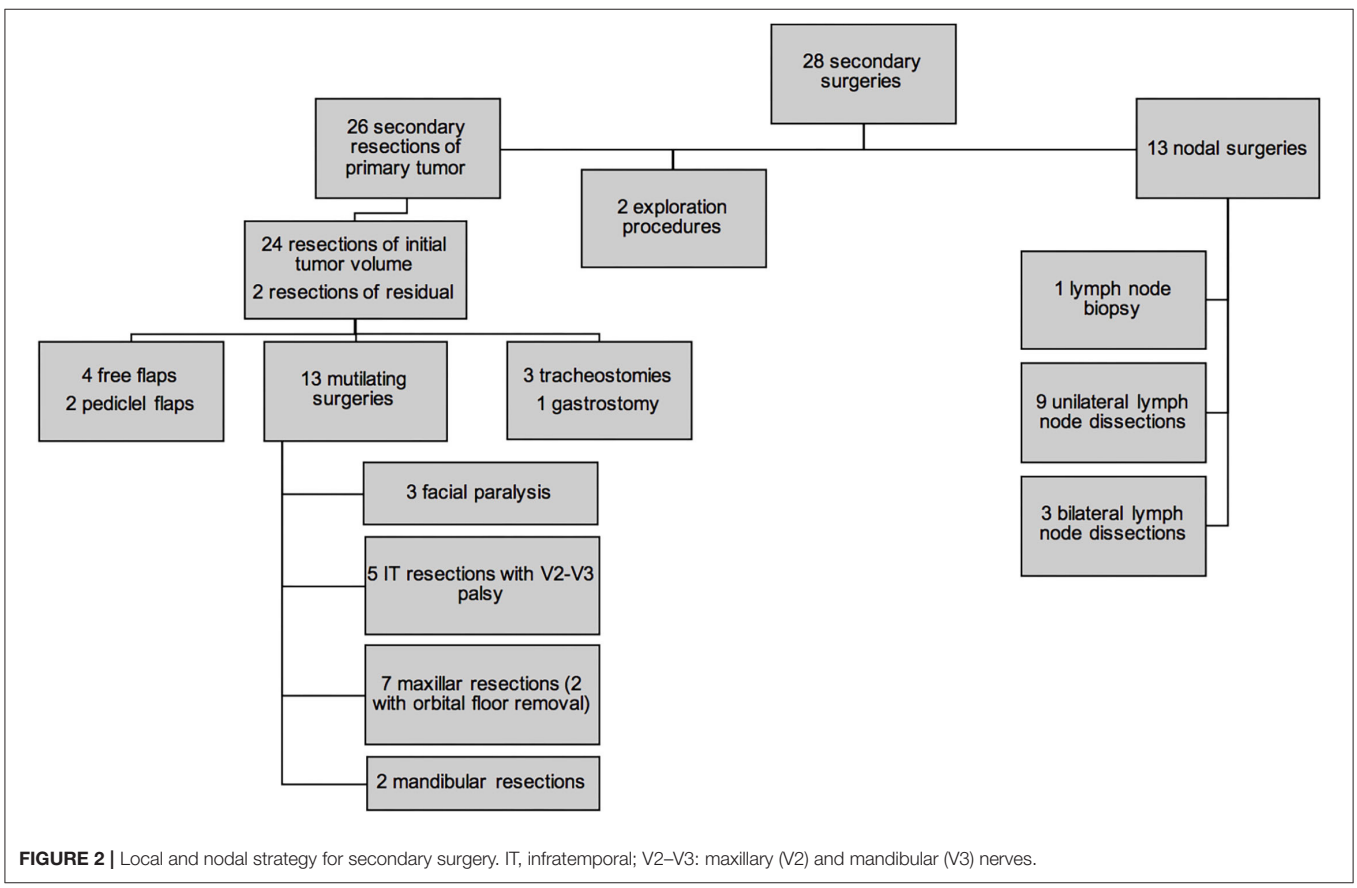


FIGURE 2 | Local and nodal strategy for secondary surgery. IT, infratemporal; V2–V3: maxillary (V2) and mandibular (V3) nerves.

of the infratemporal fossa; 5 infratemporal fossa resections and 7 maxillary resections with orbital floor removal in 2 cases. Four resections were reconstructed by free flap (2 *latissimus dorsi* muscle flap, 1 scapular dorsal flap, 1 thoracodorsal artery perforator free flap) and two by a pedicle flap (1 temporalis muscle flap and 1 Abbe flap). Four surgeries required a transient tracheotomy and 1 patient required a transient gastrostomy after arytenoidectomy due to choking. Of the 26 patients who underwent SRPT, 10 patients (38.5%) had microscopically negative margins (R0). For 14 patients (54%), the margins were microscopically positive (R1). In 2 cases (7.7%), the excision was macroscopically incomplete (R2). The residual lesions were located in the cavernous sinus and in the temporomandibular joint next to the resection margins in the other. There were more patients under 10 years of age who underwent an SRPT than patients older than 10 years of age ($p = 0.002$) (**Figure 3**). There was no difference between patients who underwent SRPT and patients who did not according to initial T and N status, tumor size, and tumor location.

Thirteen cervical LN surgeries were performed (7 N0 patients, 6 N1 patients): 9 unilateral and 3 bilateral LN dissections, and 1 single retropharyngeal LN resection. Among these 13 patients, 8 had a PM and 5 non-PM ARMS. None of the 3 patients with a tumor limited to the orbit had secondary LN surgery. In 4 patients (30.77%), histological analysis revealed LN metastases (3 PM and 1 non-PM ARMS). No N0 patient at the time of diagnosis was pN1. Among the 19 patients with pathological LN assessment (initial staging and/or secondary nodal surgery), 1 patient displayed an LN relapse vs. 4 patients among the 29 patients without LN assessment ($p = 0.635$).

Radiation Therapy

Forty-three patients (90%) had RT, including 42 external beam RT and 1 iridium-192 brachytherapy. Among the 42 patients treated with external beam RT, 33 received IMRT and 5 PBT. For 4 patients, the external RT technique was not specified. Five patients did not receive RT treatment due to their very young age and/or complete second surgery. Their median age was 14 ± 18 months (minimum 4 months; maximum 48 months). For the 42 patients treated with external RT, 25 irradiation fields targeted only the primary tumor site, whereas 16 fields included the primary tumor site and LN chains. For the patient who displayed a nodal progression after secondary surgery and before the end of chemotherapy, the irradiation field targeted only LN areas. Of the 41 irradiation fields targeting the primary tumor, 32 included the initial tumor volume and 5 concerned the residual tumor volume after chemotherapy and/or surgery. The median radiation dose of external RT was 50.4 Grays ± 4.5 (range 38.6–56) and 44.8 Grays ± 5.2 (range 41.4–54.4) for primary tumor site (data available $n = 38$) and nodes (data available $n = 15$), respectively.

Events

For alive patients, the median follow-up was 7.4 years (standard deviation 3.2 years; minimum 2.2 years; maximum 13.2 years). An event occurred in 16 patients (33.3%) (**Table 2**). All were related to disease failure: 3 patients had local relapse, 1 local and

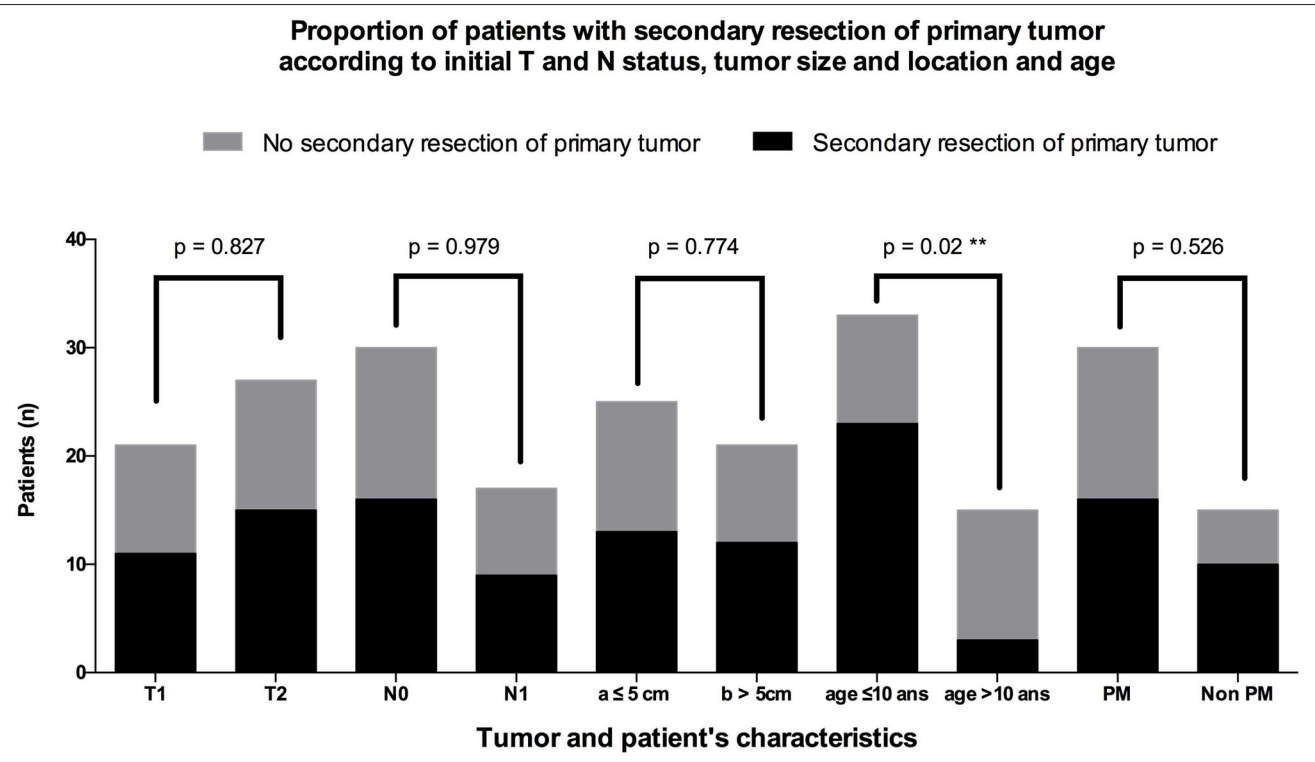


FIGURE 3 | Repartition of operated and non-operated patients according to the initial T and N status, tumor size and location, and patient age.

TABLE 2 | Details of events according to the tumor location.

	Events						
	Local relapses	**Nodal relapses**	**Local and nodal relapses**	**Metastatic relapses**	**Local and metastatic relapses**	**Progressions***	**All ($n = 16$)**
Orbit ($n = 3$)	0/3 (0%)	0/3 (0%)	0/3 (0%)	0/3 (0%)	0/3 (0%)	0/3 (0%)	0/3 (0%)
Non PM ($n = 15$)	0/15 (0%)	2/15 (13%)	1/15 (7%)	2/15 (13%)	1/15 (7%)	1/15 (7%)	7/15 (47%)
PM ($n = 30$)	3/30 (10%)	1/30 (3%)	0/30 (0%)	4/30 (13%)	0/30 (0%)	1/30 (3%)	9/30 (30%)
All ($n = 48$)	3/48 (6%)	3/48 (6%)	1/48 (2%)	6/48 (13%)	1/48 (2%)	2/48 (4%)	16/48 (33%)

*One local and one nodal progression.
PM, parameningeal.

LN relapse, 3 LN relapse, 6 metastatic relapse, and 1 local relapse associated with metastases. A local progression was observed in 1 patient and in another one who initially had no adenopathy on imaging (N0), LN metastases appeared during chemotherapy. No in-transit LN metastasis was observed. In total, local, regional LN, and metastatic relapse/progression represented 40, 31, and 44% of events, respectively. The median delay between diagnosis and event was 20 ± 12 months (range 7–45 months). Ten patients (62%) died, and the 6 other ones were disease-free at their most recent visit. Among the 10 patients who displayed a local or nodal event, 5 had a PM ARMS and 5 a non-PM ARMS (**Table 3**).

Among the 5 patients who had a nodal event either a relapse ($n = 4$) or LN progression ($n = 1$), 2 were N1 at diagnosis and 3 were N0; 4 had a PET-CT at diagnosis, one of which showed signs suggestive of LN metastasis. The 2 N0 patients who displayed a nodal relapse had undergone a PET-CT at diagnosis. One patient (7.7%) over the 13 who underwent a nodal secondary surgery presented with a nodal relapse vs. 4 (11.4%) over the 35 patients who did not. No patient who had a secondary nodal surgery presented with metastatic relapse compared to 7 patients out of 35 (20%) without secondary LN surgery. Radiation field targeted the primary tumor in 7 cases, both the primary tumor and LN chains in 2 cases, and only LN chains in 1 case (patient with nodal progression). There were 5 in-field relapses (one of which was probably due to patient's refusal to receive the full initially planned radiation dose), 1 nodal marginal relapse, and 1 nodal out-field relapse. Among the 5 patients who did not receive RT, 1 had a local and nodal relapse and another one died of a local and metastatic relapse.

Univariate Analysis of Relapses According to Initial Characteristics and Treatment

Of the 16 patients who displayed an event, none had an initial tumor localized to the orbit, 7 had a non-PM tumor, and 9 had a PM tumor (**Table 4**). The median age of patients with relapse was 10.2 ± 7.3 years (minimum 4 months; maximum 20.8 years). Of these 16 patients, 14 had a tumor expressing an FT, and for the other two, these data were not available. No patient whose tumor did not express FT had relapse or progression. Among the 16 tumors that relapsed, 6 were T1 and 10 were T2 at diagnosis; 11 were N0 and 5 were N1. In univariate analysis, age over 10 years and tumor expression of a PAX3/PAX7-FOXO1 FT were significant risk factors of event ($p = 0.048$ and $p = 0.004$, respectively).

The existence of LN surgery was the only therapeutic modality associated with a lower risk of event occurrence in univariate analysis (Fisher's test exact, $p = 0.036$). No patient who had a secondary nodal surgery presented with metastatic relapse compared to 7 patients out of 35 (20%) without secondary LN surgery. No relapse was observed in patients who underwent both secondary surgery and postoperative RT (**Figure 4**). The two N1 patients who underwent LN dissection without RT had no nodal relapse (**Figure 5**).

Survival Analysis

Thirty-eight patients (79.2%) were alive at the last visit date, and 10 patients (20.8%) were deceased. For alive patients, the median follow-up was 7.4 years (standard deviation 3.2 years; minimum 2.2 years; maximum 13.2 years). Of the 32 patients on first complete remission, 22 had at least 5 years of follow-up; median follow-up was 6.1 years (standard deviation 3.1 years; range 2.2–13.2 years). The 6 patients on second complete remission had at least 5 years of follow-up; median follow-up was 9.8 years (standard deviation 2.4 years; range 6.1–11.8 years). The 5-year OS was 78% [95% confidence interval (95% CI), 63–88%]. The 5-year EFS was 66% (95% CI, 51–78%) (**Figure 6**).

In univariate analysis, sex, tumor and nodal stage, size, IRS stage, initial tumor location, resection margins, and radiation therapy did not influence OS and EFS (**Table 5**). Patients under 10 years had better OS than patients older than 10 years ($p = 0.002$) and better EFS ($p = 0.050$) (**Figure 7**). The 5-year OS was 87% (95% CI, 69–95%) for patients under 10 years of age and 54% (95% CI, 32–72%) for patients older than 10 years. The 5-year EFS was 75% (95% CI, 56–87%) in those under 10 years of age and 47% (95% CI, 21–69%) in those older than 10 years. Patients with tumors expressing a PAX3/PAX7-FOXO1 FT had an OS of 75% (95% CI, 56–87%) vs. 100% for those not expressing it ($p = 0.071$) (**Figure 8**). Patients with FT-negative tumors had significantly better EFS than those expressing a FT with 100% 5-year EFS vs. 56% (95% CI, 38–72%) ($p = 0.011$).

For PM ARMS, the 5-year OS was 88% (95% CI, 59–97%) for patients who underwent SRPT vs. 63% (95% CI, 32–83%) in those who did not (**Figure 9**) ($p = 0.176$). The EFS was significantly higher ($p = 0.036$) in patients with PM ARMS who underwent SRPT compared to those who did not. The 5-year EFS in operated

TABLE 3 | Details of local and nodal treatments for the 10 patients who displayed an event.

Age (years and months)	Tumor location		TNM	Secondary surgery		Radiation therapy (RT)		Event	Relapse location in relationship with RT field	Last status
				Local (margins)	Nodal (pN)	Local RT	Nodal RT			
3y 2m	Non-PM	Arytenoid	T2N0	Initial tumor resection (R1)	–	Initial tumor volume	–	Nodal relapse	Out-field	Complete remission
8m	Non-PM	Nasal ala	T1N0	Initial tumor resection (R1)	–	–	–	Local and nodal relapse	–	Complete remission
7y 4m	Non-PM	Nasal ala	T1N1	–	Unilateral lymph node dissection (pN1)	Initial tumor volume	Unilateral cervical	Nodal relapse	Marginal	Dead
13y	PM	Maxillary sinus	T2N0	–	–	Initial tumor volume	–	Local relapse	In-field	Dead
1y 9m	PM	Orbit with bony erosion	T2N0	–	–	Initial tumor volume	–	Local relapse	In-field	Complete remission
19y 7m	PM	Maxillary sinus	T2N0	–	–	Residual tumor volume	–	Local relapse	In-field	Complete remission
4m	Non-PM	Retroauricular scalp	T1N0	Initial tumor resection (R0)	–	–	–	Local and metastatic relapse	–	Dead
3y 10m	Non-PM	Upper lip	T1N0	Initial tumor resection (R1)	–	–	Unilateral cervical (after nodal progression)	Nodal progression (before end of induction chemotherapy)	Nodal progression before RT (no local or nodal relapse after RT)	Complete remission
13y 4m	PM	Ethmoidal sinus	T2N1	–	–	Initial tumor volume	Unilateral cervical and retropharyngeal	Nodal relapse	In-field	Dead
20y 9m	PM	Orbit with bony erosion	T2N0	–	–	Initial tumor volume	–	Local progression	In-field (patient refused to receive the initially planned radiation dose)	Dead

PM, parameningeal; RT, radiation therapy.

TABLE 4 | Univariate analysis of events according to patients and tumor characteristics and treatment for the 48 HN-ARMSs.

		n	%	Complete remission without event	Event	*p*
Age						**0.048**
	0–10 years	33	6.25%	25	8	
	>10 years	15	31.25%	7	8	
Sex						1.000
	Female	15	31.25%	10	5	
	Male	33	68.75%	22	11	
Histology						0.546
	ARMS	46	95.83%	30	16	
	Solid ARMS	2	4.17%	2	0	
PAX3/PAX7-FOXO1 FT expression						**0.004**
	Yes	33	68.75%	19	14	
	No	12	25.00%	12	0	
	Investigation not done	3	6.25%	1	2	
Tumor stage at diagnosis						0.537
	T1	21	43.75%	15	6	
	T2	27	56.25%	17	10	
Size at diagnosis						0.685
	a <5 cm	25	52.08%	17	8	
	B >5 cm	21	43.75%	13	8	
	x: unavailable	2	4.17%	2	0	
Nodal stage at diagnosis						0.770
	N0	31	64.58%	20	11	
	N1	17	35.42%	12	5	
IRS group						0.849
	I	0	0%			
	IIa	3	6.25%	2	1	
	IIb	0	0%			
	IIc	0	0%			
	IIIa	38	79.17%	26	12	
	IIIb	7	14.58%	4	3	
	IV	0	0%			
Tumor location						0.279
	Orbit	3	6.25%	3	0	
	Non-parameningeal	15	31.25%	8	7	
	Parameningeal	30	62.5%	21	9	
Aggressiveness patterns for PM tumors ($n = 30$)						
	Cranial nerve palsy	10	33.33%	7	3	1.000
	Skull base erosion	21	70%	15	6	1.000
	Intracranial extension	6	20%	4	2	1.000
Secondary resection of primary tumor						0.306
	Yes	26	54.17%	19	7	
	No	22	45.83%	13	9	
Nodal secondary surgery						0.036
	Yes	13	27.08%	12	1	
	No	35	72.92%	20	15	
Radiation therapy						1.000
	Yes	43	89.58%	29	14	
	No	5	10.42%	3	2	

ARMS, alveolar rhabdomyosarcoma; TF, fusion transcript; PM, parameningeal. Bold text indicates a statistically significant difference.

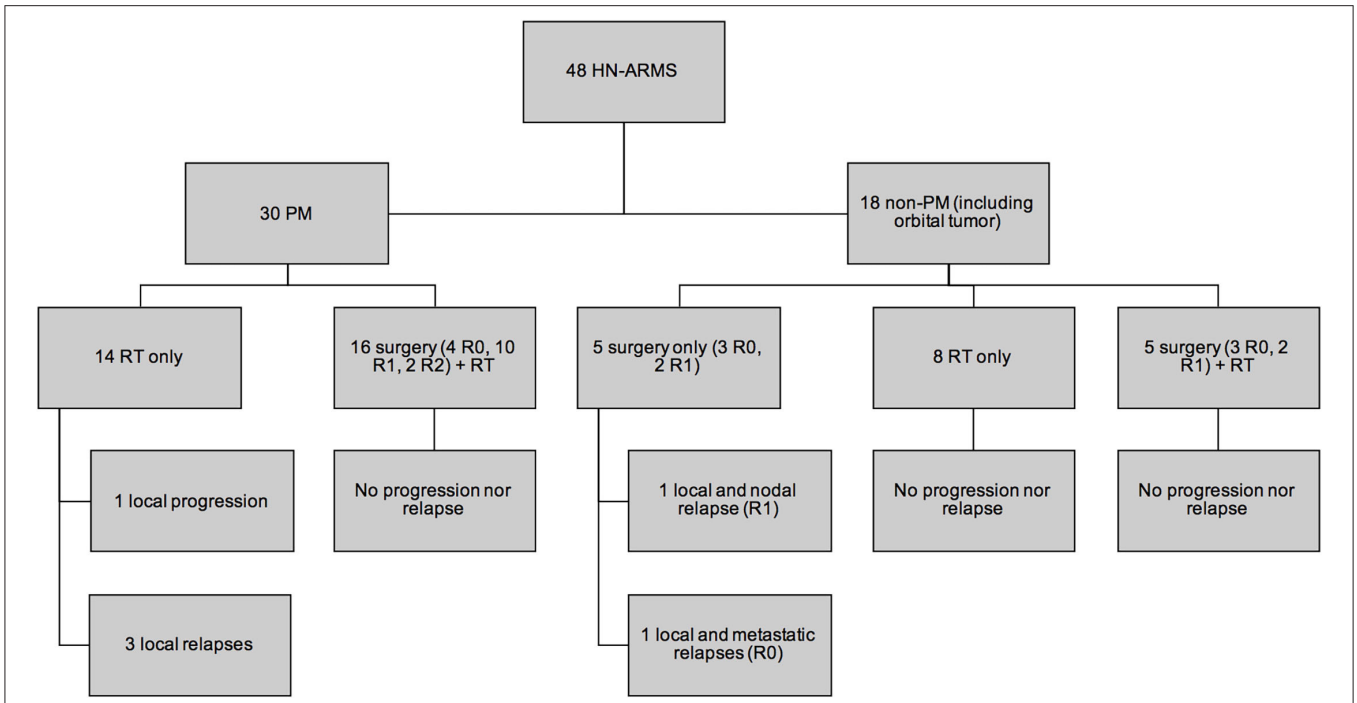


FIGURE 4 | Local control according to the local treatments (surgery and radiation therapy).

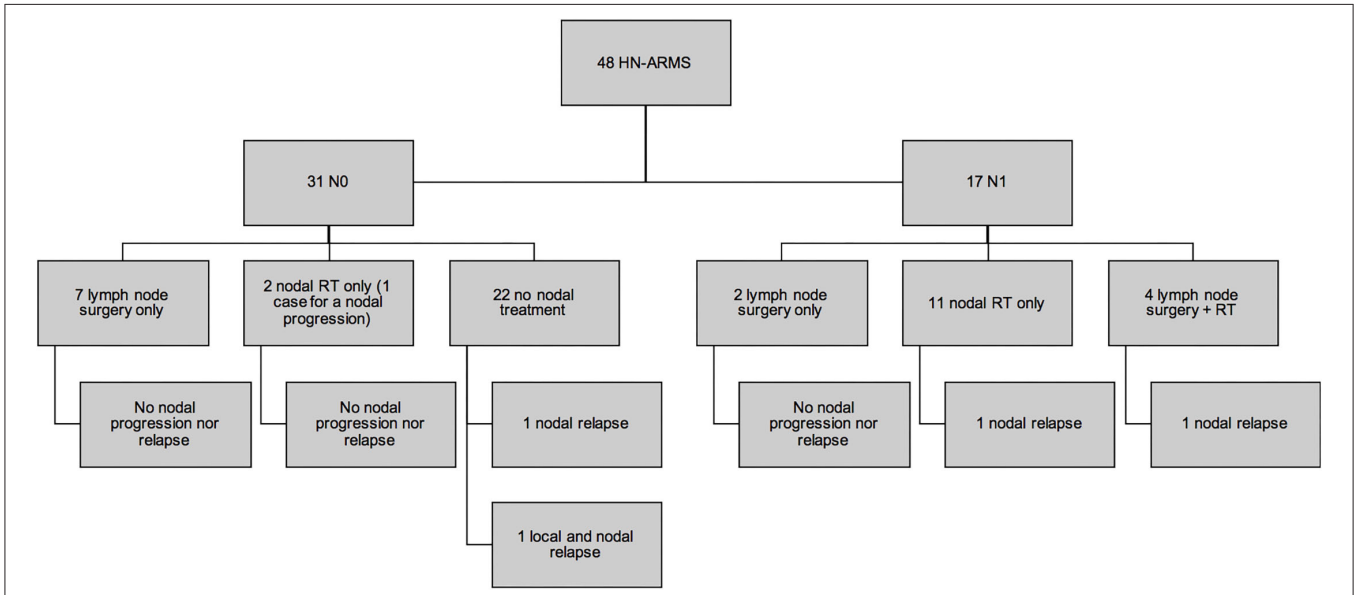


FIGURE 5 | Nodal control according to the lymph node chain treatments (surgery and radiation therapy).

PM tumors was 88% (95% CI, 59–97%) vs. 50% (95% CI, 23–72%) in non-operated patients. The 5-year OS was 92% (95% CI, 57–99%) for patients who underwent LN surgery vs. 73% (95% CI, 54–85%) in those who did not ($p = 0.210$) (**Figure 10**). EFS was significantly higher for patients who underwent LN surgery with 5-year EFS at 92% (95% CI, 57–99%) compared to those who did not with 5-year EFS at 56% (95% CI, 38–71%) ($p = 0.034$).

DISCUSSION

RMSs, although rare in the general population, are among the most common tumors in children. The alveolar histological subtype is associated with a poorer prognosis that justifies a high burden of therapy. Surgery is theoretically reserved for patients with resectable negative margin

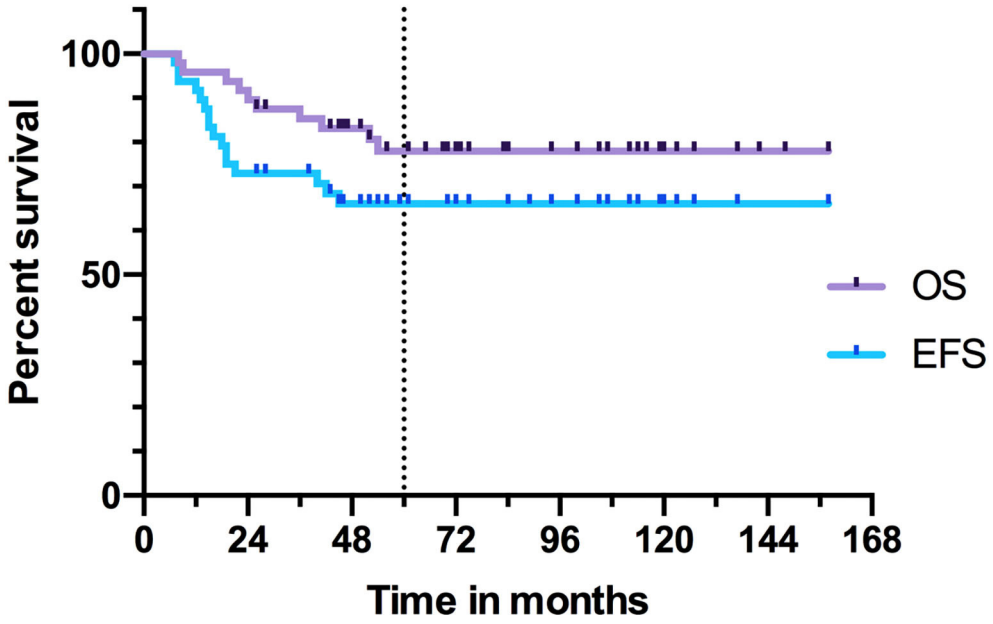


FIGURE 6 | Overall survival and event-free survival of the 48 patients with head and neck alveolar rhabdomyosarcoma (HN-ARMS).

tumor and remains a carcinological, functional, and aesthetic challenge.

Characteristics of the Population and of the Tumors

Characteristics of our population are comparable to those of Ludmir et al. (6) and Radzikowska et al. (21) series (with 14 pediatric HN-ARMS and 36 pediatric HN-RMS cases, respectively) concerning the sex ratio (respective percentages of boys in these 3 series: 69, 64, 67%) and for the median age (respective values for these 3 series: 6, 7, and 7 years). Initial tumor location was PM in 62.5% of the cases in our series, which is comparable to the 57% observed by Ludmir et al. (6) and to the 67% observed by Radzikowska et al. (21). Intracranial extension of PM ARMS was present in 20% of our cases and in 14.3% of cases in the series by Ludmir et al. (6). The percentage of tumors expressing a PAX3-PAX7/FOXO1 FT was 68.75% in our cohort, which is similar to the 67% observed by Bradley et al. (22) in a series of 24 pediatric PM ARMS. The proportion of LN involvement at diagnosis (N1) was slightly lower in our study (35.4%) compared to that (42.9%) of Ludmir et al. This difference could be explained by the exclusion from our study of patients with metastatic disease at the time of diagnosis. Initial assessment is essential, in particular, to determine the nodal extension of the disease. Several studies suggest the benefit of performing a PET-CT at the time of diagnosis given its high sensitivity and specificity (23–26). However, at variance with these studies, and in accordance with the publication of Ludmir et al. (6), we did not observe any association between the inclusion of a PET-CT in the diagnostic workup and patients' initial N status.

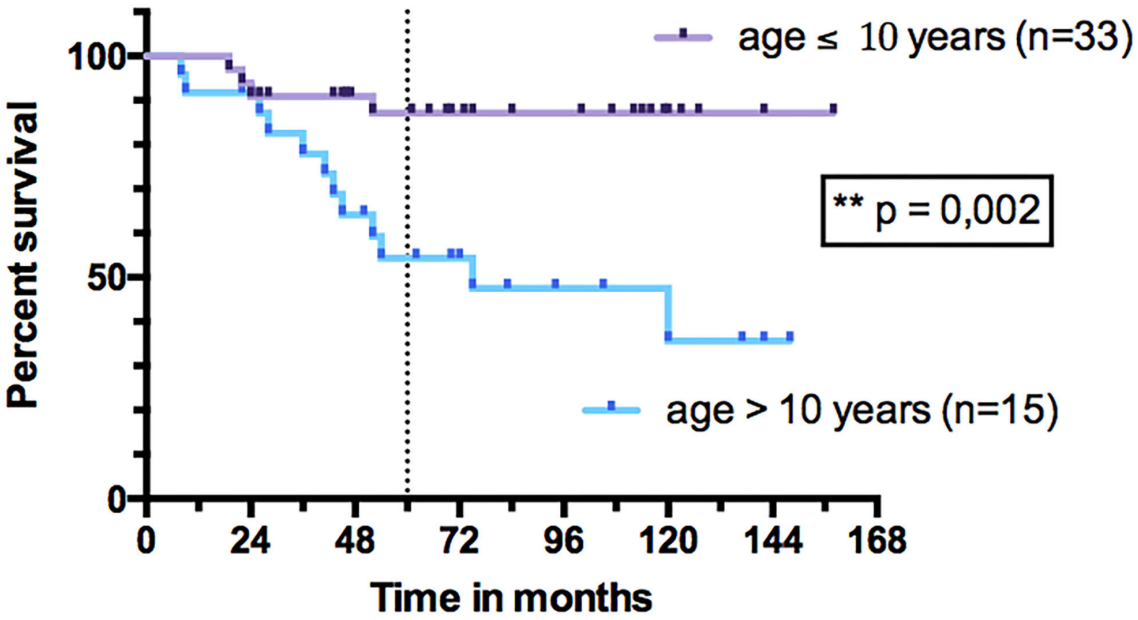
Survival and Prognostic Factors

The 5-year OS and EFS of our series (respectively, 78 and 66%) appear to be higher than those observed by Dantonello et al. (27) in a series of 235 pediatric ARMS of any location (58 and 47%, respectively). This difference could suggest a better prognosis for HN-ARMS compared to other locations. Our 5-year OS and EFS are higher than the series of 14 pediatric HN-ARMS (respectively, 45 and 25%) of Ludmir et al. (6). This could be explained by the higher percentage of FT-negative tumor in our series (25%) compared to that (14%) of Ludmir et al. Indeed, in univariate analysis, tumor expression of a PAX3/PAX7-FOXO1 FT was a significant risk factor for relapse ($p = 0.004$). This is consistent with other large series, which suggest using the FT as a prognostic factor to reassign FT-negative patients to a lower treatment group. Children under 10 years of age had a better prognosis in our series, with 5-year OS and EFS of 87 and 75%, respectively. In univariate analysis, age over 10 years was an event risk factor ($p = 0.048$). This observation was also made by several authors for RMS of any location (27, 28) and more specifically for HN-ARMS (15). Considering these risk factors, therapeutic burden may be adapted—with less systematic radiotherapy in case of adequate and complete SPRT performed by a referent surgical team—for patients under 10 years of age. In a series of 140 localized non-PM RMS including 40 RMSA from 1984 to 2004, Orbach et al. (29) observed a 5-year OS of 66% and a 5-year

TABLE 5 | Univariate analysis of 5-year OS and EFS according to patients and tumors' characteristics and treatment modalities for the 48 HN-ARMSs.

Univariate analysis		5-year overall survival (CI)	*p*-value	5-year event-free survival (CI)	*p*-value
Sex			0.988		0.993
	Male	78% (59–89%)		66% (48–80%)	
	Female	79% (48–93%)		64% (33–84%)	
Age (years)			0.002		**0.050**
	≤10	87% (69–95%)		75% (56–87%)	
	>10	54% (32–72%)		47% (21–69%)	
Tumor stage			0.352		0.580
	T1	84% (59–95%)		70% (44–85%)	
	T2	73% (51–86%)		63% (42–78%)	
Nodal stage			0.701		0.702
	N0	80% (60–90%)		64% (44–78%)	
	N1	75% (46–90%)		70% (42–86%)	
Size (cm)			0.283		0.667
	a ≤5	81% (57–93%)		66% (43–82%)	
	b >5	71% (47–86%)		62% (38–79%)	
IRS stage			0.291		0.801
	IIa	100%		67% (5–95%)	
	IIIa	81% (65–91%)		68% (50–80%)	
	IIIb	51% (12–81%)		57% (17–84%)	
Tumor location			0.622		0.532
	Non-PM (including orbital)	82% (54–94%)		58% (31–77%)	
	PM	76% (56–88%)		70% (50–83%)	
Local aggressiveness for PM			0.809		0.961
	Skull base erosion	76% (52–89%)		71% (47–86%)	
	No skull base erosion	76% (33–94%)		67% (28–88%)	
			0.413		0.661
	Intracranial extension	67% (19–90%)		67% (19–90%)	
	No intracranial extension	78% (54–90%)		71% (48–85%)	
			0.452		0.797
	Cranial nerve palsy	70% (33–89%)		70% (33–89%)	
	No cranial nerve palsy	78% (52–91%)		70% (45–85%)	
FT expression			0.071		**0.011**
	Yes	75% (56–87%)		56% (38–72%)	
	No	100%		100%	
Secondary resection of primary tumor			0.348		0.323
	Yes	84% (62–94%)		72% (51–86%)	
	No	71% (47–86%)		58% (35–76%)	
			0.176		**0.036**
	PM ARMS operated	88% (59–97%)		88% (59–97%)	
	PM ARMS non-operated	63% (32–83%)		50% (23–72%)	
			0.711		0.281
	Non-PM ARMS operated	77% (34–94%)		44% (12–73%)	
	Non-PM ARMS unoperated	88% (39–98%)		73% (28–93%)	
Resection margins			0.277		0.696
	R0	67% (27–88%)		70% (33–89%)	
	R1	93% (59–99%)		71% (41–88%)	
	R2	100%		100%	
Nodal secondary surgery			0.210		**0.034**
	Yes	92% (57–99%)		92% (57–99%)	
	No	73% (54–85%)		56% (38–71%)	
RT			0.958		0.768
	Yes	78% (62–88%)		67% (51–79%)	
	No	75% (13–96%)		53% (7–86%)	

CI, confidence interval; PM, parameningeal; ARMS, alveolar rhabdomyosarcoma; FT, fusion transcript; RT, radiation therapy. Bold text indicates a statistically significant difference.



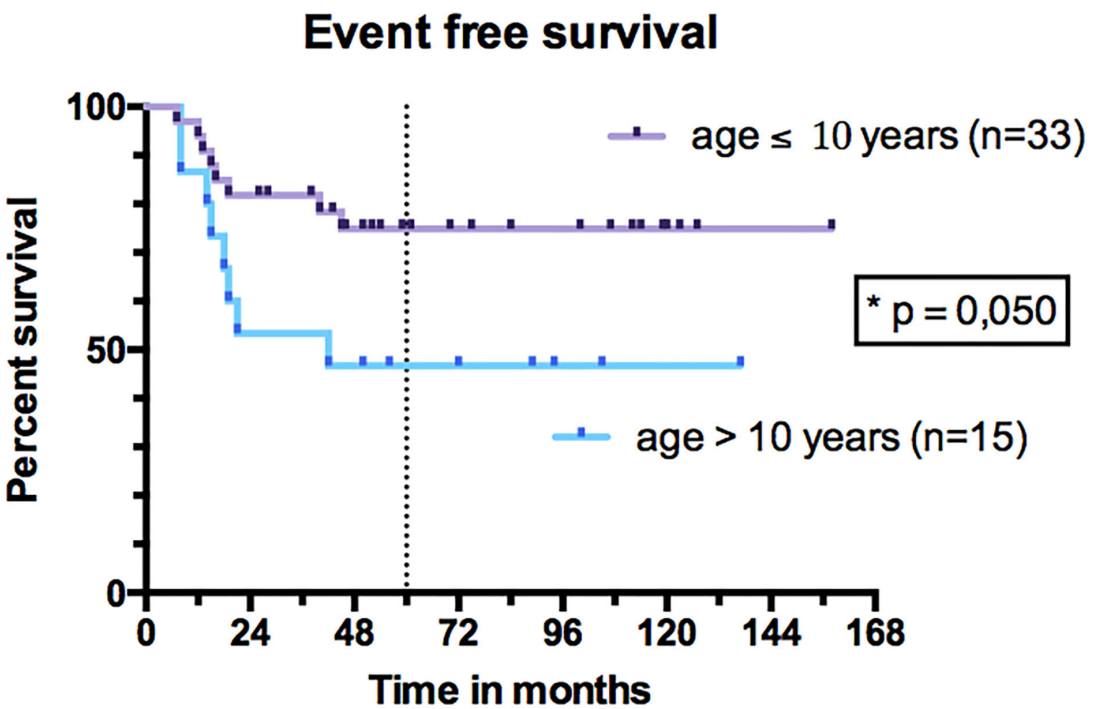
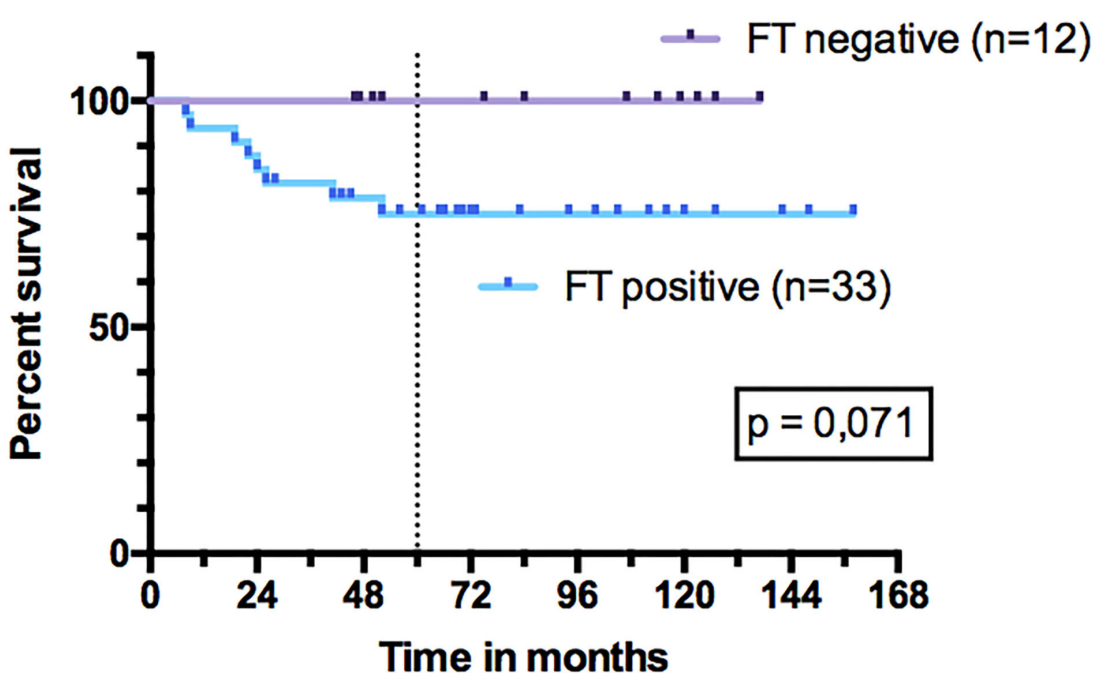


FIGURE 7 | Overall survival and event-free survival of patients under 10 years and patients over 10 years.



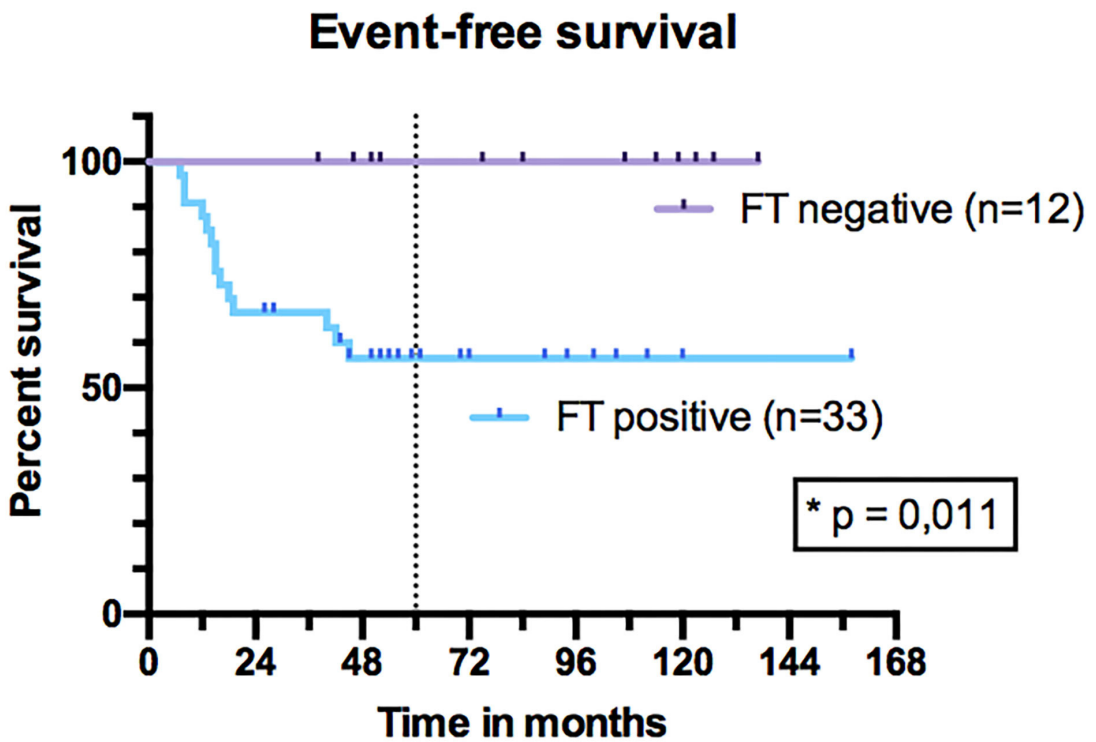


FIGURE 8 | Overall survival and event-free survival of patients with fusion transcript (FT) expression and without FT expression.

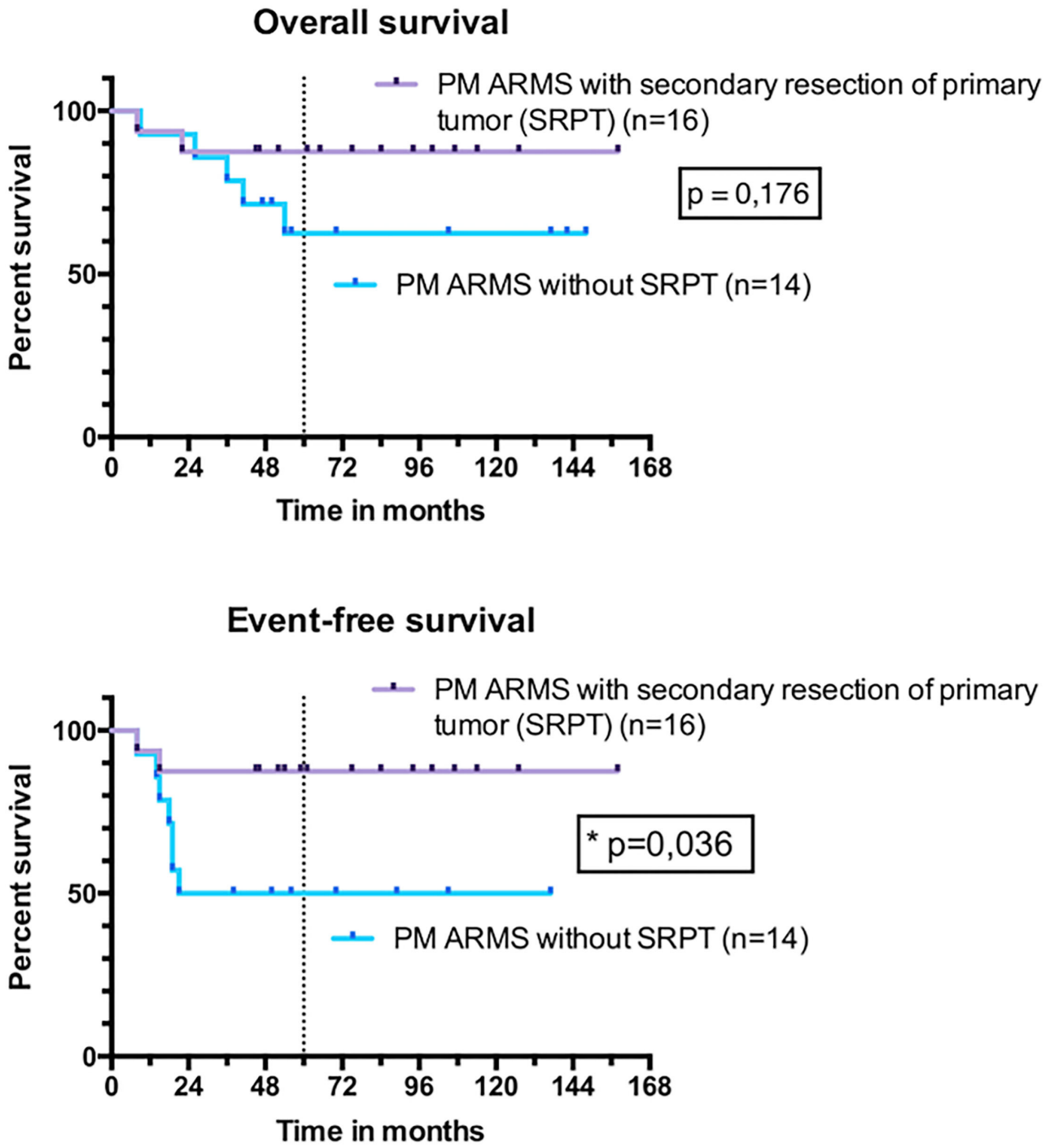


FIGURE 9 | Overall survival and event-free survival of patients with parameningeal alveolar rhabdomyosarcoma (PM ARMS) who had undergone a secondary resection of primary tumor and patients who did not.

EFS of 51%. This difference with the OS and EFS observed in our series seems all the more significant as the one by Orbach et al. (29) includes a majority of ERMSs that have a better prognosis than ARMS. Neither can this difference be explained by patients' ages [median age 5 years in the series by Orbach et al. (29) and 6.2 years in ours] or by the percentages of operated patients (64 and 54%, respectively). These differences in OS and EFS could be linked to the improvement in the quality and the tolerance of the

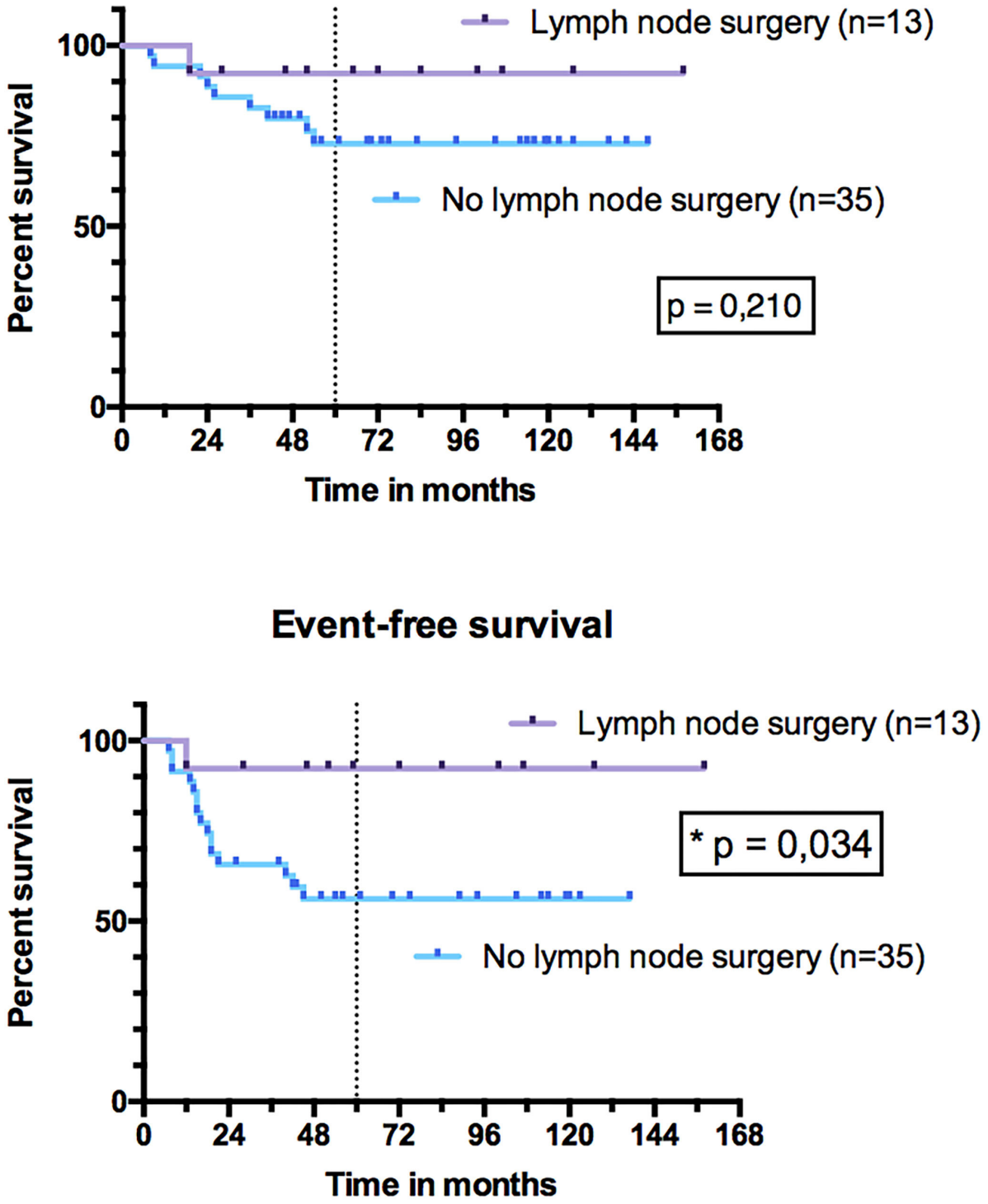


FIGURE 10 | Overall survival and event-free survival of patients with head and neck alveolar rhabdomyosarcoma (HN-ARMS) who underwent a lymph node dissection compared to those who did not.

systemic treatment, as well as to a greater efficiency of the local and regional treatment with conformational and proton beam radiation therapy and the development of free flaps, allowing large surgical resections for "ghost surgeries." Several authors suggest that OS and EFS are better in patients who underwent surgical resection in addition to treatment with chemotherapy and RT (15, 30–32). Multidisciplinary approach including "ghost surgery" for PM sarcoma is feasible and yields promising local control. This strategy may help to avoid RT or limit the RT field for young children and improve local control for these unfavorable PM sites (33). Indeed, with RT only, PM RMSs have bad outcomes, with survival of only about 60% in the more recent studies, which has to be warranted by future studies. For the 30 PM-ARMS of our series, the EFS was significantly better after SRPT ($p = 0.036$). This difference in favor of surgery was not observed for non-PM tumors, nor for all locations combined, nor for OS. Patients under 10 years of age, with better prognosis, were significantly more operated on ($p = 0.002$) in the hope of avoiding radiation therapy in some younger children. This might partly explain the difference in favor of surgery. In our series, most primary surgeries were performed without respecting the usual oncologic surgery principles (safety resection margins), explaining that none of the patients were IRS I. However, there was no significant difference in OS and EFS as a function of the IRS group ($p = 0.291$ and $p = 0.801$, respectively). Despite this absence of correlation between incomplete PRPT and events, PRPT is not indicated in the management of HN-ARMS if a negative margin resection is not possible because it compromises the evolution assessment.

In our series, 4 resections needed a free flap reconstruction, most often requiring a multidisciplinary team specialized in this kind of complex surgical procedure, and 13 SRPTs were mutilating (3 facial paralysis, 5 infratemporal fossa resections, 7 maxillary resections, and 2 mandibular resections). The large proportion of patients who underwent SRPT (54%) as a core of initial therapy can be explained by improvements in perioperative management as well as surgical techniques in children in the last few years. Postoperative morbidity has also been reduced—thanks to the development of combined minimally invasive endoscopic and transcranial or transfacial approaches and free-flap reconstructive possibilities, which limit functional and cosmetic sequelae (34–36). In the retrospective study of 92 HN-RMS (both ERMS and ARMS) by Dombrowski et al. (16), surgery was associated with a reduced risk of mortality after adjusting for TNM staging and location of the tumor ($p = 0.05$). Furthermore, in our series, the quality of the resection assessed by the resection margins (R0, R1, or R2) was not correlated with OS and EFS. Yunteng et al. (37) also made this observation on his series of 51 HN-RMS ($p = 0.86$). This might be due to the fact that RT, which is proposed in most cases after surgery, helps control any postoperative tumor remnant, especially in anatomical areas where it is impossible to operate with safe resection margins such as cavernous sinus or skull base foramina. In our series, only 5 patients did not receive RT because of long-term sequelae of radiation for very young children.

In our series, performing LN dissection was associated with better EFS ($p = 0.034$). However, patients who underwent an LN secondary surgery had a lower rate of LN relapse (7.7%) than those who did not (11.4%), but the difference concerned more metastatic relapses (0% in case of LN secondary surgery and 20% in the absence of it). We suggest that LN dissection does not increase so much the burden of therapy, especially if it is carried out at the same time as secondary surgery of the primary tumor. More importantly, in the future, negative neck dissection (pN0) could justify to not perform radiation therapy on LN areas. In their series of 14 HN-ARMSs, Ludmir et al. (6) observed 57% of LN relapses vs. 8.3% in our series. These two series are mainly different in terms of rates of N1 patients at diagnosis, rates of FT-positive tumors as described before, and therapeutic strategy [SRPT for 26 patients in our series compared to medical therapy with chemotherapy and RT in the series by Ludmir et al. (6)], which could explain the difference in LN relapse rates. No difference in OS and EFS was brought out according to RT. This could be explained by the small size of our cohort and the low number of patients who did not receive RT. Indeed, in a meta-analysis including 1,105 PM RMSs, Merks et al. (38) found a poorer 10-year OS for non-irradiated patients (40.8%) than for irradiated patients (68.5%).

Limitations of the Study

Although our population is histologically homogeneous, the proportion of tumors not expressing PAX3/PAX7-FOXO1 fusion (25%) may be a bias for the analysis of ARMS survival. Indeed, it is now well-recognized that about 20–25% of ARMSs do not express FOXO1 fusion conferring specific clinic and biologic characteristics with inferior outcomes (39). Despite the difference of prognosis of these 2 molecular subtypes, FT-positive and FT-negative tumors, our cohort reflects the real-life experience in France. Moreover, the RMS2005 study recommended RMS prognostic stratification and therapeutic decision based on histology only. Of course, in the current era, FOXO1 fusion instead of histology is used even if a minority of tumors are still histologically classified as "true" ARMS lacking the canonical PAX-FOXO1 fusion but had new molecular alterations (40). In addition, the small size of the population ($n = 48$ patients) limits the statistical test power. Diversity of locations in HN region and of therapeutic strategies complicates the results' interpretation. Finally, the morbidity of the various treatments has not been exhaustively studied and could have been underevaluated. In conclusion, management of HN-ARMS is an oncological, radiotherapeutic, and surgical challenge. The initial staging is essential to best adapt the systemic and locoregional treatment. SRPT could improve the EFS of PM tumors, and LN surgery could improve EFS. SRPT must respect the principles of oncologic surgery while limiting mutilating procedures in order to preserve the quality of life of patients. Further larger international analyses of HN ARMS, including not only pediatric oncologists but also HN surgeons and RT physicians, are needed to confirm these findings.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary

material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

All participating Centres were required to obtain written approval from their local authorities and Ethical Committees, as well as written informed consent from patients or their parents or legal guardians.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

REFERENCES

1. Brady A-C, Rao KA, Lane R, Garvin L, Sola JE, Perez EA. Increased lymph node ratio predicts poor survival in pediatric rhabdomyosarcoma. *J Pediatr Surg*. (2019) 55:369–75. doi: 10.1016/j.jpedsurg.2019.09.006
2. Leiner J, Le Loarer F. The current landscape of rhabdomyosarcomas: an update. *Virchows Arch*. (2020) 476:97–108. doi: 10.1007/s00428-019-02676-9
3. Selfe J, Olmos D, Al-Saadi R, Thway K, Chisholm J, Kelsey A, et al. Impact of fusion gene status versus histology on risk-stratification for rhabdomyosarcoma: retrospective analyses of patients on UK trials. *Pediatr Blood Cancer*. (2017) 64:10.1002/pbc.26386. doi: 10.1002/pbc.26386
4. Dantonello TM, Stark M, Timmermann B, Fuchs J, Selle B, Linderkamp C, et al. Tumour volume reduction after neoadjuvant chemotherapy impacts outcome in localised embryonal rhabdomyosarcoma: response in embryonal rhabdomyosarcoma. *Pediatr Blood Cancer*. (2015) 62:16–23. doi: 10.1002/pbc.25207
5. Dziuba I, Kurzawa P, Dopierała M, Larque AB, Januszkiewicz-Lewandowska D. Rhabdomyosarcoma in children - current pathologic and molecular classification. *Pol J Pathol*. (2018) 69:20–32. doi: 10.5114/pjp.2018.75333
6. Ludmir EB, Paulino AC, Grosshans DR, McAleer MF, McGovern SL, Huh WW, et al. Regional nodal control for head and neck alveolar rhabdomyosarcoma. *Int J Radiat Oncol Biol Phys*. (2018) 101:169–76. doi: 10.1016/j.ijrobp.2018.01.052
7. McCarville MB, Christie R, Daw NC, Spunt SL, Kaste SC. PET/CT in the evaluation of childhood sarcomas. *Am J Roentgenol*. (2005) 184:1293–304. doi: 10.2214/ajr.184.4.01841293
8. Arnold MA, Anderson JR, Gastier-Foster JM, Barr FG, Skapek SX, Hawkins DS, et al. Histology, fusion status, and outcome in alveolar rhabdomyosarcoma with low-risk clinical features: a report from the children's oncology group. *Pediatr Blood Cancer*. (2016) 63:634–9. doi: 10.1002/pbc.25862
9. Skapek SX, Ferrari A, Gupta AA, Lupo PJ, Butler E, Shipley J, et al. Rhabdomyosarcoma. *Nat Rev Dis Primer*. (2019) 5:1. doi: 10.1038/s41572-018-0051-2
10. Fredericks WJ, Galili N, Mukhopadhyay S, Rovera G, Bennicelli J, Barr FG, et al. The PAX3-FKHR fusion protein created by the t(2;13) translocation in alveolar rhabdomyosarcomas is a more potent transcriptional activator than PAX3. *Mol Cell Biol*. (1995) 15:1522–35. doi: 10.1128/MCB.15.3.1522
11. Davis RJ, D'Cruz CM, Lovell MA, Biegel JA, Barr FG. Fusion of PAX7 to FKHR by the variant t(1;13)(p36;q14) translocation in alveolar rhabdomyosarcoma. *Cancer Res*. (1994) 54:2869–72.
12. Wachtel M, Dettling M, Koscielniak E, Stegmaier S, Treuner J, Simon-Klingenstein K, et al. Gene expression signatures identify rhabdomyosarcoma subtypes and detect a novel t(2;2)(q35;p23) translocation fusing PAX3 to NCOA1. *Cancer Res*. (2004) 64:5539–45. doi: 10.1158/0008-5472.CAN-04-0844
13. Kim JR, Yoon HM, Koh K-N, Jung AY, Cho YA, Lee JS. Rhabdomyosarcoma in children and adolescents: patterns and risk factors of distant metastasis. *Am J Roentgenol*. (2017) 209:409–16. doi: 10.2214/AJR.16.17466
14. Dantonello TM, Int-Veen C, Schuck A, Seitz G, Leuschner I, Nathrath M, et al. Survival following disease recurrence of primary localized alveolar rhabdomyosarcoma: recurrent alveolar rhabdomyosarcoma outcomes. *Pediatr Blood Cancer*. (2013) 60:1267–73. doi: 10.1002/pbc.24488
15. Lee RJ, Lee KK, Lin T, Arshi A, Lee SA, Christensen RE. Rhabdomyosarcoma of the head and neck: impact of demographic and clinicopathologic factors on survival. *Oral Surg Oral Med Oral Pathol Oral Radiol*. (2017) 124:271–9. doi: 10.1016/j.oooo.2017.05.507
16. Dombrowski ND, Wolter NE, Robson CD, Kawai K, Irace AL, Vargas SO, et al. Role of surgery in rhabdomyosarcoma of the head and neck in children. *Laryngoscope*. (2021) 131:E984–92. doi: 10.1002/lary.28785
17. Bisogno G, Jenney M, Bergeron C, Gallego Melcón S, Ferrari A, Oberlin O, et al. Addition of dose-intensified doxorubicin to standard chemotherapy for rhabdomyosarcoma (EpSSG RMS 2005): a multicentre, open-label, randomised controlled, phase 3 trial. *Lancet Oncol*. (2018) 19:1061–71. doi: 10.1016/S1470-2045(18)30337-1
18. Lawrence W, Anderson JR, Gehan EA, Maurer H. Pretreatment TNM staging of childhood rhabdomyosarcoma: a report of the Intergroup Rhabdomyosarcoma Study Group. Children's Cancer Study Group. Pediatric Oncology Group. *Cancer*. (1997) 80:1165–70. doi: 10.1002/(SICI)1097-0142(19970915)80:6<1165::AID-CNCR21>3.0.CO;2-5
19. Raney RB, Maurer HM, Anderson JR, Andrassy RJ, Donaldson SS, Qualman SJ, et al. The intergroup rhabdomyosarcoma study group (IRSG): major lessons from the IRS-I through IRS-IV studies as background for the current IRS-V treatment protocols. *Sarcoma*. (2001) 5:9–15. doi: 10.1080/13577140120048890
20. Bisogno G, De Salvo GL, Bergeron C, Gallego Melcón S, Merks JH, Kelsey A, et al. Vinorelbine and continuous low-dose cyclophosphamide as maintenance chemotherapy in patients with high-risk rhabdomyosarcoma (RMS 2005): a multicentre, open-label, randomised, phase 3 trial. *Lancet Oncol*. (2019) 20:1566–75. doi: 10.1016/S1470-2045(19)30617-5
21. Radzikowska J, Kukwa W, Kukwa A, Czarnecka AM, Kawecki M, Lian F, et al. Management of pediatric head and neck rhabdomyosarcoma: a case-series of 36 patients. *Oncol Lett*. (2016) 12:3555–62. doi: 10.3892/ol.2016.5072
22. Bradley JA, Indelicato DJ, Uezono H, Morris CG, Sandler E, de Soto H, et al. Patterns of failure in parameningeal alveolar rhabdomyosarcoma. *Int J Radiat Oncol*. (2020). 107:325–33. doi: 10.1016/j.ijrobp.2020.01.035
23. Häußler SM, Stromberger C, Olze H, Seifert G, Knopke S, Böttcher A. Head and neck rhabdomyosarcoma in children: a 20-year retrospective study at a tertiary referral center. *J Cancer Res Clin Oncol*. (2018) 144:371–9. doi: 10.1007/s00432-017-2544-x
24. Eugène T, Ansquer C, Oudoux A, Corradini N, Carlier T, Thomas C, et al. Apport de la TEP/TDM au 18FDG dans la stadification initiale et l'évaluation précoce de la réponse thérapeutique des rhabdomyosarcomes pédiatriques. *Méd Nucl*. (2010) 34:655–63. doi: 10.1016/j.mednuc.2010.10.007
25. Federico SM, Spunt SL, Krasin MJ, Billup CA, Wu J, Shulkin B, et al. Comparison of PET-CT and conventional imaging in staging pediatric rhabdomyosarcoma. *Pediatr Blood Cancer*. (2013) 60:1128–34. doi: 10.1002/pbc.24430
26. Norman G, Fayter D, Lewis-Light K, Chisholm J, McHugh K, Levine D, et al. An emerging evidence base for PET-CT in the management of childhood rhabdomyosarcoma: systematic review. *BMJ Open*. (2015) 5:e006030. doi: 10.1136/bmjopen-2014-006030
27. Dantonello TM, Int-Veen C, Winkler P, Leuschner I, Schuck A, Schmidt BF, et al. Initial patient characteristics can predict pattern and risk of relapse in localized rhabdomyosarcoma. *J Clin Oncol*. (2008) 26:406–13. doi: 10.1200/JCO.2007.12.2382
28. Perez EA, Kassira N, Cheung MC, Koniaris LG, Neville HL, Sola JE. Rhabdomyosarcoma in children: a SEER population based study. *J Surg Res*. (2011) 170:e243–51. doi: 10.1016/j.jss.2011.03.001

29. Orbach D, Mosseri V, Gallego S, Kelsey A, Devalck C, Brenann B, et al. Nonparameningeal head and neck rhabdomyosarcoma in children and adolescents: lessons from the consecutive international society of pediatric oncology malignant mesenchymal tumor studies: head and neck rhabdomyosarcoma in children. *Head Neck*. (2017) 39:24–31. doi: 10.1002/hed.24547
30. Unsal AA, Chung SY, Unsal AB, Baredes S, Eloy JA. A population-based analysis of survival for sinonasal rhabdomyosarcoma. *Otolaryngol Neck Surg*. (2017) 157:142–9. doi: 10.1177/0194599817696292
31. Gillespie MB, Marshall DT, Day TA, Mitchell AO, White DR, Barredo JC. Pediatric rhabdomyosarcoma of the head and neck. *Curr Treat Options Oncol*. (2006) 7:13–22. doi: 10.1007/s11864-006-0028-3
32. Glosli H, Bisogno G, Kelsey A, Chisholm JC, Gaze M, Kolb F, et al. Non-parameningeal head and neck rhabdomyosarcoma in children, adolescents, and young adults: experience of the European paediatric Soft tissue sarcoma Study Group (EpSSG) – RMS2005 study. *Eur J Cancer*. (2021) 151:84–93. doi: 10.1016/j.ejca.2021.04.007
33. Minard-Colin V, Kolb F, Saint-Rose C, Fayard F, Janot F, Rey A, et al. Impact of extensive surgery in multidisciplinary approach of pterygopalatine/infratemporal fossa soft tissue sarcoma: pterygopalatine/infratemporal fossa sarcoma. *Pediatr Blood Cancer*. (2013) 60:928–34. doi: 10.1002/pbc.24374
34. Herrmann BW, Sotelo-Avila C, Eisenbeis JF. Pediatric sinonasal rhabdomyosarcoma: three cases and a review of the literature. *Am J Otolaryngol*. (2003) 24:174–80. doi: 10.1016/S0196-0709(03)00025-5
35. Rutland JW, Gill CM, Ladner T, Goldrich D, Villavisanis DF, Devarajan A, et al. Surgical outcomes in patients with endoscopic versus transcranial approach for skull base malignancies: a 10-year institutional experience. *Br J Neurosurg*. (2020) 1–7. doi: 10.1080/02688697.2020.1779659 [Epub ahead of print].
36. Markiewicz MR, Ruiz RL, Pirgousis P, Bryan Bell R, Dierks EJ, Edwards SP, et al. Microvascular free tissue transfer for head and neck reconstruction in children: part I. *J Craniofac Surg*. (2016) 27:846–56. doi: 10.1097/SCS.0000000000002515
37. Yunteng W, Xuhui M, Guoxin R, Wei G. Radical surgery for head and neck rhabdomyosarcoma failed primary chemotherapy. *J Craniofac Surg*. (2019) 30:e113–6. doi: 10.1097/SCS.0000000000005019
38. Merks JHM, De Salvo GL, Bergeron C, Bisogno G, De Paoli A, Ferrari A, et al. Parameningeal rhabdomyosarcoma in pediatric age: results of a pooled analysis from North American and European cooperative groups. *Ann Oncol*. (2014) 25:231–6. doi: 10.1093/annonc/mdt426
39. Missiaglia E, Williamson D, Chisholm J, Wirapati P, Pierron G, Petel F, et al. *PAX3/FOXO1* fusion gene status is the key prognostic molecular marker in rhabdomyosarcoma and significantly improves current risk stratification. *J Clin Oncol*. (2012) 30:1670–7. doi: 10.1200/JCO.2011.38.5591
40. Di Carlo D, Chargari C, Scoazec J-Y, Cotteret S, Felix A, Moalla S, et al. PAX3-NCOA1 alveolar rhabdomyosarcoma of the tongue: a rare entity with challenging diagnosis and management. *Pediatr Blood Cancer*. (2021) 68:e29288. doi: 10.1002/pbc.29288

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
published: 08 March 2022
doi: 10.3389/fsurg.2022.844810

Special Considerations for Tympanoplasty Type I in the Oncological Pediatric Population: A Case-Control Study

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Introduction: Although cutting-edges antineoplastic therapies increase survival in children with malignancies, the optimal surgical strategy to address associated comorbidities such as chronic tympanic membrane perforation is still poorly documented. The aim of this study is to evaluate the outcomes of type I tympanoplasty in pediatric cancer survivors who received chemo and/or radiotherapy to the skull and to identify potential associated risk factors.

Methods: This case-control study included medical records review of oncologic patients (age < 21) treated at the same Academic medical oncologic center between March 2015 and July 2021 and referred for conductive hearing loss and chronic tympanic membrane perforation. Patients and middle ear status-related variables were analyzed, and outcomes were compared with matched peers without any history of malignancies.

Results: A total of seven pediatric cancer survivors and seven paired children without any history of malignancies were included in this report. The mean age at tympanoplasty type I surgery was 10.2 years (range = 4.3–19.9; median = 7.9 years) for the pediatric cancer survivors' group and 10.1 years (range = 5.5–19.2; median = 7.9 years) in the control group. Three pediatric cancer patients had received chemotherapy alone, one patient had radiotherapy to the skull base, and three patients had received chemoradiotherapy. On average, surgery was performed 3.9 years after chemo and/or radiotherapy termination, except for 1 patient for whom the tympanoplasty was performed during chemotherapy treatment. A retroauricular approach was used for one of the pediatric cancer patients, a transcanal approach was performed in one other and five patients benefited from an otoendoscopic approach. Tragal perichondrium with cartilage was used in most of the pediatric cancer survivor cases (four out seven cases) while xenograft (Biodesign) and Temporalis fascia without cartilage graft were used in five out of the seven control cases. Rate of tympanic membrane perforation recurrence was similar between groups (28.6%). Mean functional gain for air conduction Pure Tone Average (AC PTA) was 2.6 and 7.7 dB HL for the oncologic and control group, respectively. Mean postoperative air-bone gap (ABG) was 10.7 dB HL [median = 8.7; inter-quartile range (*IQR*) = 13.8] for the oncologic cohort and 10.1 dB HL (median = 10.7; *IQR* = 9.6) for the control group.

OPEN ACCESS

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Specialty section:
This article was submitted to Pediatric Surgery, a section of the journal Frontiers in Surgery

Received: *28 December 2021*
Accepted: *07 February 2022*
Published: *08 March 2022*

Citation:
Richard C, Baker E and Wood J (2022) Special Considerations for Tympanoplasty Type I in the Oncological Pediatric Population: A Case-Control Study. Front. Surg. 9:844810. doi: 10.3389/fsurg.2022.844810

Discussion: Chemo- and chemoradiotherapy to the skull are associated with damages to the inner and middle ear structures with secondary eustachian tube dysfunction and chronic middle ear effusion. Although healing abilities and immunological defenses are compromised as part of the expected effects of antineoplastic therapies, type I tympanoplasty can be safe and effective in this population. While different approaches may be considered, otoendoscopy showed excellent results with less morbidity in this vulnerable population.

Keywords: pediatric oncology, radiotherapy, chemotherapy, audiologic, otology, tympanoplasty, hearing loss

INTRODUCTION

With advances in chemotherapeutic agents and radiation modalities, survival prognosis has tremendously improved for children with malignancies (1). Besides these fantastic steps toward improved survival rate, the associated morbidities add an undesirable burden to the oncologic journey.

With a higher incidence of upper airways infections, anatomical peculiarities of the eustachian tube during childhood, adenoid hypertrophy, biofilm formation (2) among other factors, the pediatric population is at higher risks for chronic otitis media with effusion (OME). OME is the most common pediatric ear pathology, leading to a significant morbidity in this population. Although symptoms are usually unspecific, persistent OME causes hearing impairment, reportedly permanent in 2–35 per 10,000 (3). While controversies remain in the adult population as to the optimal management of radiation induced middle ear effusion, the cohort of children with malignancies follows the recommendations intended for the general pediatric population. General pediatric population guidelines recommend ventilation tube insertion in OME lasting $\geq$3 months, and/or with any associated impairments and/or with increased risk for speech and language development compromise (4).

Notwithstanding recent advances with targeted chemotherapy to specific molecular tumor profiles (5) and refinement of radiotherapy (6) to improve both effectiveness and safety, children with malignancies are at higher risks for middle ear pathologies compared to their healthy peers. Chemotherapy raises the risk for infection-related complications especially at the level of the upper respiratory tract (7). Radiotherapy can alter eustachian tube function and middle ear homeostasis (altered ciliary function, hyperreactivity in secretion) (8) while surgery may damage the parapharyngeal structures (9). Although the most prevalent, OME is not the only cause of conductive hearing loss in radiation-exposed children (10, 11). Others etiologies include chronic suppurative otitis media, tympanic membrane perforation (TMP), fibrotic changes of the middle ear mucosa, and/or ossicular necrosis (11). While OME is most frequent during radiation therapy, the mucosal damages to the middle ear (12, 13) associated with persistent ET dysfunction can lead to persistent OME after RT completion (11). Although ventilation tube placement will help with symptoms, the underlying cause may persist and compromise the outcomes of local procedures. One associated comorbidity in the pediatric oncologic population is represented by hearing loss that can be sensorineural (impairment at the level of the inner ear and/or subcortical-cortical structures), conductive (external and middle ear dysfunction), or mixed (both the sensorineural and conductive systems are affected). While radiation- and/or chemotherapy-induced damages to the inner ear are well-documented, current literature regarding the effects on middle ear and related surgeries is parse.

Therefore, we decided to conduct this case-control analysis to appraise the outcomes and peculiarities of type I tympanoplasty in the oncologic pediatric population.

MATERIALS AND METHODS

The St. Jude Children's Research Hospital Institutional Review Board and Le Bonheur Children's Hospital Institutional Review Board approved this retrospective study and its related protocol (# 21-0799). Patients with malignancies who receive chemo- or chemoradiotherapy and underwent surgical treatment for TMP between March 2015 and September 2021 were eligible for inclusion. Potential control peers were identified from the Pediatric Otolaryngology Head and Neck surgery database, spanning year 2012 to 2021. Both the oncologic and control groups were operated on by the same surgical team. Only children with history of type 1 tympanoplasty were included. In the present study, we referred to as type 1 tympanoplasty of any tympanic membrane reconstruction performed by lifting a formal tympanomeatal flap in a middle ear with normal ossicular chain status. Those diagnosed with previous history of tympanoplasty on the same ear, and/or history of cholesteatoma, and/or ossicular chain abnormality, and/or who underwent surgery after 21 years of age were excluded. Additionally, all patients were required to have a minimum of one postoperative clinical follow-up with pre- and postoperative audiometric data available to be included in the study. Each oncologic patient identified was matched with a control peer using the following criteria: type of surgery (type 1 tympanoplasty), age at surgery ($\pm$1.5 years), and size of tympanic perforation. Patient demographics, medical histories, and prior ear surgeries were recorded. Audiometric testing was performed by experienced audiologists using a pure-tone audiometer in a sound-proof booth, and thresholds were determined from 0.25 to 4 KHz. Hearing sensitivity within the speech frequencies was recorded according to the Academy of Otolaryngology-Head and Neck

Surgery standards with four-tone air conduction (AC) pure-tone averages (PTA) obtained from AC thresholds collected at 0.5, 1, 2, and 3 kHz. Any missing values from the 3 kHz were replaced by the average value of the 2 and 4 kHz thresholds. The air-bone gap (ABG) was measured as the difference between air and bone conduction thresholds. The primary surgical outcome was recurrence rate for the oncological group and their matched peers. Surgical technique, including the approach, grafting material, and technique were collected.

Descriptive statistics are provided. Due to the skewness of the datasets, median, mean, and inter-quartile range (IQR) using quartile inclusive values are provided. Wilcoxon Rank Sum Test was applied for statistical analysis. Statistics were performed using R software version 4.0.4 9 [R Core Team (2013)]. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org/). The p-values < 0.05 were used as cut-off for statistical significance.

RESULTS

Patients' Profile

We identified seven patients with malignancies who underwent type 1 tympanoplasty under 21 years of age. Among the 2,620 pediatric patients of non-oncology from the database who underwent tympanoplasty surgery, mostly were excluded based on the previous exclusion criteria. Seven non-oncologic patient controls were identified as best match based on their age at surgery, surgical technique, and operating surgical team. The mean age at tympanoplasty type I surgery was 10.2 (range = 4.3–19.9; median = 7.9 years) for the pediatric cancer survivors' group and 10.1 (range = 5.5–19.2; median = 7.9 years) in the control group. General characteristics and otologic history for the oncologic patients and their match are presented in **Table 1**. Etiology of the TMP was ventilation tube placement in five and in four of the oncologic and control patients, respectively (**Table 1**). All ventilation tubes placement occurred after primary tumor diagnosis and during oncologic treatment. The time between last set of ventilation tube placement and perforation diagnosis was not statistically different between the two groups ($W = 3$; $p > 0.05$), with a mean time of 1.6 ± 1.06 years (range = 0.6–3.6 years) and 5.4 ± 3.06 years (range = 1.2–8.3 years) for the oncologic and control group, respectively. The median time from perforation diagnosis to surgery was 14.7 months (range = 5.5–35.7 months; $IQR = 14.1$) for the oncologic cohort and 13.1 months (range = 4.6–20.9 months; $IQR = 3.9$) for the control group. Of note, neither underlying sinonasal infection nor recurrent upper airways infections were evidence in any of the patients included in either group.

Oncologic Treatments

The mean age at primary tumor diagnosis was 4.95 ± 4.04 years (range = 0.5–13.7 years; median = 3.5 years) and the time from diagnosis to treatment start was 18.6 weeks (range = 0.1–69.3 weeks; median = 6.7 weeks). Two children presented with a history of leukemia and received chemotherapy regimens including methotrexate. Cisplatin was part of the chemotherapy regimen for two cases (#1 and 6) and carboplatin for one case (#5). Chemoradiation was considered for three other patients. One patient presented with a chordoma and underwent surgery followed by radiotherapy to the clivus. The mean radiation dose to the cranium was 54.6 Gy and lasted from 26 to 61 days divided on 5 days weekly. Steroids was added to the drug regimens in two cases (#1 and 2) (**Table 2**).

Surgery and Timelines

Age at surgery did not significantly differ across groups ($W = 25$; $p > 0.05$), with 10.2 ± 5.3 years (range = 4.3–19.9 years; median = 7.9 years; $IQR = 6.8$ years) in the oncologic group and 10.1 ± 4.5 years (range = 5.5–19.2 years; median = 7.9 years; $IQR = 5.2$ years) in the control group. Time from the end of chemotherapy to surgery varied from 1.2 to 15.8 years (mean = 4.42 years; median = 1.86 years; $IQR = 0.66$ year) and time from the end of radiotherapy to surgery varied from 1.5 to 16.5 years (mean = 5.5 years; median = 2.01 year; $IQR = 4.2$ years), **Table 2**. Preoperative size of the TMP varied from 15 to 95% with a median of 30% for both groups ($IQR = 20$ for both groups, **Table 3**). All grafts were placed in underlay with a transcanal approach for three cases (two controls and one oncologic patient), a retroauricular approach for three others (two controls and one oncologic patient) and an otoendoscopic approach was used for the others ($N = 8$). In five out of the seven oncologic patients, cartilage was part of the graft materials whereas it was used in only two of the control patients, **Table 3**. All ears were dry at the time of surgery. However, two oncologic patient and two controls demonstrated inflammation of the middle ear mucosa during surgery (#5 and #6, C#3 and C#6).

Audiometric Status

The mean preoperative AC PTA was 24 dB HL (range = 11.2–41.2 dB; median = 22.5 dB; $IQR = 12.2$) for the oncologic group and 24.7 dB HL (range = 8.1–37.5 dB; median = 26.2 dB; $IQR = 9.4$) for the control group. Preoperative PTA was not significantly different between the oncologic and control groups ($W = 11$; $p > 0.05$).

Mean follow-up time after tympanoplasty was 17.4 months (range = 1.4–63.7 months; median = 6.97; $IQR = 19.2$) for the oncologic population and 22.8 months (range = 1.6–79.1 months; median = 5.7; $IQR = 29.2$) for controls. Bilateral chronic middle ear inflammation was reported in two oncologic patients (#1 and #5). However, the small sample size prevented us from drawing any conclusion or reaching any statistical significance. For both groups, the last AC PTA and ABG recorded had improved from preoperative data. The mean postoperative AC-PTA was 21.4 dB HL (range = 5–33.7 dB; median = 21.2 dB; $IQR = 7.5$) for the oncologic group and 17 dB HL (range = 4.4–26.9 dB; median = 15.6 dB; $IQR = 7.05$) for the control group. Mean functional gain for AC PTA was not significantly different between groups, with 2.6 and 7.7 dB HL for the oncologic and control group, respectively ($W = 30$; $p > 0.05$). Mean postoperative ABG was 10.7 dB HL (median = 8.7; $IQR = 13.8$) for the oncologic cohort and 10.1 dB HL (median = 10.7; $IQR = 9.6$) for the control group, **Figure 1**. The mean ABG functional gain for the oncologic group was

TABLE 1 | Patients' characteristics and otologic history.

	Gender	Ethnicity	Primary malignancy	Ear surgery before cancer diagnosis	Previous ear surgery	Number of PET sets	Previous adenoidectomy	Associated syndrome	TMP etiology
1	F	White, non-hispanic	Medulloblastoma	No	PET	3	No	No	*Tube-Related*
C #1	F	White	NA	NA	PET	1	Yes	No	*Tube-Related*
2	M	White, Hispanic	Left parapharyngeal RMS, with skull base and orbital extension	No	PET	1	No	No	*Tube-Related*
C #2	F	Unavailable	NA	NA	no	NA	No	No	Chronic Otitis Media
3	M	White, non-hispanic	B-cell ALL	No	PET	1	Yes 01/25/2019	No	*Tube-Related*
C #3	M	Unavailable	NA	NA	PET	5	Yes	No	*Tube-Related*
4	M	White, non-hispanic	Pre-B ALL	No	No	0	No	No	Unknown
C #4			NA	NA	PET	1	No	Ehlers Danlos	*Tube-Related*
5	F	Black	Optic pathway glioma	No	PET	1	No	NF1	*Tube-Related*
C #5	F	White, non-hispanic	NA	NA	no	NA	No	No	Draining AOM
6	M	White, non-hispanic	Neuroblastoma	No	PET and T tube	2	No	No	*Tube-Related*
C #6	F	Asian decent	NA	NA	PET	5	No	CLP	*Tube-Related*
7	M	Black and white	Chordoma	No	No	No	No	No	Unknown
C #7	F	White	NA	NA	PET	1	No	No	Post traumatic

TMP, tympanic membrane perforation; C #, control patient; AOM, acute otitis media; CLP, cleft lip and palate.

not significantly different from the control group with 2.4 and 9.5 dB, respectively ($W = 22$; $p > 0.05$). Adherence to national cisplatin ototoxicity monitoring guidelines were observed in this study with serial pre-, per-, and post-treatment audiograms. However, children from the oncologic group who were not at risk for chemo-induced hearing loss had hearing monitoring during antineoplastic therapy but not always immediately after completion. For instance, #2 had his preoperative audiogram during chemotherapy that revealed an ABG = 1.9 dB HL, and a postoperative ABG of 15 dB HL with an AC PTA of 30 dB HL with a healed eardrum. Patient #5 was diagnosed with neurofibromatosis type I, enlargement of the brainstem, and bilateral optic pathway glioma. Although the patient's preoperative AC PTA was 22.5 dB HL, delays in conduction patterns were observed on the auditory brainstem responses and the patient had been fitted with hearing aids at 4 years of age and was receiving early speech and language therapy. For this patient, the surgery aimed at assisting with hearing aid adaptation by providing a dry ear, limiting the impact of chronic infection, and hearing loss on speech and language development and the quality of academic activities.

Four out of the seven oncologic patients presented with high-frequencies sensorineural hearing loss and an AC threshold ≥ 55 dB HL at 4 KHz (range 45–80 dB HL; mean = 58.75 dB HL). The sensorineural component of hearing loss was cisplatin-induced for two patients (#1 and 6) and radiation-induced for one (#2) who received radiation to the ipsilateral infratemporal fossa.

No difference was noted in PTA outcomes between the oncologic and control group at postoperative follow-up.

Complications

No graft lateralization, blunting nor cholesteatoma was reported during follow-up. One oncologic patient #1 presented with a small retraction pocket anterior to the malleus for which the team elected for close monitoring. For this patient, temporalis fascia was the material graft used. Two patients from each group presented with a recurrent TMP (28.6%, **Table 3**). Recurrence time ranged from 1.5 to 5.3 months for the oncologic group vs. 1.7–3.1 months for the control group. For both oncologic patients, a cartilage was used during surgery for additional reinforcement. Patient #4 had his surgery 1.2 year after treatment completion and did not feature any specific signs of inflammation during surgery. Patient #5 was referred for tympanoplasty at an early age (4.4 years old) due to concern with chronic otorrhea and hearing aid adjustment. The team elected for tympanoplasty with a cartilage graft to provide an additional layer resistant

TABLE 2 | Lines of treatments and timeline to surgery.

#	Primary	Age at tumor diagnosis (years)	End of chemotherapy-surgery (weeks)	End of radiotherapy-surgery (weeks)	First round chemotherapy		Second round chemotherapy		Third and fourth rounds chemotherapy	Radiotherapy			
					Regimen (high dose)	Duration (weeks)	Regimen (high dose)	Duration (weeks)	Regimen/ duration	Field	Radiation dosage (Gy)	RT type	Duration (weeks)
1	Medulloblastoma	2.5	97	89	Methotrexate, cisplatin, cyclophosphamide, and vincristine	15	Topotecan and cyclophosphamide	9	NA	Skull & Spine	54	Photon CSI	4
2	Left parapharyngeal RMS, extension to the skull base and orbit	5.25	65	120	Vincristine/irinotecan	63	NA	NA	NA	Left infratemporal fossa)	36	Proton	7
3	B-cell ALL	3.5	100	NA	Methotrexate and mercaptopurine	5	Mercaptopurine	125	NA	NA	NA	NA	NA
4	PreB ALL	6.8	63	NA	Methotrexate and mercaptopurine	128	NA	NA	NA	NA	NA	NA	NA
5	Optic pathway glioma	0.5	current chemotherapy	NA	Vincristine, carboplatin and temozolomide	83	Vinblastine	98	Selumetinib from 2017 to 2020 (147 weeks) and resumed in 2021	NA	NA	NA	NA
6	Neuroblastoma	2.4	825	860	Cyclophosphamide, doxorubicin, etoposide, cisplatin, melphalan, topotecan, tretinoin	72	NA	NA	NA	Abdomen	53	Photon CSI	6
7	Chordoma	13.7	NA	80	NA	NA	NA	NA	NA	Clivus	73.8	Proton	9

TABLE 3 | Otologic procedures.

	Age at surgery (years)	Side	At surgery					Post-surgery			
			TMP location	TMP size (%)	Surgical approach	Surgical technique	Graft	TMP	TMP timing (months)	TMP location	TMP size (%)
1	5.0	Left	Inferior	40	Transcanal	Underlay	TF	-	-	-	-
C #1	6.7	Left	Inferior posterior	30	Transcanal	Underlay	TF +Xenograft	-	-	-	-
2	7.9	Left	Subtotal	95	Retroauricular	Underlay	TF +Cartilage	-	-	-	-
C #2	7.9	Left	Inferior	80	Retroauricular	Underlay	TF	+	3.1	Inferior	10
3	7.9	Right	Anterior-inferior	20	Otoendoscopy	Underlay	Perichondrium +Cartilage	-	-	-	-
C #3	7.3	Left	Anterior-inferior	25	Retroauricular	Underlay	TF	-	-	-	-
4	10.7	Right	Anterior-inferior	40	Otoendoscopy	Underlay	Perichondrium +Cartilage	+	1.5	Central	10
C #4	11.2	Left	Central	40	Otoendoscopy	Underlay	Xenograft	+	1.7	Central	20
5	4.4	Left	Central-inferior	50	Otoendoscopy	Underlay	Perichondrium +Cartilage	+	5.3	Central	50
C #5	5.5	Left	Inferior	60	Otoendoscopy	Underlay	Perichondrium +Cartilage	-	-	-	-
6	19.9	Left	Anterior	30	Otoendoscopy	Underlay	Perichondrium +Cartilage	-	-	-	-
C #6	19.2	Right	Inferior	30	Otoendoscopy	Underlay	Perichondrium +Cartilage	-	-	-	-
7	15.8	Left	Anterior	15	Otoendoscopy	Underlay	Xenograft	-	-	-	-
C #7	13.2	Left	Posterior	30	Transcanal	Underlay	Xenograft	-	-	-	-

C, control patient; TMP, tympanic membrane perforation; TMP timing (months), time from surgery to first diagnosis of TMP recurrence; TF, temporalis fascia.

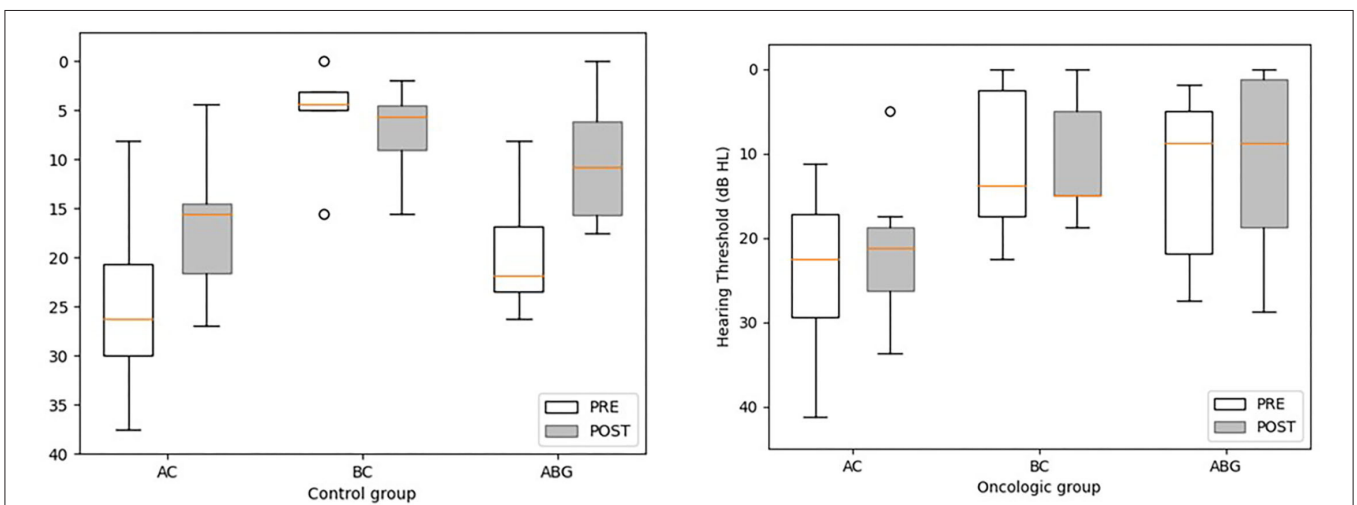

FIGURE 1 | Pre-and post-treatment pure tone average thresholds in air conduction (AC), bone conduction (BC), and air-bone gap (ABG) for the oncologic and control groups. Statistical differences were analyzed using the Wilcoxon Rank Sum test. The postoperative BC PTA is missing for two children from the oncologic group (#5 and 7) and for 1 control patient (C#6). No values met the significance threshold of ≤ 0.05. A tendency was observed for the control group for postoperative AC PTA and ABG in comparison to preoperative values ($V = 25$ and $p = 0.078$ for AC PAT; $V = 20$, $p = 0.06$ for ABG).

to negative middle ear pressures (**Table 2**). She presented with a recurrent tympanic membrane perforation that failed two subsequent tympanoplasties.

Postoperative Changes in Middle Ear Status

Patients #2 and #5 had an episode of postoperative middle ear effusion and patient #1 presented with recurrent episodes of postoperative left maxillary sinusitis with left middle ear effusion noted on serial control imaging for his primary tumor (left parapharyngeal rhabdomyosarcoma).

DISCUSSION

Type I tympanoplasty is nowadays a well-described surgery providing children with an improved hearing and dry ear. However, the oncologic population raises new challenges with a drug- and/or radiation-induced middle ear homeostasis disruption, delayed healing, and immune-system compromise. This is the first pediatric case-control study focusing on the potential factors affecting hearing and surgical outcomes in the oncologic population. This retrospective case series with matched controls provide a review and analysis of our experience with this vulnerable population. To our knowledge, no previous study has compared graft success and audiometric outcomes in this subset of patients.

The generally accepted definition of success encompasses the graft integrity and postoperative gain of more than 10 dB and neither OME recurrence nor atelectasis (14). Overall, surgical and hearing outcomes observed in this study did not significantly differ from the controls and from the general litterature (15–22). While comparing to the generally restrictive criteria (closure of the tympanic perforation with ABG $\leq$ 20 dB and an aerated middle space), four out of seven oncologic patients (57.1%) had a successful type I tympanoplasty, a rate slightly inferior to the report from Isaacson and Melaku (23). However, given the peculiarities of the presently reported oncologic population, and the disparities in age, TMP size and location, these criteria are difficult to apply to this study. When focusing on the rate of graft uptake, the oncologic population had the same rate as the control group (71.4%), but inferior to the mean weighted closure rate reported for pediatric tympanoplasty was 83.4% (24).

One of the main concerns when dealing with the oncologic population is to assess the optimized timing of surgery. The potential role of age as a prognosis factor of success is still subject to controversies (14, 25–27). However, the erratic eustachian tube function coupled with immunological immaturity of early childhood is one of the arguments for some teams justifying to delay tympanoplasty until 6 years of age in the general population (14). Although limited in size, no effect of age was observed in our oncologic cohort. Beyond the hearing improvement, the goal of type I tympanoplasty in children with malignancies is improve their quality of life by limiting the impact of the associated comorbidities. Type I tympanoplasty is also intended to help control otorrhea and assist with hearing aid fitting (11, 28). Early postoperative graft failure, within the first 3 months, is most commonly secondary to inadequate graft positioning, postoperative infection or pressure-related incident (i.e., early postoperative blowing) (29). However, a delayed failure (>3 months) is in most cases secondary to an underlying middle ear pathology. Effects of chemotherapy on the middle ear can be mediated through different ways. The pre-clinical studies showed the negative impact of chemotherapeutic agents on the immunologic status and on the wound healing process (30). The chemo-induced immune deficiency disrupts the middle ear homeostasis which is exposed higher risks for local infections (7), while its effects on cell division will impede fibroblasts proliferation (31), and subsequently impacts the course of TM healing. Among the different chemotherapies reported, vinblastine an alkaloid chemotherapeutic agent has been shown to affect microtubules affects tumors by impeding their cellular migration (32). Another concern for our oncologic population was the potential impact of the different chemotherapy regimens on wound healing, among which methotrexate has been well-reported (30). Although the limited number of patients prevents us from formulating any conclusions, both of our oncologic cases were either in an ongoing- or early post-chemotherapy phase.

Another factor that may jeopardize of graft uptake in the oncologic population is radiation. The effects of cranial radiation are reportedly notable with 82.5% of patient presenting with abnormal eustachian tube function and related middle ear dysfunction and with conductive hearing loss in one-third of patients (33, 34). In response to radiation, the TM thickens (35), middle ear mucosa undergoes edematous process with impaired gas exchanges, eustachian tube dysfunction resulting in a negative pressure and subsequent middle ear effusion (36). Radiation to the skull induces damages to the osteocytes and blood supply (37), and triggers repetitive inflammatory responses (38). All these changes are usually transient, lasting a couple of months (33). The timeline in recovery may explain the high rate of success observed for our radiated patients for whom the procedure was at distance from treatment completion ($\geq$80 weeks). Two oncologic patients and two controls demonstrated inflammation of the middle ear mucosa during surgery (#5 and #6, C#3 and C#6), of whom only patients #5 presented with a postoperative effusion. The two other patients presenting with postoperative middle ear effusion (#1 and #2) were not reported with an inflamed middle ear mucosa at the time of the surgery. Based on our limited oncologic cohort, we cannot draw any conclusions whether a therapeutic mastoidectomy should be performed in case of inflamed middle ear status. However, based on the literature in non-oncologic patients, performing a therapeutic mastoidectomy does not improve the outcomes in patients with chronic otitis media (39). Moreover, in case of oncologic patients, their altered wound healing processes could increase the mastoidectomy-related morbidity.

Although **t**emporalis fascia is easily accessible and reliable as a graft material, the peculiarities of the oncologic middle ear supported the surgeon's choice of adding an extra layer of support with cartilage for most of the oncologic cases with a TMP $\geq$ 20% which is more restrictive than in the 50% reported in the literature (40). One exception was patient #1 for whom no cartilage was used for extra resistance. This patient presented with a retraction pocket within 3 months

post-surgery. This finding corroborates previous report on the disrupted middle ear homeostasis secondary to antineoplastic therapies, placing the patient at higher risk for retraction pocket when considering temporalis fascia without any other support material (29). Another concern in our population is whether the radiated cartilage is an adequate graft material. Radiation-induced changes to the cartilage have been poorly studied. Although no graft failure was observed in our radiated patients, observations of scant cartilage matrix with decreased number of viable chondrocytes have been reported (41). When considering auditory outcomes, cartilage addition (0.5–1 mm width) when well-positioned without any direct contact with the sulcus has shown to have minimal impact on sound transmission (42–44). Although not significant, the control group tended to have better hearing outcomes (mean AC PTA and ABG functional gains) than the patients of oncology. Such results may be influenced by various factors among which the recurrence of middle ear effusion and/or the use of cartilage grafts. Surgical approach to the middle ear may vary, with a recent trend toward the use of otoendoscopes with an overall endoscopic success rate of 86.5% in the literature, increasing with the addition of a concurrent cartilage graft. The endoscopic tympanoplasty technique was refined and established itself as a recognized minimally invasive approach that limits the impact on the external auditory canal skin while providing an excellent view for graft positioning. It allows for better visualization in cases with tortuous bony canal or bony overhangs thus minimizing the rate of canalplasty (45–47) and avoiding its additional burden to a radiated bone. Endoscopic approach provides similar results to a microscopic approach on cochlear function, whether graft material is considered (48). The only limitation to endoscopic surgery is the ability of the surgeon with one-handed procedures and the need for an endoscope holders by some surgeons that may increase the exposure time of the middle ear to high temperature (49). Whether to choose an endoscopic or microscopic route, the postauricular approach, is more a matter of TMP size and surgeon's preference and training. In our experience, a more minimally invasive approach should be considered in pediatric oncologic patients presenting with a TMP $< 50\%$. By avoiding the need for a postauricular approach and canalplasty, the endoscopic approach allows for shorter operative times (46) that are advantageous in children especially in case of malignancies.

Given the substantial risk for TMP recurrence and the associated morbidity and impact of revision surgery, we believe reporting case in the specific subset of pediatric oncologic patients is critical. Although definitive conclusions are difficult to draw regarding the success rate of functional otologic surgery following chemo- or chemoradiotherapy; based on our institutional experience, type I tympanoplasty appeared to be safe and effective for more than half of the patients of oncology. There is a need for more reports in the oncologic population in order to better counsel patients and families. The clinician needs to be counseled on the possibility of TMP recurrence and the need for close long-term follow-up. However, we do believe that this functional surgery can improve their quality of life. Collecting further data will provide support for clinicians to discuss strategic choice in terms of timing, approaches, and graft material choices.

LIMITATIONS

The main limitation of this case-control study is its limited sample size. Over quantity, we elected for rigorous inclusion criteria in order to better evaluate the probability of success of the therapeutic intervention. To ensure reproducibility of techniques, we only included surgery performed by the team of surgeons and excluded previous cases for which surgical technique may have varied causing additional bias to the outcome's evaluation. Moreover, oncological cases with a history of type 1 tympanoplasty are rare and poorly documented. Unfortunately, such drastic criteria in such a limited cohort prevented us from matching all patients for the type of graft and surgical approach. Another limitation of this study is the use of cartilage graft for five oncologic patients, which may have prevented an adequate postoperative evaluation of the middle ear status, with potential missed middle ear effusions.

CONCLUSION

Adequate timing and optimized strategies may improve the surgical outcomes in this population. This study provides the pediatric otolaryngologist with an insight to quantify the probability of success for an oncologic patient and material to discuss the intervention with a patient and its family members.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the St. Jude Children's Research Hospital Institutional Review Board and Le Bonheur Children's Hospital Institutional Review Board approved this retrospective study and its related protocol (# 21-0799). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

JW and CR: study conception and design. EB, CR, and JW: data collection, analysis and interpretation of results, and draft manuscript preparation. All authors reviewed the results and approved the final version of the manuscript.

ACKNOWLEDGMENTS

We would like to thank Dr. Sheyn and Dr. Rangel for their assistance in IRB forms preparation. We would like to thank all the families and patients who agreed to participate in this study with the only goal of helping other children with malignancies.

REFERENCES

1. Ward E, DeSantis C, Robbins A, Kohler B, Jemal A. Childhood and adolescent cancer statistics, 2014. *CA Cancer J Clin.* (2014) 64:83–103. doi: 10.3322/caac.21219
2. Gok U, Bulut Y, Keles E, Yalcin S, Doymaz MZ. Bacteriological and PCR analysis of clinical material aspirated from otitis media with effusions. *Int J Pediatr Otorhinolaryngol.* (2001) 60:49–54. doi: 10.1016/S0165-5876(01)00510-9
3. Lee JY, Kim SH, Song CI, Kim YR, Kim YJ, Choi JH. Risk factors for persistent otitis media with effusion in children: a case-control study. *Yeungnam Univ J Med.* (2018) 35:70–5. doi: 10.12701/yujm.2018.35.1.70
4. Rosenfeld RM, Schwartz SR, Pynnonen MA, Tunkel DE, Hussey HM, Fichera JS, et al. Clinical practice guideline: tympanostomy tubes in children. *Otolaryngol Head Neck Surg.* (2013) 149(1 Suppl.):S1–35. doi: 10.1177/0194599813490141
5. Le Tourneau C, Borcoman E, Kamal M. Molecular profiling in precision medicine oncology. *Nat Med.* (2019) 25:711–2. doi: 10.1038/s41591-019-0442-2
6. Kucha N, Soni TP, Jakhotia N, Patni M, Singh DK, Gupta AK, et al. A prospective, comparative analysis of acute toxicity profile between three-dimensional conformal radiotherapy (3DCRT) and intensity-modulated radiotherapy (IMRT) in locally advanced head and neck cancer patients. *Cancer Treat Res Commun.* (2020) 25:100223. doi: 10.1016/j.ctarc.2020.100223
7. Inaba H, Pei D, Wolf J, Howard SC, Hayden RT, Go M, et al. Infection-related complications during treatment for childhood acute lymphoblastic leukemia. *Ann Oncol.* (2017) 28:386–92. doi: 10.1093/annonc/mdw557
8. Ohashi Y, Nakai Y, Esaki Y, Ikeoka H, Koshimo H, Onoyama Y. Acute effects of irradiation on middle ear mucosa. *Ann Otol Rhinol Laryngol.* (1988) 97:173–8. doi: 10.1177/000348948809700215
9. Redaelli de Zinis LO, Parrinello G, Schreiber A, Nicolai P. Middle ear effusion in patients with sinonasal cancer treated by surgery with or without radiotherapy. *Otolaryngol Head Neck Surg.* (2013) 148:619–24. doi: 10.1177/0194599812474798
10. Jereczek-Fossa BA, Zarowski A, Milani F, Orecchia R. Radiotherapy-induced ear toxicity. *Cancer Treat Rev.* (2003) 29:417–30. doi: 10.1016/S0305-7372(03)00066-5
11. Nader ME, Gidley PW. Challenges of hearing rehabilitation after radiation and chemotherapy. *J Neurol Surg B Skull Base.* (2019) 80:214–24. doi: 10.1055/s-0039-1677865
12. Elwany S. Delayed ultrastructural radiation induced changes in the human mesotympanic middle ear mucosa. *J Laryngol Otol.* (1985) 99:343–53. doi: 10.1017/S002221510009681X
13. Magnuson K, Franzén L, Henriksson R, Gustafsson H, Hellström S. Structural changes in the middle ear tissues of the rat after fractionated irradiation. *Eur Arch Otorhinolaryngol.* (1993) 250:92–6. doi: 10.1007/BF00179305
14. Boronat-Echeverría NE, Reyes-García E, Sevilla-Delgado Y, Aguirre-Mariscal H, Mejía-Aranguré JM. Prognostic factors of successful tympanoplasty in pediatric patients: a cohort study. *BMC Pediatr.* (2012) 12:67. doi: 10.1186/1471-2431-12-67
15. Çayir S, Kayabaşi S. Type 1 tympanoplasty in pediatric patients: comparison of fascia and perichondrium grafts. *Int J Pediatr Otorhinolaryngol.* (2019) 121:95–8. doi: 10.1016/j.ijporl.2019.03.007
16. Baklaci D, Guler I, Kuzucu I, Kum RO, Ozcan M. Type 1 tympanoplasty in pediatric patients: a review of 102 cases. *BMC Pediatr.* (2018) 18:345. doi: 10.1186/s12887-018-1326-1
17. Vrabec JT, Deskin RW, Grady JJ. Meta-analysis of pediatric tympanoplasty. *Arch Otolaryngol Head Neck Surg.* (1999) 125:530–4. doi: 10.1001/archotol.125.5.530
18. Pignataro L, Grillo Della Berta L, Capaccio P, Zaghis A. Myringoplasty in children: anatomical and functional results. *J Laryngol Otol.* (2001) 115:369–73. doi: 10.1258/0022215011907893
19. Umapathy N, Dekker PJ. Myringoplasty: is it worth performing in children? *Arch Otolaryngol Head Neck Surg.* (2003) 129:1053–5. doi: 10.1001/archotol.129.10.1053
20. Sckolnick JS, Mantle B, Li J, Chi DH. Pediatric myringoplasty: factors that affect success-a retrospective study. *Laryngoscope.* (2008) 118:723–9. doi: 10.1097/MLG.0b013e31815f8e2f
21. Lau T, Tos M. When to do tympanoplasty in children? *Adv Otorhinolaryngol.* (1988) 40:156–61. doi: 10.1159/000415686
22. MacDonald RR, Lusk RP, Muntz HR. Fasciaform myringoplasty in children. *Arch Otolaryngol Head Neck Surg.* (1994) 120:138–43. doi: 10.1001/archotol.1994.01880260010003
23. Isaacson G, Melaku A. Results of pediatric tympanoplasty on short-term surgical missions. *Laryngoscope.* (2016) 126:1464–9. doi: 10.1002/lary.25486
24. Hardman J, Muzaffar J, Nankivell P, Coulson C. Tympanoplasty for chronic tympanic membrane perforation in children: systematic review and meta-analysis. *Otol Neurotol.* (2015) 36:796–804. doi: 10.1097/MAO.0000000000000767
25. Yegin Y, Çelik M, Altintaş A, Çolak C, Kayhan FT. Do the angle and length of the eustachian tube affect the success rate of pediatric cartilage type 1 tympanoplasty? *J Craniofac Surg.* (2017) 28:e227–31. doi: 10.1097/SCS.0000000000003434
26. Chandrasekhar SS, House JW, Devgan U. Pediatric tympanoplasty. A 10-year experience. *Arch Otolaryngol Head Neck Surg.* (1995) 121:873–8. doi: 10.1001/archotol.1995.01890080041008
27. Denoyelle F, Roger G, Ducroz V, Escudier E, Fauroux B, Garabedian EN. Results of tympanoplasty in children with primary ciliary dyskinesia. *Archiv Otolaryngol Head Neck Surg.* (1998) 124:177–9. doi: 10.1001/archotol.124.2.177
28. Hsu YC, Su CY. Tympanoplasty for chronic otitis media in post-irradiated nasopharyngeal carcinoma patients. *Ann Otol Rhinol Laryngol.* (2006) 115:330–3. doi: 10.1177/000348940611500502
29. Demirci S, Tuzuner A, Karadas H, Acikgoz C, Caylan R, Samim EE. Comparison of temporal muscle fascia and cartilage grafts in pediatric tympanoplasties. *Am J Otolaryngol.* (2014) 35:796–9. doi: 10.1016/j.amjoto.2014.07.011
30. Shamberger RC, Devereux DF, Brennan MF. The effect of chemotherapeutic agents on wound healing. *Int Adv Surg Oncol.* (1981) 4:15–58.
31. Makuszewska M, Bonda T, Cieślińska M, Bialuk I, Winnicka MM, Niemczyk K. Expression of collagen type III in healing tympanic membrane. *Int J Pediatr Otorhinolaryngol.* (2020) 136:110196. doi: 10.1016/j.ijporl.2020.110196
32. Wang X, Decker CC, Zechner L, Krstin S, Wink M. In vitro wound healing of tumor cells: inhibition of cell migration by selected cytotoxic alkaloids. *BMC Pharmacol Toxicol.* (2019) 20:4. doi: 10.1186/s40360-018-0284-4
33. Negm H, Mosleh M, Hosni N, Fathy HA, Fahmy N. Affection of the middle ear after radiotherapy for head and neck tumors. *Egypt J Otolaryngol.* (2014) 30:5–9. doi: 10.4103/1012-5574.127184
34. Upadhya I, Jariwala N, Datar J. Ototoxic effects of irradiation. *Indian J Otolaryngol Head Neck Surg.* (2011) 63:151–4. doi: 10.1007/s12070-011-0142-9
35. Coplan J, Post EM, Richman RA, Grimes CT. Hearing loss after therapy with radiation. *Am J Dis Child.* (1981) 135:1066–7. doi: 10.1001/archpedi.1981.02130350066021
36. Borsanyi SJ, Blanchard CL. Ionizing radiation and the ear. *J Am Med Assoc.* (1962) 181:958–61. doi: 10.1001/jama.1962.03050370026006
37. Stone HB, Coleman CN, Anscher MS, McBride WH. Effects of radiation on normal tissue: consequences and mechanisms. *Lancet Oncol.* (2003) 4:529–36. doi: 10.1016/S1470-2045(03)01191-4
38. Haubner F, Ohmann E, Pohl F, Strutz J, Gassner HG. Wound healing after radiation therapy: review of the literature. *Radiat Oncol.* (2012) 7:162. doi: 10.1186/1748-717X-7-162
39. Trinidade A, Page JC, Dornhoffer JL. Therapeutic mastoidectomy in the management of noncholesteatomatous chronic otitis media: literature review and cost analysis. *Otolaryngol Head Neck Surg.* (2016) 155:914–22. doi: 10.1177/0194599816662438
40. Naina P, Pokharel A, Syed KA, John M, Varghese AM, Kurien M, et al. Three point assessment protocol for tympanoplasty outcomes: a retrospective analysis. *Int Arch Otorhinolaryngol.* (2020) 24:e438–43. doi: 10.1055/s-0039-3402432
41. Lambert EM, Gunn GB, Gidley PW. Effects of radiation on the temporal bone in patients with head and neck cancer. *Head Neck.* (2016) 38:1428–35. doi: 10.1002/hed.24267

42. Aarnisalo AA, Cheng JT, Ravicz ME, Hulli N, Harrington EJ, Hernandez-Montes MS, et al. Middle ear mechanics of cartilage tympanoplasty evaluated by laser holography and vibrometry. *Otol Neurotol.* (2009) 30:1209–14. doi: 10.1097/MAO.0b013e3181bc398e
43. Eldaebes MMAS, Landry TG, Bance ML. Repair of subtotal tympanic membrane perforations: a temporal bone study of several tympanoplasty materials. *PLoS ONE.* (2019) 14:e0222728. doi: 10.1371/journal.pone.0222728
44. Mohamad SH, Khan I, Hussain SSM. Is cartilage tympanoplasty more effective than fascia tympanoplasty? A systematic review. *Otol Neurotol.* (2012) 33:699–705. doi: 10.1097/MAO.0b013e318254fbc2
45. Lade H, Choudhary SR, Vashishth A. Endoscopic vs microscopic myringoplasty: a different perspective. *Eur Arch Otorhinolaryngol.* (2014) 271:1897–902. doi: 10.1007/s00405-013-2673-z
46. Schwam ZG, Cosetti MK. Endoscopic myringoplasty and type I tympanoplasty. *Otolaryngol Clin North Am.* (2021) 54:75–88. doi: 10.1016/j.otc.2020.09.010
47. Manna S, Kaul VF, Gray ML, Wanna GB. Endoscopic versus microscopic middle ear surgery: a meta-analysis of outcomes following tympanoplasty and stapes surgery. *Otol Neurotol.* (2019) 40:983–93. doi: 10.1097/MAO.0000000000002353
48. Botti C, Fermi M, Amorosa L, Ghidini A, Bianchin G, Presutti L, et al. Cochlear function after type-1 tympanoplasty: endoscopic versus microscopic approach, a comparative study. *Eur Arch Otorhinolaryngol.* (2020) 277:361–6. doi: 10.1007/s00405-019-05706-z
49. Ozturan O, Dogan R, Eren SB, Aksoy F. Intraoperative thermal safety of endoscopic ear surgery utilizing a holder. *Am J Otolaryngol.* (2018) 39:585–91. doi: 10.1016/j.amjoto.2018.07.001

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

STUDY PROTOCOL
published: 10 March 2022
doi: 10.3389/fped.2022.836230

A Phase II Trial of a Personalized, Dose-Intense Administration Schedule of ^{177}Lutetium-DOTATATE in Children With Primary Refractory or Relapsed High-Risk Neuroblastoma–LuDO-N

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OPEN ACCESS

Edited by:
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Maria Carmen Garganese, Bambino Gesù Children's Hospital (IRCCS), Italy

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Specialty section:
This article was submitted to Pediatric Surgery, a section of the journal Frontiers in Pediatrics

Received: *15 December 2021*
Accepted: *31 January 2022*
Published: *10 March 2022*

Citation:
Sundquist F, Georgantzi K, Jarvis KB, Brok J, Koskenvuo M, Rascon J, van Noesel M, Grybäck P, Nilsson J, Braat A, Sundin M, Wessman S, Herold N, Hjorth L, Kogner P, Granberg D, Gaze M and Stenman J (2022) A Phase II Trial of a Personalized, Dose-Intense Administration Schedule of ^{177}Lutetium-DOTATATE in Children With Primary Refractory or Relapsed High-Risk Neuroblastoma–LuDO-N. Front. Pediatr. 10:836230. doi: 10.3389/fped.2022.836230

Background: Half the children with high-risk neuroblastoma die with widespread metastases. Molecular radiotherapy is an attractive systemic treatment for this relatively radiosensitive tumor. ^{131}I-mIBG is the most widely used form in current use, but is not universally effective. Clinical trials of ^{177}Lutetium DOTATATE have so far had disappointing results, possibly because the administered activity was too low, and the courses were spread over too long a period of time, for a rapidly proliferating tumor. We have devised an alternative administration schedule to overcome these limitations. This involves two high-activity administrations of single agent ^{177}Lu-DOTATATE given 2 weeks apart, prescribed as a personalized whole body radiation absorbed dose, rather than a fixed administered activity. "A phase II trial of ^{177}Lutetium-DOTATATE in children with primary refractory or relapsed high-risk neuroblastoma - LuDO-N" (EudraCT No: 2020-004445-36, ClinicalTrials.gov Identifier: NCT04903899) evaluates this new dosing schedule.

Methods: The LuDO-N trial is a phase II, open label, multi-center, single arm, two stage design clinical trial. Children aged 18 months to 18 years are eligible. The trial is conducted by the Nordic Society for Pediatric Hematology and Oncology (NOPHO) and it has been endorsed by SIOPEN (https://www.siopen.net). The Karolinska University Hospital, is the sponsor of the LuDO-N trial, which is conducted in collaboration with Advanced Accelerator Applications, a Novartis company. All Scandinavian countries, Lithuania and the Netherlands participate in the trial and the UK has voiced an interest in joining in 2022.

Results: The pediatric use of the Investigational Medicinal Product (IMP) ^{177}Lu-DOTATATE, as well as non-IMPs SomaKit TOC® (^{68}Ga-DOTATOC) and LysaKare® amino acid solution for renal protection, have been approved for pediatric use, within the LuDO-N Trial by the European Medicines Agency (EMA). The trial is currently recruiting. Recruitment is estimated to be finalized within 3–5 years.

Discussion: In this paper we present the protocol of the LuDO-N Trial. The rationale and design of the trial are discussed in relation to other ongoing, or planned trials with similar objectives. Further, we discuss the rapid development of targeted radiopharmaceutical therapy and the future perspectives for developing novel therapies for high-risk neuroblastoma and other pediatric solid tumors.

Keywords: neuroblastoma, relapse, refractory, ^{177}Lu-DOTATATE, PRRT, radiopharmaceutical, therapy, high-risk

INTRODUCTION

Neuroblastoma (NBL) is a malignant disease that most commonly occurs in early childhood, and displays a wide heterogeneity regarding biological features, clinical presentation, morbidity, and mortality (1). It derives from neural crest cells, precursors of the sympatico-adrenergic system, manifesting in the adrenal glands and/or along the para-vertebral sympathetic ganglia (1). With an incidence of ∼10 cases per million children under 15 years of age, approximately 1 in 7,000 children are affected (2). NBL is risk-stratified in terms of historical outcome in low-, intermediate- or high-risk based on age, stage, histologic classification, grade of tumor differentiation, MYCN amplification and chromosome 11q deletion status, according to The International Neuroblastoma Risk Group (INRG) Classification System (3). At least half of NBL patients are high-risk (HR). Treatment of HR-NBL generally consists of (i) an induction phase with intensive neoadjuvant chemotherapy, (ii) establishment of local control by surgery and radiotherapy, (iii) systemic remission consolidation therapy with high-dose chemotherapy and autologous stem cell reinfusion, and (iv) maintenance therapy with anti-GD2 antibodies and retinoic acid (3–5). In spite of this multimodal treatment, survival has remained at ∼50% in HR-NBL. In case of a relapse, primary refractory disease or ultra-high-risk features, the outcome is dismal (1, 3, 4, 6). Historically treatment options for relapsed or refractory disease have focused on further cytotoxic chemotherapy with unsatisfying outcomes, partly due to chemo-resistance and poor bone marrow reserve after prior therapies and bone marrow disease (7). There is no consensus regarding the most effective therapy in this setting and currently two other European trials are focusing on this group of patients: The VERITAS trial comparing the combination of cytotoxic chemotherapy and intravenous targeted ^{131}I-mIBG radiotherapy with high-dose cytotoxic chemotherapy; *(VERITAS trial, Institute Gustave Roussy, Paris, France, ClinicalTrials.gov Identifier: NCT03165292)* and the MINIVAN trial that compares different combinations of anti-PD1 and anti-GD2 therapy and intravenous targeted ^{131}I-mIBG radiotherapy, *(MINIVAN trial, University Hospital Southampton NHS Foundation Trust, UK, ClinicalTrials.gov Identifier: NCT02914405)*. In addition, the BEACON trial comparing the combination of cytotoxic chemotherapy and anti-VEGF therapy with cytotoxic chemotherapy alone; *(BEACON trial, University of Birmingham, ClinicalTrials.gov Identifier: NCT02308527)* was recently finalized. Also, a multinational trial evaluating the combination of Naxitamab (anti-GD2) and GM-CSF (YmAbs Therapeutics, *ClinicalTrials.gov Identifier: NCT03363373)* is currently recruiting in several European countries.

Neuroblastoma is known to be a radiosensitive disease, although if the disease is localized in several different sites external beam radiotherapy is not suitable. Therefore, targeted molecular radiotherapy is a more attractive treatment option in this setting, as it delivers tumor specific radiation and can target multiple sites of disease from the same administration (8–14). Studies have shown that 85–90% of neuroblastomas express the noradrenaline transporter molecule and can be targeted with the catecholamine analog, meta-iodobenzylguanidine (mIBG), labeled with ^{123}I for imaging and with the β-emitter ^{131}I for therapy. Molecular radiotherapy with ^{131}I-mIBG has been used

in relapsed and refractory neuroblastoma since the mid 1980's (15). Published data includes results from Phase I, Phase II trials and Pilot Studies with reported response rates ranging from 0 to 60% but there has been a wide variation in the administered radiation doses and methods of response assessment (16–20). The dose limiting toxicity for ^{131}I-mIBG is confined to the bone marrow and attempts to increase the response rate to ^{131}I-mIBG have included dose escalation and the use of radio-sensitizing agents, for example Topotecan. Both of these approaches require stem cell support to circumvent myelotoxicity (21, 22).

Another alternative target for molecular radiotherapy in neuroblastoma is the somatostatin receptors. Prior studies during the 1990's characterized the expression of somatostatin receptors in neuroblastoma cell lines and *in vivo* scintigraphic tumor imaging suggested putative therapeutic applications. Further studies confirmed that somatostatin analog therapy was effective in neuroblastoma xenografts *in vivo* (23–27). Recent studies based on immunohistochemistry have demonstrated expression of all known somatostatin receptors SSTR 1–5 on primary neuroblastoma (28). More specifically, SSTR 2 is expressed in the majority of neuroblastomas, even in recurrent tumors and in primary refractory disease (29). This can be utilized according to the same principle as ^{123}I- and ^{131}I-mIBG, by targeting SSTR with somatostatin analogs that can be labeled with ^{68}Ga for imaging and with the β-emitter ^{177}Lu for therapy. ^{177}Lu-DOTATATE is composed of a somatostatin analog (TATE) and a chelator (DOTA) that binds the radio-isotope Lutetium-177. TATE binds to somatostatin receptors 1–5 (30), the binding between TATE and SSTR-2 having the highest affinity. The cytotoxic effect of Lutetium is primarily caused by emission of β-radiation. The effective range of the β-radiation is about 0.67 mm, causing a local effect on approximately 10^6 cells. About 10% of the radiation energy emitted is γ radiation that can be detected by SPECT imaging and utilized for dosimetry by determination of radiation dosage to the tumor target sites and to organs at risk. PET/CT, using the tracer ^{68}Ga-DOTATATE or DOTATOC, another somatostatin analog that binds to SSTR receptors, can be used for identifying patients eligible for treatment with ^{177}Lu-DOTATATE and this method can also be used for evaluating the response to treatment (31). ^{177}Lu-DOTATATE therapy is thus, theoretically analogous to the well-established mIBG-therapy, but it utilizes a different targeting molecule and also a different radio-isotope for delivering beta radiation specifically to the tumor lesions.

^{177}Lu-DOTATATE targeting the SSTRs has been shown to be effective in the treatment of neuroendocrine tumors in adults. This type of tumor-specific radiation therapy, called peptide receptor radionuclide therapy (PRRT), has recently been established as second-line treatment for grade 1 and 2 midgut neuroendocrine tumors (NET), that progress after first-line treatment with octreotide (32). ^{177}Lu-DOTATATE has been well-tolerated in adult patients with a low incidence of hematological and renal toxicity, which are the main reported side effects. Renal irradiation is compounded by proximal tubular reabsorption of the radiolabeled somatostatin analog and co-administration of amino acid solution has been shown to reduce the risk of renal damage, as has limiting the estimated cumulative radiation dose to the kidneys to 23 Gy or less. The administration of cationic amino acids saturates the renal tubular uptake of proteins/peptides and amino acids, thereby reducing the exposure to the radionuclide (33–35). Severe delayed complications such as secondary malignancies and renal insufficiency has been reported in adult cohorts, but in general, severe side effects are rare. Brabander et al. (36) found that 1.5% of patients developed myelodysplastic syndrome and 0.7% developed acute leukemia during a median follow-up of 5 years after Lu-DOTATATE treatment in a cohort of 610 patients. No therapy-related long-term renal or hepatic failure occurred. A study by Garske-Roman et al. (37) found similar frequencies in a cohort of 200 patients and a median follow-up of 31 months. In this cohort 4% of patients developed grade 2 and 0.5% grade 4 renal toxicity (30, 36, 37). In a recent report from the NETTER-1 Trial, 2% of 111 patients developed myelodysplastic syndrome, whereas no cases of acute myeloid leukemia were reported. One patient developed diffuse large B-cell lymphoma during long-term follow-up, deemed not related to the ^{177}Lu-DOTATATE treatment. The long-term renal function was similar in treatment and control groups, but the number of evaluable patients was small (38).

Pilot studies on pediatric patients, performed by Gains et al. at the University College London Hospitals NHS Trust, UK (UCLH) and by Kong et al. the Royal Children's Hospital, Melbourne, Australia (RCH) have shown that ^{68}Ga-DOTATOC or DOTATATE PET/CT is a feasible method to identify patients with neuroblastoma that can benefit PRRT with ^{177}Lu-DOTATATE. Both of these studies reported promising results from treating children with refractory or relapsed neuroblastoma with ^{177}Lu-DOTATATE, some of which achieving remission that lasted for several years (39, 40). A phase IIa LuDO trial at UCLH, did not, however, show an objective response among 14 evaluable patients (41). In this study, dose limiting toxicity was observed only in one patient and the investigators speculate that this may have been due to concurrent use of myelosuppressive antibiotics, rather than an actual side effect of the trial treatment. The measured renal radiation dose was lower than the objective in all cases and the median value was only about 70% of the 23 Gy objective. The main reason for this was the rapid disease progression in many of the study subjects, indicating that with an intensified dosing schedule, the administered activity could have been substantially increased in most patients. Despite the negative result, the investigators conclude that ^{177}Lu-DOTATATE may have value as a treatment for neuroblastoma and propose further clinical trials to be conducted (29). The design of the LuDO-N Trial, described in this paper, builds on the experience of the recent LuDO-Trial.

METHODS AND ANALYSIS

Study Design and Setting

The LUDO-N Trial (EudraCT number: 2020-004445-36, ClinicalTrials.gov Identifier: NCT04903899) is a prospective, non-blinded, open-label, single arm two stage, multicenter phase II trial for pediatric patients between 18 months and 18 years old with relapsed or refractory high-risk neuroblastoma. Patients

are recruited from the Nordic Society of Pediatric Hematology and Oncology (NOPHO) catchment area, including the Nordic countries and Lithuania, and from the Netherlands with a combined population of roughly 50 million. The LuDO-N Trial is sponsored by the Karolinska University Hospital (Stockholm, Sweden). Study centers have been established in each of the participating countries and the ^{177}Lu-DOTATATE treatment will be given at the Karolinska University Hospital, Stockholm Sweden, the Princess Máxima Center, Utrecht, The Netherland and possibly at a later stage at the University College London Hospitals, London, UK. The trial aims to confirm the dose and assesses the response to ^{177}Lu-DOTATATE (provided by Advanced Accelerator Applications, a Novartis company) as a single agent for treatment of relapsed or refractory high-risk NBL in children. The pediatric use of the Investigational Medicinal Product (IMP) ^{177}Lu-DOTATATE, as well as non-IMPs SomaKit TOC® (^{68}Ga-DOTATOC) and LysaKare® amino acid solution for renal protection, have been approved for the LuDO-N Trial by the European Medicines Agency (EMA). Competent authority and ethical approvals and are being applied for separately in each of the participating countries. Fourteen patients will be recruited in the first stage and provided a response, as defined by the Revised International Neuroblastoma Response Criteria (INRC) 1 month after completion of therapy, is seen in 3 or more of these patients, a further 10 will be enrolled in the second stage (42, 43). Accrual is expected to last for up to 60 months. Follow-up continues until 5 years after the last ^{177}Lu-DOTATATE treatment or death, of all included patients, whichever occurs first. At writing, the LuDO-N Trial has opened for recruitment in Sweden and Norway and the first patient has recently received ^{177}Lu-DOTATATE treatment within the trial.

Consent and Screening

Participants are screened and recruited by the National Principal Investigator (PI), or delegate, in each of the participating countries. It is the responsibility of the national PI to give oral and written information about the trial and to obtain written informed consent for each patient to be registered. No trial specific procedure is carried out prior to the consent form being signed by the patient and/or parents or legal guardians. All patients undergo the following assessments as part of screening. Please see **Tables 1**, **2**.

TABLE 1 | Screening.

Screening	Method	Timeframe
Imaging	^{123}I-MIBG	Within 2 months prior to trial treatment
	^{68}Ga DOTATOC PET	Within 2 months prior to registration and within 2 weeks prior to trial treatment
	MRI/CT	Within 2 months prior to trial treatment
Bone marrow	Aspirate and trephines According to INSS guidelines	Within 2 months prior to trial treatment
Kidney function	GFR according to recognized local method eGFR	Within 2 months prior registration Prior to trial treatment
Clinical status	Clinical status, vital sign, weight, and performance status.	Within a week prior to registration and prior to each treatment
Urine catecholamine metabolites	Dopamine, HVA, VMA	Within 2 months prior to trial treatment
Blood tests	Hematology, biochemistry	Within a week prior to trial treatment
Pregnancy test		Within a week prior to Cycle 1 dosing
Peripheral blood stem cells		Available at start of trial treatment

Radiation Safety

Radiation exposure is governed by Swedish national legislation, as defined in the Swedish Radiation Safety Authority regulations SSMFS 2018:1 and 2018:5, in accordance with the European Council Directive 2013/59/EURATOM. It is recognized that children receiving molecular radiotherapy will require care and support from adults whilst receiving their treatment. Children receiving ^{177}Lu-DOTATATE will be radioactive and will also have radioactive bodily products such as urine, saliva and vomit, all of which represent a potential radiation hazard to those adults caring for them during their treatment. Potential Supporting Persons will be informed of the risks of radiation exposure and trained in radiation hygiene and appropriate precautions to keep their personal radiation exposure as low as reasonably achievable – the ALARA principle. Patients will require specific medical interventions during their treatment from doctors, nurses and other health care professionals. These individuals will therefore receive some radiation during these tasks. Health care professionals are governed by annual radiation limits and it is essential, in keeping with the ALARA principle, that they do not receive avoidable radiation. Therefore, Supporting Persons are asked to undertake all normal child-care tasks such as washing, dressing, feeding, entertainment and support during the first 24 h after ^{177}Lu-DOTATATE administration.

Study Interventions

A baseline ^{68}Ga-DOTATOC PET/CT is performed within 2 weeks, prior to ^{177}Lu-DOTATATE treatment. A total of two doses of ^{177}Lu-DOTATATE are administered intravenously 2–4 weeks apart. A weight-based activity of 200 MBq kg^{-1} is used for the first dose. The activity of the second dose is calculated based on whole body activity scans as well as SPECT CT scans to determine the absorbed kidney dose. The aim is to administer ^{177}Lu-DOTATATE corresponding to a whole-body dose of 1,2 Gy, with a cumulative whole-body dose of about 2,4 Gy over two courses, and not exceeding a cumulative renal dose of 23 Gy, in order to avoid renal toxicity (41). Please see **Table 3**. LysaKare® amino acid solution for renal protection is administered as a 4-h infusion of 20 ml/kg, which corresponds to typical fluid resuscitation dose in children. This

TABLE 2 | Inclusion and Exclusion criteria.

Rule	Criterium	Definition
Inclusion	Pathology	• Histologically confirmed diagnosis of neuroblastoma
	Stage	• Relapsed or primary refractory high-risk neuroblastoma: INSS stage 4 disease or INRGSS stage M disease
	Age	• Age >18 months and <18 years
	Life expectancy	• >3 months
	Performance status	• Karnofsky >50% or Lansky >50%
	Prior treatment	• 2 weeks since prior treatment • Recovered from prior hematological toxicity • Recovered from major surgery
	Diagnostic imaging	• Uptake in the primary tumor or metastatic tumor deposits on ^{68}Ga-DOTATATE PET/CT exceeding the liver uptake and performed within two months prior to registration • ^{123}I-mIBG scintigraphy to be performed within two months prior to registration • CT or MRI of the primary tumor and bulky metastatic sites within 2 months prior to registration
	Biochemistry	Hematology: • Hemoglobin, If Hb is <120 g/L then patient will receive a blood transfusion prior to commencing trial treatment • Absolute neutrophil granulocyte count > 1.0 x 10^9/L • Absolute platelet count > 100 x 10^9/L Biochemistry: • Bilirubin within 1.5 x ULN • ALT within 2.5 x ULN • AST within 2.5 x ULN • GGT within 5 x ULN • ALP within 5 x ULN • Glomerular filtration rate >50 mL/min/1.73m^2 assessed by a recognized method, such as inulin, ^{51}Cr-EDTA, ^{99m}Tc-DTPA or Iohexol clearance and performed within 2 months prior to registration • Urinary catecholamine metabolites measured within 2 months prior to registration
	Peripheral blood stem cells	• A minimum of 4 x 10^6 CD34 + cells/kg (optimally 6 x10^6 CD34+ cells/kg) must be available for each study subject prior to registering
	Consent	• Written informed consent from patient and/or parent(s) or legal guardian(s) in accordance with national regulations, prior to registration or any trial-related screening procedures
Exclusion	Pregnant	• Pregnant or lactating patient
	Condition	• Not fit enough to undergo proposed study treatment, as assessed by national PI.
	Concurrent treatment	• Concurrent treatment with any anti-tumor agents
	Prior treatment	• Treatment with long-acting somatostatin analogs within 30 days prior the administration of ^{177}Lu-DOTATATE • Prior treatment with other radiolabeled somatostatin analogs
	Allergy	• Hypersensitivity to any component of the investigational drug ^{177}Lu-DOTATATE
	Compliance	• Any psychological, familial, sociological or geographical condition potentially hampering compliance with the study protocol and follow-up schedule

dose translates to the established adult dose of 1,000 ml in a child weighing 50 kg.

Study Outcomes

Main Study Objective

To assess response to single agent ^{177}Lutetium-DOTATATE treatment in patients with relapsed or refractory high-risk neuroblastoma.

Secondary Study Objectives

- To assess long term survival and response.
- To assess treatment-related toxicity.

Sub Study Objectives

- To correlate tumor dosimetry with response.
- To correlate somatostatin type 2 receptor (SSTR-2) expression with ^{68}Ga-DOTATOC PET/CT uptake.
- To correlate the uptake on ^{68}Ga-DOTATOC PET/CT with response to ^{177}Lu-DOTATATE therapy.

Adverse Events

An adverse event (AE) is any untoward medical occurrence or experience in a patient administered with an investigational product and which does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (such as a rash or an abnormal laboratory finding) symptom, or disease temporally associated with the use of the protocol treatment. Any AE occurring during the reporting period is to be reported in the eCRF. The Investigator should assess the seriousness and causality (relatedness) of all AEs experienced by the patient and document the assessments in the patient records. Each AE should be classified with the severity grade in accordance with the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 5.0 (https://ctep.

TABLE 3 | Administration schedule.

Day	Action	Definition
−14– −1	^{68}Ga-DOTATOC PET/CT	Baseline
0	^{177}Lu-DOTATATE **dose 1**	Dosage: **200** MBq kg^{-1}
+1–7	SPECT/CT	Minimum of 3 images
+1–14	Assessment of clinical status/condition/biochemistry	According to trial protocol, see **Supplementary**
+14–28	^{177}Lu-DOTATATE **dose 2**	Dosage: **x** MBq kg^{-1}, **x** calculated from SPECT/CT, target maximum dose **≤2,4 Gy to whole-body** (calculated from image data) AND **≤2,4 Gy to whole-body** (calculated from dose rate survey readings) AND **≤23 Gy to either kidney** (mean dose)

cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf). An AE not included in the CTCAE should be graded by an Investigator and recorded on the AE Form using a scale of (1) mild, (2) moderate or (3) severe. For each sign/symptom, the highest grade observed since the last visit should be recorded. Any Serious Adverse Event (SAE) occurring during the reporting period must be reported by the site investigator. Prompt initial reporting is required within 24 h after the investigator first becoming aware of the event. On becoming aware that a patient has experienced a SAE, the Investigator (or delegate) must complete, date and sign a trial specific SAE report. The report should be faxed together with a SAE fax cover sheet to Clinical Trials Office (CTO), Center for Clinical Cancer Studies (CKC), Karolinska University Hospital.

Data Collection

Trial data is collected and entered into the eCRF system PheedIt provided by Clinical Trials Office (CTO), Center for Clinical Cancer Studies (CKC), Karolinska University Hospital. eCRFs are required to be completed for each subject enrolled in the trial and all continuously collected data will be entered in the eCRF in a timely manner, within 1 month. All data must be entered in English. The PI is responsible for ensuring the accuracy, completeness and legibility of the data recorded in the eCRFs. Study monitors, data manager or the study statistician will review data according to the Monitoring Plan and Data Management Plan. Queries that are sent to site will require response and confirmation or correction of the data by delegated site staff. The Data Management Plan provides detailed information about data collection and data management throughout the trial.

Data Quality and Monitoring

The source documents consist of the medical records and any additional relevant documentation for trial purpose. This documentation will contain trial information such as trial number, date of informed consent, trial assignment and the name of the trial PI.

Source data for all variables will be defined in a source data log, which will be stored in the Investigator's Site File. Specific trial information such as trial title, trial number, date of informed consent, trial treatment and that the patient adheres to the inclusion and exclusion criteria should be recorded in the medical record.

Site Set-Up and Initiation

Prior to site activation of each participating site must have an Investigator Site File with all essential documents in place, including authority approvals, Clinical Study Site Agreements and a signed delegations log. Key members of the site research team will be required to attend either a meeting or a teleconference covering aspects of the trial aim and design, protocol procedures, Adverse Event reporting, collection and reporting of data and record keeping.

Trial Monitoring

The trial quality will be assured by trial monitoring in accordance with ICH-GCP of the trial sites in all participating countries. The monitoring will be carried out in accordance with the risk assessment and a common Monitoring Plan prepared by the monitoring unit in Sweden. At least three monitoring visits will be performed; the site initiation visit, a visit after the first included patient and a close-out visit. Additional on-site monitoring visits or more extensive source data verification may be triggered for example by poor CRF return, poor data quality, low/high SAE reporting rates, excessive number of patient withdrawals or deviations. The data recorded in the eCRFs will be controlled for consistency with the source data/hospital records during the monitoring (source data verification). Any discrepancies of data will be documented and explained in the monitoring reports.

Audit and Inspection

The Investigator and site staff will permit trial-related monitoring, audits, ethical review, and regulatory inspection(s) at their site, providing direct access to source data/documents.

Statistical Analysis Plan

The sample size calculation is based on a Simon Two Stage Minimax design and the primary outcome measure of response rate assessed by the Revised International Neuroblastoma Response Criteria at 1 month after completion of therapy (42, 43). A complete response + partial response rate of 40% or more is defined as the acceptable level of response. A response of 20% or less would be considered unacceptably low. The probability of obtaining a false negative result, β (i.e., incorrectly rejecting for further study a treatment with a true response rate of ≥ 40%) is set at 20%. The probability of obtaining a false positive result, α (i.e., incorrectly accepting for further study a treatment that has a true response rate ≤20%) is set at 10%. Stage 1 requires 14 eligible and evaluable patients, with a minimum of 3 to be responders to proceed to stage 2. A further 10 patients will be recruited in Stage 2. A minimum of 8 responses out of 24 patients would be considered success and indicate that the treatment is active and should go onto further studies to evaluate efficacy (while taking account of toxicity). A standard

Simon 2 stage has been chosen over a single stage (e.g., A'Hern) design in order to allow the trial to be stopped sooner, and to prevent unnecessary exposure of patients to radiotherapy, if insufficient responses are seen. Simon Optimal (3 responses /12 patients after first stage, 8/25 at end) and Minimax (3/14, 8/24) designs gives very similar numbers, so the decision to choose the latter is largely arbitrary (one patient fewer in total, but slightly more conservative after first stage). Sample size calculations were performed using Sample Size Tables for Clinical Trials software by Machin and Campbell. Loss to follow-up is likely to be minimal; all patients are referred from clinicians at established oncology centers with excellent close relations and regular communication or the patient's continued care is at the recruiting hospital. In addition, it is current standard practice to follow-up patients until they are 18 and then refer them to adult late effect physicians (according to long term follow-up guidelines from Children's Cancer and Leukemia Group (CCLG) and other relevant guidelines).

Analysis

Response to treatment is assessed using the Revised International Neuroblastoma Response Criteria (INRC). Overall response is defined as complete response, partial response, minor response, stable disease, or progressive disease. Baseline assessment is carried out within 2 months prior to registration and an additional ^{68}Ga-DOTATOC PET/CT is performed within 2 weeks prior to treatment. Response evaluation is performed 1 month and 4 months post treatment. Overall response integrates tumor response in the primary tumor, soft tissue and bone metastases, and bone marrow. Primary and metastatic soft tissue sites are assessed by MRI or CT, ^{68}Ga-DOTATOC PET/CT and ^{123}I-mIBG imaging. Target and non-target lesions are defined and scored according to the RECIST 1.1 guidelines. Assessment of bone marrow involvement is done by evaluation of bilateral aspirates and bilateral trephine biopsies from a total of four sampled sites. The analysis of the primary outcome measure is carried out on an eligible and evaluable patient basis. Patients who are registered, but for any reason did not complete treatment and/or the first response evaluation at 1 month after EOT, are deemed not evaluable, and so will be excluded from the primary analysis. Outcomes for all patients will be reported on an intention-to-treat basis. Descriptive statistics are used to summarize baseline characteristics, treatment, report harms, and response outcomes.

Primary Outcomes Measure

- Response by the Revised International Neuroblastoma Response Criteria and RECIST 1.1 guidelines at 1 month after completion of therapy (42, 43).

Secondary Outcomes Measures

- Response by the Revised International Neuroblastoma Response Criteria and RECIST 1.1 guidelines at 4 months after completion of therapy.
- Progression-free Survival (PFS), defined as the time from registration until objective tumor progression or death or to date of censoring for patients who do not experience the event during trial follow-up.
- Overall Survival (OS), defined as the time from registration into the trial until date of death (death from any cause) or to date of censoring for patients who do not experience the event during trial follow-up.
- Hematological and renal toxicity according to CTCAE 5.0.

Sub Study Measures

- Tumor and risk organ dosimetry (SPECT/CT), defined as the absorbed dose in Gray that the tumor sites and organs of risk receive following each administration of ^{177}Lu-DOTATATE.
- Semi-quantitative analysis of the expression of SSTR-2 on immunohistochemistry in the primary tumor histology. The results will be correlated with tumor uptake on ^{68}Ga-DOTATOC PET/CT.
- The uptake on ^{68}Ga-DOTATOC PET/CT measured by SUV_{max} (maximum standardized uptake value). Pre-treatment SUV_{max} values will be correlated to the response to ^{177}Lu-DOTATATE therapy.

Planned Interim Analysis and Timelines

The two-stage design will ensure that the study is terminated after the first stage if there is insufficient evidence of therapeutic activity. If any unexpected severe adverse events (CTC grades 3, 4) are encountered, consideration will be given to suspending the entry of new patients pending clarification of a causal relationship, and the trial may be stopped if unacceptable toxicities are observed. An interim analysis will be performed after inclusion of the first 14 patients. The main analysis of all outcome measures is planned at 6 months after completion of treatment of the last patient. In addition, subsequent analysis of all survival outcome measures will be conducted 5 years after completion of treatment of the last patient.

Current Trial Status

Active in Sweden and Norway, recruiting since May 2021.

DISCUSSION

PRRT with ^{177}Lu-DOTATATE is an attractive new option with potentially less adverse effects and a significantly lesser exposure to radiation for persons participating in the care of the patient, as compared to ^{131}I-mIBG -therapy (36, 37, 44). Contributing factors to these findings have not been exactly defined, but probably include a lesser extent of accumulation of ^{177}Lu-DOTATATE in the bone marrow, as well as a shorter biological half-life and a smaller proportion of gamma-radiation emitted by ^{177}Lu as compared to ^{131}I. While pilot trials on ^{177}Lu-DOTATATE at UCLH, London and RCH, Melbourne have shown promising results, the phase IIa LuDO trial recently conducted at UCLH concluded that ^{177}Lu-DOTATATE in children was safe, but ineffective at the given dose and dosing schedule (39–41). In the LuDO trial the administration schedule was based on what was shown to be effective in an adult neuroendocrine tumor (NET) setting with a fixed administered activity every 2 months over a period of 6 months and to be

within safety limits for renal and hematological toxicity. While no objective responses were seen by the standard criteria, interim assessment did show a reduction in the mIBG semiquantitative score in three patients, indicating some effect of treatment, even if it was not sustained. Compared to NET, neuroblastoma is a rapidly proliferating tumor and the intervals between administered activity may have allowed re-population that would have masked any objective responses. While the aim was to deliver a maximum kidney dose of 23 Gy to all patients, the median kidney dose actually delivered was only 16,5 Gy with this fixed activity schedule (41). The aim of the current LuDO-N trial is to establish whether ^{177}Lu-DOTATATE can be effective as a single agent, in the treatment of relapsed or primary refractory high-risk neuroblastoma, when the dose schedule is intensified to two doses delivered 2 weeks apart and the administered activity is optimized/personalized by dosimetry. Due to an anticipated increased risk of myelotoxicity, secondary to the short recovery interval between doses, autologous hematopoietic stem cells are available as stem cell support in all patients. The intensified dosing schedule is similar to the one used successfully in previous studies on ^{131}I-mIBG in relapsed or primary refractory high-risk neuroblastoma and it allows for an increased dose rate as compared to the LuDO trial, without increasing the cumulative radiation dose (22).

To our knowledge, there are currently two similar clinical trials on radiopharmaceutical therapy in neuroblastoma. One is under preparation: Safety Evaluation of Peptide Receptor Radionuclide Therapy (PRRT) With ^{177}Lu-DOTA0-Tyr3-Octreotate for Refractory or Recurrent Metastatic Neuroblastoma Expressing Somatostatin Receptors (NEUROBLU 02), Institut Universitaire du Cancer Toulouse – Oncopole, Toulouse, France (ClinicalTrials.gov Identifier: NCT03966651). The NEUROBLU 02 trial uses a dose escalation design to assess the effective dose and the highest dose of ^{177}Lu-DOTATATE, that can be given safely without the need for stem cell re-infusion. In order to make results from the NEUROBLU 2 and LuDO-N trials comparable, the dosimetry and response evaluation protocols of these two trials have been harmonized. Another similar multi-center trial in the USA, is already recruiting: ^{67}Cu-SARTATE™ Peptide Receptor Radionuclide Therapy Administered to Pediatric Patients with High-Risk, Relapsed, Refractory Neuroblastoma (ClinicalTrials.gov Identifier: NCT04023331). The trial is sponsored by Clarity Pharmaceuticals Ltd. SARTATE, similarly to DOTATATE, binds to the SSTR-2 receptor and the cytotoxic effect of ^{67}Cu is, also similarly to ^{177}Lu, caused by β-radiation. Imaging and response evaluation is performed using another copper isotope ^{64}Cu. (45, 46). In addition, ^{131}I-mIBG has been randomized as first line treatment, in two of the treatment arms of the currently ongoing Children's Oncology Group (COG) ANBL 1531 Trial (ClinicalTrials.gov Identifier: NCT03126916), in an effort to target the metastatic disease in an early stage of the treatment. The ANBL 1531 trial uses a weight-based dosing of ^{131}I-mIBG (47).

While the crossfire effect of β-emitters like ^{131}I, ^{177}Lu or ^{67}Cu, can be beneficial for treatment of bulky disease in a refractory disease or relapse setting, there is a concern, that the radiation energy might not be sufficient to cause a cytotoxic effect in single, or small clusters of metastatic cells, containing only a limited number of target antigens (48). In this situation, most of the radiation would be dispersed to surrounding healthy tissues and subsequently, the radiation dose to the metastatic cells may be insufficient. The higher linear energy transfer (LET) and significantly shorter path length of the α-particle emission from ^{213}Bi or ^{225}Ac, might be preferable for effective depletion of micro-metastasis, since a lethal dose to a small number of cells, can be delivered from only 1–20 α-particle traversals of the cell nucleus (48–50). Switching from PRRT based on ^{131}I, ^{177}Lu or ^{67}Cu to targeted α-particle therapy (TAT) would, however, include a loss of the cross-fire effect caused by β-emitters, due to the significantly shorter path length of the α-particles compared to β-radiation. This is highly relevant in the context of the macroscopic and microscopic tumor heterogeneity that has been described in neuroblastoma, carrying the subsequent risk that while some tumor cells would be effectively targeted and sterilized, others might escape completely unharmed (45, 46). One possibility for delivering targeted α-particle therapy to bone metastases is to utilize osteomimetic radionuclides, such as ^{223}Ra-dichloride (Xofigo®) that incorporates into newly formed bone matrix within osteoblastic metastatic lesions (51), but this might not be a feasible approach in a growing child. Alternatively, combinations of radionuclide conjugates with different targeting molecules could be explored. While there are many targeting options for neuroblastoma, the combination of ^{177}Lu-DOTATATE with the already established ^{131}I-mIBG therapy, could be a viable alternative for a future follow-up trial aimed at overcoming the problem of tumor heterogeneity.

CONCLUSION

Overall survival in high-risk neuroblastoma is about 50% and long-term survival in the setting of primary refractory disease or relapse is exceedingly rare. Novel therapies, such as ALK-inhibitors can offer a possibility to reach secondary remission in a selected group of patients, but for the vast majority of cases there are currently no effective treatment options. We estimate that the potential benefits of ^{177}Lu-DOTATATE treatment with an intensified dosing schedule, outweigh the risks in this specific group of pediatric patients with primary refractory or relapsed high-risk neuroblastoma, in whom established options for curative treatment have been exhausted. We hope that the results from the LuDO-N trial and similar trials on radiopharmaceutical therapy for high-risk neuroblastoma, will generate valuable knowledge, leading to effective therapeutic options that can significantly improve survival in the future.

ETHICS STATEMENT

The trial will be conducted according to the trial protocol, ICH-GCP, EU-directive (2001/20/EC) and applicable regulatory requirements and in accordance with the principles described in the latest version of the Declaration of Helsinki, available through

the World Medical Association (WMA) website: (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/). The patient information and informed consent-form will incorporate wording that complies with relevant data protection and privacy legislation. The protocol and patient material will be submitted to and approved by the National Ethics Committees prior to implementation. It is the responsibility of the National Coordinating Investigators to ensure that all subsequent amendments gain the necessary local approval. This does not affect the individual clinicians' responsibility to take immediate action if thought necessary to protect the health and interest of individual patients.

AUTHOR CONTRIBUTIONS

FS, PG, JN, MS, SW, NH, LH, PK, DG, MG, and JS conceptualized and participated in drafting the LuDO-N trial protocol. KG, KJ, JB, JR, MK, and MN contributed with valuable feedback on the trial protocol, as well as with drafting national authority applications in order to enable implementation of the trial in their respective countries. AB contributed to the dosimetry and response evaluation protocols and was responsible for setting up a system for central review of the imaging. FS, KG, MG, and JS drafted the manuscript. All authors participated in critical review of the manuscript prior to submission.

FUNDING

The LuDO-N trial has been supported by funding from the Swedish Cancer Society, the Swedish Childhood Cancer Fund, Region Stockholm and Applied Accelerator Applications / Novartis. In addition, all investigational and non-investigational products have been provided free of charge for the LuDO-N trial by Applied Accelerator Applications / Novartis. MG was supported by the National Institute for Health Research, University College London Hospitals, Biomedical Research Center and the Radiation Research Unit at the Cancer Research UK City of London Center Award (C7893/A28990).

ACKNOWLEDGMENTS

We are deeply thankful to Sanna Nyström and Claudia Maes at the Center for Clinical Cancer Studies at the Karolinska University Hospital for providing expert assistance in generating the eCRF and in administrative management of the trial, as well as to Helena Aaröe, Pernilla Callerström, Rut-Inger Enryd and Sofie Garpemo at the pediatric clinical trial unit HOPE at the Karolinska University Hospital, for providing expert assistance in the setup and clinical management of the trial, and finally to Daniela Niepel at Advanced Accelerator Applications / Novartis for valuable feedback on the manuscript.

REFERENCES

1. Matthay KK, Maris JM, Schleiermacher G, Nakagawara A, Mackall CL, Diller L, et al. Neuroblastoma. *Nat Rev Dis Primers.* (2016) 2:16078. doi: 10.1038/nrdp.2016.78
2. Ries LAG. *Cancer Incidence and Survival Among Children and Adolescents: United States Seer Program, 1975–1995*. Bethesda, MD: National Cancer Institute (1999).
3. Ladenstein R, Pötschger U, Pearson ADJ, Brock P, Luksch R, Castel V, et al. Busulfan and melphalan versus carboplatin, etoposide, and melphalan as high-dose chemotherapy for high-risk neuroblastoma (Hr-Nbl1/Siopen): an international, randomised, multi-arm, open-label, phase 3 trial. *Lancet Oncol.* (2017) 18:500–14. doi: 10.1016/S1470-2045(17)30070-0
4. von Allmen D, Davidoff AM, London WB, Van Ryn C, Haas-Kogan DA, Kreissman SG, et al. Impact of extent of resection on local control and survival in patients from the cog A3973 study with high-risk neuroblastoma. *J Clin Oncol.* (2017) 35:208–16. doi: 10.1200/JCO.2016.67.2642
5. Holmes K, Pötschger U, Pearson ADJ, Sarnacki S, Cecchetto G, Gomez-Chacon J, et al. Influence of surgical excision on the survival of patients with stage 4 high-risk neuroblastoma: a report from the Hr-Nbl1/siopen study. *J Clin Oncol.* (2020) 38:2902–15. doi: 10.1200/JCO.19.03117
6. Basta NO, Halliday GC, Makin G, Birch J, Feltbower R, Bown N, et al. Factors associated with recurrence and survival length following relapse in patients with neuroblastoma. *Br J Cancer.* (2016) 115:1048–57. doi: 10.1038/bjc.2016.302
7. Herd F, Basta NO, McNally RJQ, Tweddle DA. A systematic review of re-induction chemotherapy for children with relapsed high-risk neuroblastoma. *Eur J Cancer.* (2019) 111:50–8. doi: 10.1016/j.ejca.2018.12.032
8. Gatcombe HG, Marcus RB Jr, Katzenstein HM, Tighiouart M, Esiashvili N. Excellent Local Control from Radiation Therapy for High-Risk Neuroblastoma. *Int J Radiat Oncol Biol Phys.* (2009) 74:1549–54. doi: 10.1016/j.ijrobp.2008.10.069
9. Robbins JR, Krasin MJ, Pai Panandiker AS, Watkins A, Wu J, Santana VM, et al. Radiation therapy as part of local control of metastatic neuroblastoma: the St Jude children's research hospital experience. *J Pediatr Surg.* (2010) 45:678–86. doi: 10.1016/j.jpedsurg.2009.11.003
10. Casey DL, Kushner BH, Cheung NK, Modak S, LaQuaglia MP, Wolden SL. Local control with 21-Gy radiation therapy for high-risk neuroblastoma. *Int J Radiat Oncol Biol Phys.* (2016) 96:393–400. doi: 10.1016/j.ijrobp.2016.05.020
11. Stenman J, Herold N, Svensson PJ, Kogner P. Improved local control by extensive surgery in high-risk neuroblastoma may be dependent on adjuvant radiotherapy. *J Clin Oncol.* (2017) 35:1965–6. doi: 10.1200/JCO.2016.72.1175
12. Aldridge MD, Peet C, Wan S, Shankar A, Gains JE, Bomanji JB, et al. Paediatric molecular radiotherapy: challenges and opportunities. *Clin Oncol.* (2021) 33:80–91. doi: 10.1016/j.clon.2020.11.007
13. Arumugam S, Manning-Cork NJ, Gains JE, Boterberg T, Gaze MN. The evidence for external beam radiotherapy in high-risk neuroblastoma of childhood: a systematic review. *Clin Oncol.* (2019) 31:182–90. doi: 10.1016/j.clon.2018.11.031
14. Davis L, Smith AL, Aldridge MD, Foulkes J, Peet C, Wan S, et al. Personalisation of molecular radiotherapy through optimisation of theragnostics. *J Pers Med.* (2020) 10:174. doi: 10.3390/jpm10040174
15. Wilson JS, Gains JE, Moroz V, Wheatley K, Gaze MN. A systematic review of 131i-meta iodobenzylguanidine molecular radiotherapy for neuroblastoma. *Eur J Cancer.* (2014) 50:801–15. doi: 10.1016/j.ejca.2013.11.016
16. Hartmann O, Lumbroso JD, Lemerle M, Schlumberger M, Ricard M, Aubert B, et al. The therapeutic use of I-131 meta-iodobenzylguanidine (Mibg) in neuroblastoma: a phase Ii study in 12 patients. *Prog Clin Biol Res.* (1988) 271:655–67.
17. Lewis IJ, Lashford LS, Fielding S, Flower MA, Ackery D, Kemshead J, et al. Phase I/Ii study of 131i Mibg in chemo-resistant neuroblastoma. The United Kingdom children's cancer study group (Ukccsg). *Prog Clin Biol Res.* (1991) 366:463–9.
18. Lumbroso J, Hartmann O, Schlumberger M. Therapeutic use of [131i]metaiodobenzylguanidine in neuroblastoma: a phase Ii study in 26 patients. "Societe Francaise D'oncologie Pediatrique" and nuclear medicine co-investigators. *J Nucl Biol Med.* (1991) 35:220–3.
19. Klingebiel T, Bader P, Bares R, Beck J, Hero B, Jürgens H, et al. Treatment of neuroblastoma stage 4 with 131i-meta-iodo-benzylguanidine, high-dose chemotherapy and immunotherapy. A Pilot Study. *Eur J Cancer.* (1998) 34:1398–402. doi: 10.1016/S0959-8049(98)00130-0

20. Matthay KK, Yanik G, Messina J, Quach A, Huberty J, Cheng SC, et al. Phase Ii study on the effect of disease sites, age, and prior therapy on response to iodine-131-metaiodobenzylguanidine therapy in refractory neuroblastoma. *J Clin Oncol.* (2007) 25:1054–60. doi: 10.1200/JCO.2006.09.3484
21. Matthay KK, Quach A, Huberty J, Franc BL, Hawkins RA, Jackson H, et al. Iodine-131–metaiodobenzylguanidine double infusion with autologous stem-cell rescue for neuroblastoma: a new approaches to neuroblastoma therapy phase I study. *J Clin Oncol.* (2009) 27:1020–5. doi: 10.1200/JCO.2007.15.7628
22. Gaze MN, Chang Y-Ci, Flux GD, Mairs RJ, Saran FH, Meller ST. Feasibility of dosimetry-based high-dose 131i-meta-iodobenzylguanidine with topotecan as a radiosensitizer in children with metastatic neuroblastoma. *Cancer Biother Radiopharm.* (2005) 20:195–9. doi: 10.1089/cbr.2005.20.195
23. Maggi M, Baldi E, Finetti G, Franceschelli F, Brocchi A, Lanzillotti R, et al. Identification, characterization, and biological activity of somatostatin receptors in human neuroblastoma cell lines. *Cancer Res.* (1994) 54:124–33.
24. O'Dorisio M, Chen F, O'Dorisio T, Wray D, Qualman S. Characterization of somatostatin receptors on human neuroblastoma tumors. *Cell Growth Differ.* (1994) 5:1–8.
25. Kogner P, Borgström P, Bjellerup P, Schilling FH, Refai E, Jonsson C, et al. Somatostatin in neuroblastoma and ganglioneuroma. *Eur J Cancer.* (1997) 33:2084–9. doi: 10.1016/S0959-8049(97)00212-8
26. Schilling F, Bihl H, Jacobsson H, Ambros PF, Martinsson T, Borgström P, et al. Combined (111)in-pentetreotide scintigraphy and (123)I-Mibg scintigraphy in neuroblastoma provides prognostic information. *Med Pediatr Oncol.* (2000) 35:688–91. doi: 10.1002/1096-911x(20001201)35:6<688::aid-mpo44>3.0.co;2-7
27. Borgström P, Hassan M, Wassberg E, Refai E-, Jonsson C, Larsson SA, et al. The somatostatin analogue octreotide inhibits neuroblastoma growth *in Vivo*. *Pediatr Res.* (1997) 46:328–32. doi: 10.1203/00006450-199909000-00014
28. Georgantzi K, Tsolakis AV, Stridsberg M, Jakobson A, Christofferson R, Janson ET. Differentiated expression of somatostatin receptor subtypes in experimental models and clinical neuroblastoma. *Pediatr Blood Cancer.* (2011) 56:584–9. doi: 10.1002/pbc.22913
29. Alexander N, Marrano P, Thorner P, Naranjo A, Van Ryn C, Martinez D, et al. Prevalence and clinical correlations of somatostatin receptor-2 (Sstr2) expression in neuroblastoma. *J Pediatr Hematol Oncol.* (2019) 41:222–7. doi: 10.1097/MPH.0000000000001326
30. Reubi JC, Schär JC, Waser B, Wenger S, Heppeler A, Schmitt JS, et al. Affinity profiles for human somatostatin receptor subtypes SST1-SST5 of somatostatin radiotracers selected for scintigraphic and radiotherapeutic use. *Eur J Nucl Med.* (2000) 27:273–82.
31. Alexander N, Vali R, Ahmadzadehfar H, Shammas A, Baruchel S. Review: the role of radiolabeled dota-conjugated peptides for imaging and treatment of childhood neuroblastoma. *Curr Radiopharm.* (2018) 11:14–21. doi: 10.2174/1874471011666171215093112
32. Strosberg J, El-Haddad G, Wolin E, Hendifar A, Yao J, Chasen B, et al. Phase 3 trial of (177)lu-dotatate for midgut neuroendocrine tumors. *N Engl J Med.* (2017) 376:125–35. doi: 10.1056/NEJMoa1607427
33. Bernard BF, Krenning EP, Breeman WA, Rolleman EJ, Bakker WH, Visser TJ, et al. D-Lysine reduction of indium-111 octreotide and yttrium-90 octreotide renal uptake. *J Nucl Med.* (1997) 38:1929–33.
34. Bodei L, Cremonesi M, Zoboli S, Grana C, Bartolomei M, Rocca P, et al. Receptor-mediated radionuclide therapy with 90y-dotatoc in association with amino acid infusion: a phase I study. *Eur J Nucl Med Mol Imaging.* (2003) 30:207–16. doi: 10.1007/s00259-002-1023-y
35. Taylor CA, Shankar A, Gaze MN, Peet C, Gains JE, Wan S, et al. Renal protection during 177lutetium dotatate molecular radiotherapy in children: a proposal for safe amino acid infusional volume during peptide receptor radionuclide therapy. *Nucl Med Commun.* (2021). 43:242–6. doi: 10.1097/MNM.0000000000001497
36. Brabander T, van der Zwan WA, Teunissen JJM, Kam BLR, Feelders RA, de Herder WW, et al. Long-term efficacy, survival, and safety of [(177)Lu-Dota,Tyr]octreotate in patients with gastroenteropancreatic and bronchial neuroendocrine tumors. *Clin Cancer Res.* (2017) 23:4617–24. doi: 10.1158/1078-0432.CCR-16-2743
37. Garske-Roman U, Sandstrom M, Fross Baron K, Lundin L, Hellman P, Welin S, et al. Prospective observational study of (177)Lu-Dota-octreotate therapy in 200 patients with advanced metastasized neuroendocrine tumours (Nets): feasibility and impact of a dosimetry-guided study protocol on outcome and toxicity. *Eur J Nucl Med Mol Imaging.* (2018) 45:970–88. doi: 10.1007/s00259-018-3945-z
38. Strosberg JR, Caplin ME, Kunz PL, Ruszniewski PB, Bodei L, Hendifar A, et al. 177lu-dotatate plus long-acting octreotide versus high-dose long-acting octreotide in patients with midgut neuroendocrine tumours (Netter-1): final overall survival and long-term safety results from an open-label, randomised, controlled, phase 3 trial. *Lancet Oncol.* (2021) 22:1752–63. doi: 10.1016/S1470-2045(21)00572-6
39. Gains JE, Bomanji JB, Fersht NL, Sullivan T, D'Souza D, Sullivan KP, et al. 177lu-dotatate molecular radiotherapy for childhood neuroblastoma. *J Nucl Med.* (2011) 52:1041–7. doi: 10.2967/jnumed.110.085100
40. Kong G, Hofman MS, Murray WK, Wilson S, Wood P, Downie P, et al. Initial experience with gallium-68 dota-octreotate Pet/Ct and peptide receptor radionuclide therapy for pediatric patients with refractory metastatic neuroblastoma. *J Pediatr Hematol Oncol.* (2016) 38:87–96. doi: 10.1097/MPH.0000000000000411
41. Gains JE, Moroz V, Aldridge MD, Wan S, Wheatley K, Laidler J, et al. A phase Iia trial of molecular radiotherapy with 177-lutetium dotatate in children with primary refractory or relapsed high-risk neuroblastoma. *Eur J Nucl Med Mol Imaging.* (2020) 47:2348–57. doi: 10.1007/s00259-020-04741-x
42. Brodeur GM, Pritchard J, Berthold F, Carlsen NL, Castel V. Revisions of the international criteria for neuroblastoma diagnosis, staging and response to treatment. *J Clin Oncol.* (1993) 11:1466–77. doi: 10.1200/JCO.1993.11.8.1466
43. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised recist guideline (Version 1.1). *Eur J Cancer.* (2009) 45:228–47. doi: 10.1016/j.ejca.2008.10.026
44. Gains JE, Walker C, Sullivan TM, Waddington WA, Fersht NL, Sullivan KP, et al. Radiation exposure to comforters and carers during paediatric molecular radiotherapy. *Pediatr Blood Cancer.* (2015) 62:235–9. doi: 10.1002/pbc.25250
45. Gains JE, Aldridge MD, Mattoli MV, Bomanji JB, Biassoni L, Shankar A, et al. 68ga-dotatate and 123i-Mibg as imaging biomarkers of disease localisation in metastatic neuroblastoma: implications for molecular radiotherapy. *Nucl Med Commun.* (2020) 41:1169–77. doi: 10.1097/MNM.0000000000001265
46. Gains JE, Sebire NJ, Moroz V, Wheatley K, Gaze MN. Immunohistochemical evaluation of molecular radiotherapy target expression in neuroblastoma tissue. *Eur J Nucl Med Mol Imaging.* (2018) 45:402–11. doi: 10.1007/s00259-017-3856-4
47. Weiss BD, Yanik G, Naranjo A, Zhang FF, Fitzgerald W, Shulkin BL, et al. A safety and feasibility trial of 131 I-Mibg in newly diagnosed high-risk neuroblastoma: a children's oncology group study. *Pediatr Blood Cancer.* (2021) 68:e29117. doi: 10.1002/pbc.29117
48. Sgouros G, Bodei L, McDevitt MR, Nedrow JR. Radiopharmaceutical therapy in cancer: clinical advances and challenges. *Nat Rev Drug Discov.* (2020) 19:589–608. doi: 10.1038/s41573-020-0073-9
49. Nayak TK, Norenberg JP, Anderson TL, Prossnitz ER, Stabin MG, Atcher RW. Somatostatin-receptor-targeted alpha-emitting 213bi is therapeutically more effective than beta(-)-emitting 177lu in human pancreatic adenocarcinoma cells. *Nucl Med Biol.* (2007) 34:185–93. doi: 10.1016/j.nucmedbio.2006.11.006
50. Zhang J, Kulkarni HR, Baum RP. 225ac-dotatoc-targeted somatostatin receptor alpha-therapy in a patient with metastatic neuroendocrine tumor of the thymus, refractory to beta-radiation. *Clin Nucl Med.* (2021) 46:1030–1. doi: 10.1097/RLU.0000000000003792
51. Morris MJ, Corey E, Guise TA, Gulley JL, Kevin Kelly W, Quinn DI, et al. Radium-223 mechanism of action: implications for use in treatment combinations. *Nat Rev Urol.* (2019) 16:745–56. doi: 10.1038/s41585-019-0251-x

Conflict of Interest: This study received funding from Applied Accelerator Applications, a Novartis company. The funder had the following involvement with the study: Advice on trial design, provision of all investigational and non-investigational products, free of charge for the LuDO-N trial and review of the final manuscript.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers in Pediatrics

ORIGINAL RESEARCH
published: 21 March 2022
doi: 10.3389/fped.2022.848543

Training in Pediatric Surgical Oncology

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Pediatric Surgical Oncology is a challenging sub-speciality field requiring strong discipline, resilience and a "never quit" attitude. Pediatric cancer patients are frail, vulnerable and have life limiting illness were the skill(s) of the operating surgeon may determine "cure" and survival or untimely death from inadequate tumor resection with disease progression and / or "never event" misadventure. It could be stated therefore that a career in surgical oncology may not be for the "faint hearted" surgeon. Training in a high volume center(s) accredited in the delivery of high quality care and clinical excellence driven by inspirational leaders in surgical oncology with motivated multidisciplinary teams is key. International surgery oncology fellowship(s) add significant credits to a residents' clinical training crucially also yielding opportunities for gaining research skills. On the journey toward subspeciality accreditation the aspiring surgeon oncologist must first demonstrate skill set(s) proficiency in general pediatric surgery residency training to advance to the next phase(s). This article offers a "snap shot" overview synopsis of pediatric surgical oncology training in its broadest perspective(s) for those seeking further information and career guidance.

Keywords: pediatric surgery, surgical training, surgical oncology, innovation, university

OPEN ACCESS

Edited by:
Sabine Sarnacki,
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Reviewed by:
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Pablo Andrés Lobos,
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Specialty section:
This article was submitted to Pediatric Surgery, a section of the journal Frontiers in Pediatrics

Received: *04 January 2022*
Accepted: *03 February 2022*
Published: *21 March 2022*

Citation:
Losty PD (2022) Training in Pediatric Surgical Oncology. Front. Pediatr. 10:848543. doi: 10.3389/fped.2022.848543

"You Only Train Once?"…..

THE START OF THE JOURNEY—TRAINING IN SURGICAL ONCOLOGY—A PERSONAL STORY

Getting started or wishing to train as a pediatric surgical oncologist is often influenced by exposure to leaders and role models during early surgical residency. My own personal journey was greatly influenced by working in a university department adult surgical oncology service led by an inspiring Professor of Surgery Niall O'Higgins at University College Dublin, Ireland—**Figure 1**. The clinical service was always busy with a high case load volume, several major operating lists weekly and many excellent regularly held department academic meetings. Training was a joy and privilege in these early years at St Vincent's Hospital, Dublin, Ireland. Rotating to a Senior House Officer (SHO) post in Pediatric Surgery on the Dublin RCSI Training Scheme I was then fortunate to work

FIGURE 1 | Professor Niall O'Higgins MCh FRCS—St Vincent's Hospital, University College Dublin, Ireland.

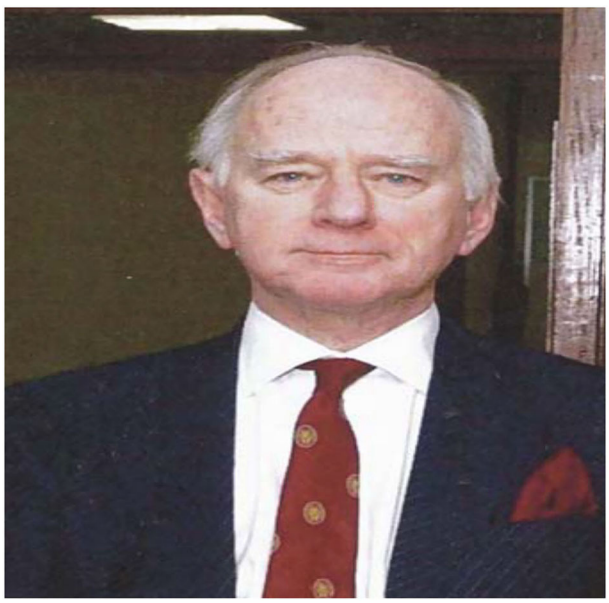

FIGURE 2 | Professor Ray Fitzgerald MA FRCS FRACS—Trinity College Dublin, Ireland.

with Ray Fitzgerald at The Children's Hospital Temple Street, Dublin who was a technically superb surgeon setting very "high standards" expected of his team and a demanding chief likely rooted from his early Royal Navy career i.e.,—"Get It Right First Time"—**Figure 2**. Residency in Pediatric Surgery in Dublin, Ireland then followed after obtaining the FRCS returning to work with Ray Fitzgerald, Barry O'Donnell and Edward Guiney—**Figures 3**–**5**. Securing a surgeon faculty post at Alder Hey Children's Hospital and University Of Liverpool transpired in the early 1990's after having completing a "much sought after" surgical research fellowship at the Massachusetts General Hospital at Harvard Medical School with Professor Pat Donahoe—**Figure 6**.

FIGURE 3 | Professor Barry O'Donnell MCh FRCS—Royal College of Surgeons in Ireland.

FIGURE 4 | Professor Edward J. Guiney MCh FRCS—University College Dublin, Ireland.

During these formative years along with completing a residency in Pediatric Surgery I had trained in General Surgery, Vascular Surgery, Thoracic Surgery, Plastic Surgery and Trauma

FIGURE 5 | Professor Ray Fitzgerald MA FRCS FRACS—Trinity College Dublin, Ireland.

FIGURE 6 | Professor Patricia Donahoe MD—Chief Of Pediatric Surgery, Massachussetts General Hospital, Harvard Medical School, Boston USA.

with many great surgeons - I firmly decided I was going to be a university pediatric surgeon with a subspeciality oncology practice. It may be said here at this point that an academic pediatric surgeon can enjoy the benefit(s) of a rewarding career where clinical job diaries with "surgical research time" are contracted and honored accordingly allowing a perfect match for surgical oncology.

SO WHAT SKILLS ARE REQUIRED?

Young surgeon(s) must be wholly mindful of the landscape in their own nations if they wish to train in pediatric surgical oncology. Where are the busy "high volume" surgery oncology centers where you can get and be assured of surgical "hands on" training? Be cognisant that pediatric cancer whilst the second leading cause of death in childhood in many developed world nations remains a rare disease and index cases notably solid tumor malignancies such as Wilms tumor (80 new cases/annum in the UK—CCLG) and neuroblastoma (100 new cases/annum in the UK—CCLG) are greatly surpassed by hematological cancers and CNS tumors. You will not want to work therefore in a surgery oncology center with a low index case load volume of solid tumors split amongst several residents and the consultant staff.

A surgical skills set acquired by spending attachements in vascular, plastic and thoracic surgery departments are invaluable (including urology) prior to a pediatric surgery residency programme before advancing to become a surgical oncologist. Technical expertise acquired in the "basic principles of surgery" here cannot be overemphasized. Surgical oncology demands the operator to have capabilities in a wide range of techniques e.g., vascular access, vessel dissection, isolation and control, vascular suture repair, reconstruction, organ sparing procedures and precision tissue handling—**Figure 7**.

Get busy then with your search when applying for these highly competitive surgery posts. At this stage if truly vocationally committed to pediatric surgical oncology also consider international fellowship training (see later). Ask yourself also what research is going on in these leading surgical centers? Will it be profitable for you? Is it exciting and worthwhile to explore? Crucially what is the track record of the surgical department(s) and their faculty staff? Do they inspire? What do staff (past & present) say about them? Are the faculty national and international leaders in their field? Do your homework. Choose (if you can) what best "fits for you."

INTERNATIONAL SURGICAL ONCOLOGY FELLOWSHIPS

Having completed pediatric surgery training hopefully in a busy hospital programme or consortium hospital network partnership with exposure to surgical oncology do then consider further opportunities with advanced progression to seeking out a surgical oncology fellowship. Currently the best programmes available for international medical graduates are located in North America. Memorial Sloan Kettering Cancer Center, New York, USA and St Jude's Hospital Memphis Tennessee for example publicize fellowship positions regularly in the major pediatric surgical journals including with other professional organizations such

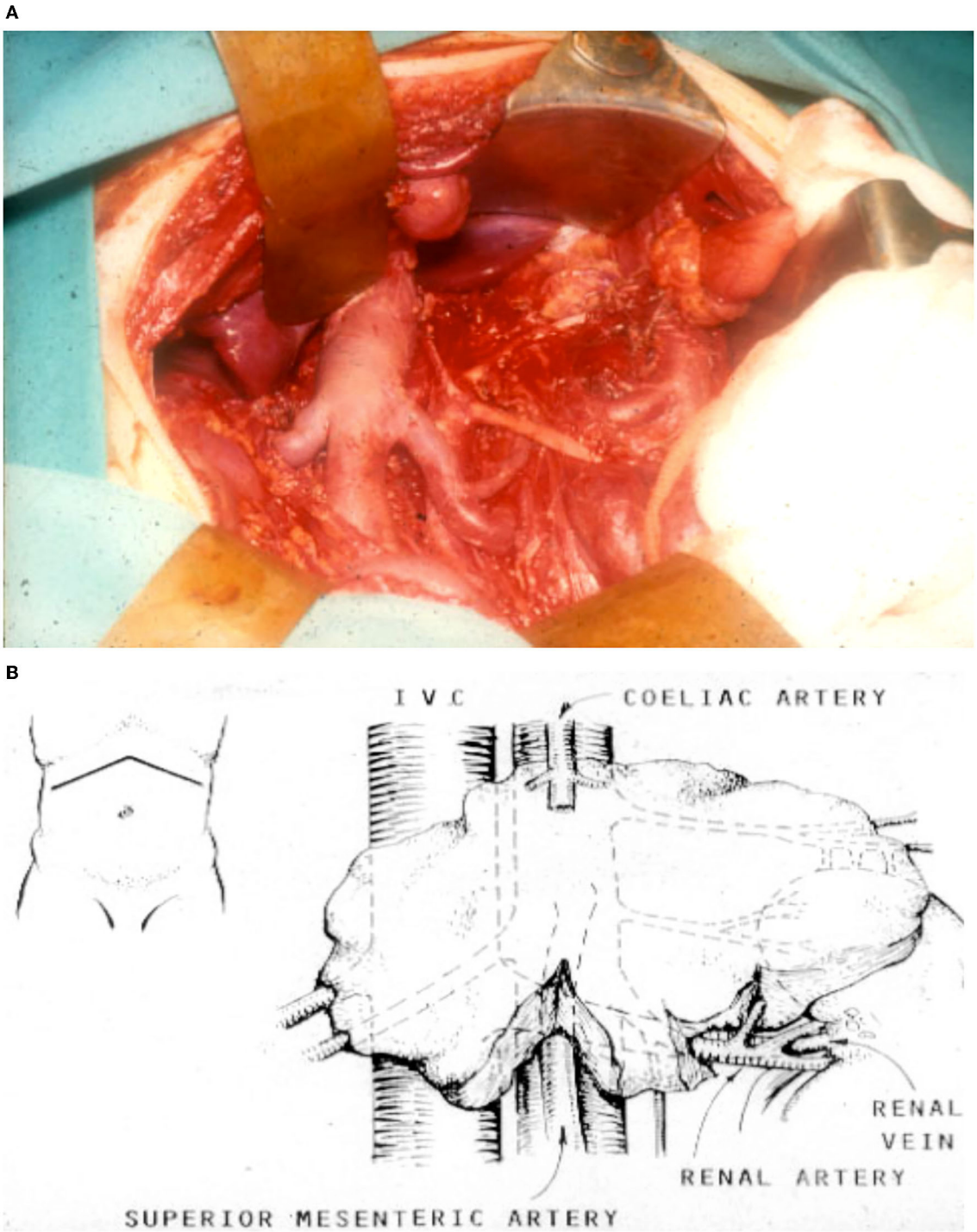

FIGURE 7 | (A) Operative field photograph following gross total resection of an abdominal neuroblastoma tumor. **(B)** Schematic image of neuroblastoma tumor. Note a precise and painstaking careful skeletonization of major vasculature is required for a successful operation.

as the International Society Of Pediatric Surgical Oncology (IPSO). It is greatly hoped in the near future further fellowships can be actively developed in other world nations through IPSO network partnerships. Different nations (i.e., in your own country) may offer varied new opportunities. So regularly check it out! ....

THE FUTURE OF PEDIATRIC SURGICAL ONCOLOGY TRAINING—WHERE TO NEXT?

In the UK currently there are too many pediatric surgery programmes (over 22 centers with a national population > 68 million) providing cancer services for children. As a result individual surgical centers manage and treat few index solid tumor malignancies to provide wholly sufficient training in pediatric surgical oncology with residents competing with consultant surgeons who now frequently share operating in teams to maintain skill competencies. Similar scenarios may exist in other world countries requiring a critical reappraisal with the considered future development of pediatric cancer surgery "super centers". As an example in the Netherlands (national population 17 million) pediatric cancer surgery (2014 –) is now restricted to a single major center located at the Princess Maxima Center in Utrech, NL. A health care model such as this concept is considered "forward thinking" in vision to create a national center of excellence teamed and "tooled up" with a highly skilled infrastructure to sustain credibility.

Perhaps the same thoughts and ideas may be uttered for other world countries with their pediatric cancer health care systems. We must shift the imbalance. Without pediatric surgical oncologists and cancer specialists working together and sharing a leadership role in health care many of our hospitals seeking to deliver world class services will suffer. Surgical training programmes working toward national ranking(s) in residency and oncology fellowship applications should also "think smart" to be competitive. The drive toward "evidence based surgical oncology" health service provision, training and innovation(s) in cancer care have never been greater than in our current times. The best hospitals in the world will reassuringly always value well-trained inspirational pediatric surgical oncology leaders. I wish you all—"Good Luck". . Get started now on your journey!.

"A Career In Surgical Oncology Is Life Long Learning"…

AUTHOR'S NOTE

PDL served as Chair UK CCLG Surgeons Group (2015–2017). PDL is currently President Elect - International Society Of Pediatric Surgical Oncology (IPSO).

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

FURTHER READING

1. Pediatric surgical oncology. In: Losty PD, La Quaglia MP, Sarnacki S, Fuchs J, Taguchi T, editors. CRC Press (2022).
2. O'Donnell B. Training in paediatric surgery. *World J Surg.* (1985) 9:316–20. doi: 10.1007/BF01656326
3. Davenport M, Jawaid WB, Losty PD. UK paediatric surgical academic output (2005-2020): a cause for concern? *J Pediatr Surg.* (2021) 56:2142–7. doi: 10.1016/j.jpedsurg.2021.07.023
4. Zenger JH, Folkman JR, Edinger SK. Making yourself indispensable. *Harv Bus Rev.* (2011) 89:84–90.
5. Harwood R, Losty PD. Training and the future delivery of UK paediatric surgery in the NHS. *BMJ.* (2021) 375:n2785. doi: 10.1136/bmj.n2785
6. Wijnen M, Hulscher J. Centralization of pediatric surgical care in the Netherlands: lessons learned. *J Pediatr Surg.* (2021). 67:178–81. doi: 10.1016/j.jpedsurg.2021.10.023
7. Losty PD. Academic paediatric surgery – Why not? *Semin Paediatr Surg.* (2021) 30:151021.
8. Losty PD. Evidence-based paediatric surgical oncology. *Semin Pediatr Surg.* (2016) 25:333–5. doi: 10.1053/j.sempedsurg.2016.09.008

Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Pediatrics

ORIGINAL RESEARCH
published: 28 April 2022
doi: 10.3389/fped.2022.840432

Considerations for Balance Between Fundamental Treatment and Improvement of Quality of Life of Pediatric Thyroid Cancer Patient: Comparative Analysis With Adult Using Propensity Score Matching

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Specialty section:
This article was submitted to Pediatric Surgery, a section of the journal Frontiers in Pediatrics

Received: *21 December 2021*
Accepted: *28 February 2022*
Published: *28 April 2022*

Citation:
You JY, An S-W, Kim HY, Park DW, Byeon HK, Patroniti S, Dionigi G and Tufano RP (2022) Considerations for Balance Between Fundamental Treatment and Improvement of Quality of Life of Pediatric Thyroid Cancer Patient: Comparative Analysis With Adult Using Propensity Score Matching. Front. Pediatr. 10:840432. doi: 10.3389/fped.2022.840432

Background: Thyroid cancer is very rarely observed in children and adolescents, some reports have shown that the long-term outcome of treatment is better than that of adult patients, despite many treatment failures or a high risk of recurrence. This study considers whether it is appropriate to treat pediatric thyroid cancer patients aggressively, as per the ATA guidelines, based on the balance between the fundamental treatment of thyroid cancer and the improvement of the long-term quality of life of pediatric patients.

Methods: A total of 1,950 patients were recruited, including 83 pediatric and 1,867 adult patients, who were diagnosed with thyroid cancer and underwent surgical treatment at one of our medical center hospitals from March 2000 to January 2020.

Results: Sixty-nine pairs of pediatric and adult patients were matched in a ratio of 1:2 through propensity score matching. When compared through propensity score matching, there was no significant difference in prognosis such as recurrence rate in children and adults at the same stage.

Conclusion: This study showed that the prognosis of both pediatric and adult patients who underwent a total thyroidectomy and lobectomy was not significantly different. If more pediatric patients can be considered for the less-aggressive lobectomy than a total thyroidectomy through various preoperative examinations and meticulous pre-diagnosis, it may be possible to properly determine the balance between improving long-term quality of life while providing fundamental cancer treatment.

Keywords: pediatric cancer, thyroid malignancy, total thyroidectomy, thyroid lobectomy, propensity score matching

INTRODUCTION

Thyroid cancer is very rarely observed in children and adolescents, and it is reported that only approximately 1.9% of new thyroid cancer patients develop it in childhood annually (1, 2). However, papillary thyroid carcinoma, which is the most common type of thyroid cancer, accounts for 90–95% of pediatric thyroid cancer; other types are reported to be very rare (3–5).

Because childhood thyroid cancer has a more extensive invasion and a higher recurrence rate than adult thyroid cancer, aggressive treatment for differentiated thyroid cancer in children is recommended, i.e., a thyroidectomy is recommended over a lobectomy (4, 6, 7). Thyroid cancer in pediatric patients is generally treated according to the American Thyroid Association (ATA) pediatric thyroid cancer guidelines, which are primarily based on Mayo clinic data from patients in the United States (8, 9). This is because very few cases are detected through screening, and most cases are in an advanced state at the time of diagnosis.

However, some reports have shown that the long-term outcome of treatment is better than that of adult patients, despite many treatment failures or a high risk of recurrence (10, 11). Compared with a lobectomy, a total thyroidectomy has a higher risk of complications, such as hypothyroidism and recurrent laryngeal nerve damage after surgery. Therefore, pediatric patients suffer from sequelae for a longer period than adults, and they are more likely to experience a reduced quality of life (12). A South Korean research team reported that a lobectomy alone is sufficient for the treatment of early cancer in pediatric patients (13). South Korea is an iodine-rich country; therefore, thyroid function tests are frequently performed, even on children. Thus, there are many cases in which thyroid cancer is diagnosed at an early stage through tests, such as ultrasound (14). At the pediatric endocrine clinic, when testing growth hormone in children, a thyroid function test is also performed. In addition, it is estimated that thyroid cancer is frequently diagnosed due to the high incidence of thyroid cancer and good access to clinics that perform thyroid ultrasound.

This study considers whether it is appropriate to treat pediatric thyroid cancer patients aggressively, as per the ATA guidelines, based on the balance between the fundamental treatment of thyroid cancer and the improvement of the long-term quality of life of pediatric patients.

MATERIALS AND METHODS

Patients

Patients who were diagnosed with thyroid cancer and underwent surgical treatment at one of three hospitals (Anam, Guro, and Ansan) of the Korea University Medical Center from March 2000 to January 2020 were recruited. Patients who underwent surgery from the Head and Neck Department of Otolaryngology or Endocrine Department of General Surgery were also included. A total of 1,950 patients were recruited, including 83 pediatric and 1,867 adult patients. According to the standard definition, children were defined as those aged younger than 20 years. Twelve pediatric patients and 479 adult patients did not have papillary thyroid cancer (PTC) based on a pathological examination, and they were excluded from the study. Additionally, two pediatric and 34 adult patients did not have baseline information (tumor size); thus, they did not have a propensity score for the propensity score matching and were also excluded (**Figure 1**). All patients were enrolled retrospectively, and clinical data were analyzed *via* chart review. This retrospective study was approved by the Institutional Review Board of the Korea University College of Medicine.

Surgical Strategy

Pediatric PTC patients were treated according to the American Thyroid Association guidelines. The guidelines recommend a total thyroidectomy because lesions are often bilateral and multifocal in pediatric patients. However, in this study, those patients who only had intrathyroidal lesions and no bilaterality underwent a lobectomy. Prophylactic central lymph node dissection was performed in all pediatric patients. A therapeutic modified radical neck dissection (MRND) was performed when N1b disease was suspected or pathologically confirmed.

Adult PTC patients were treated according to the National Comprehensive Cancer Network (NCCN) guidelines. Patients who satisfied all the following conditions underwent a lobectomy instead of a total thyroidectomy: (I) unilateral disease, (II) primary tumor size < 4 cm, (III) no extrathyroidal extension (ETE) or lympho-vascular invasion, and (IV) no clinically suspicious lymph nodes in the lateral neck. Therapeutic central and lateral neck dissections were performed on patients who were clinically suspected of having neck node metastasis.

Because the surgeons who performed thyroid cancer surgeries for pediatric and adult patients in this study had more than 10 years of experience, we assumed that there was no difference in the skill level of the surgeons and no respective bias.

Open thyroidectomies were performed on most pediatric and adult patients. Twelve adult patients underwent endoscopic thyroidectomies (9, lobectomy; 3, total), and 13 pediatric patients underwent robotic or endoscopic thyroidectomies (5, retro-auricular endoscopic lobectomy; 3, retro-auricular robotic lobectomy; 5, transoral robotic thyroidectomy). There was no significant difference in the surgical approach and method between the two patient groups.

Postoperative Treatment and Follow Up

All patients received a suppressive dose of levothyroxine after surgery and were regularly followed up through physical examinations, thyroid function tests, and neck ultrasonograms. Initially, the patients were followed up at 3 and 6 months after surgery. Subsequently, they were followed up once every year. When metastasis to the neck node or recurrence in the remaining thyroid tissues were suspected, fine needle aspiration cytology (FNA) was performed for confirmation. Computed tomography (CT), positron emission tomography (PET), and ^{131}iodine whole-body scans were also performed to assess the recurrence of PTC when necessary.

Clinicopathological Variables

The following clinicopathological variables were assessed: age, sex, body mass index (BMI), preoperative FNA cytology, type

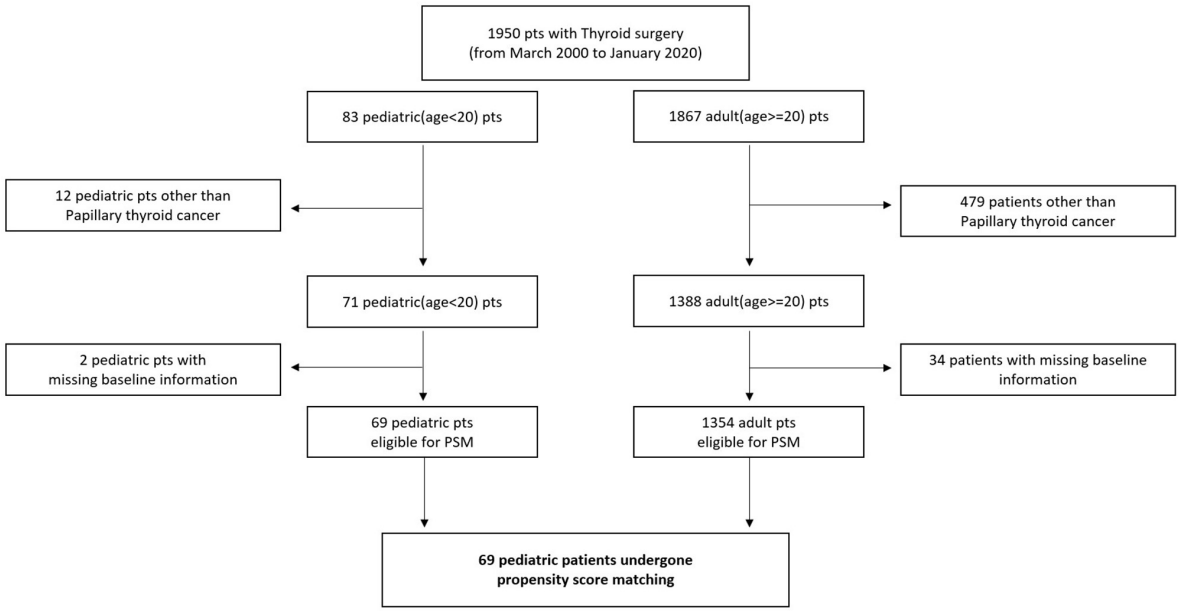

FIGURE 1 | Study design flowchart.

of surgery, radioactive iodine (RAI) therapy, recurrence, follow-up duration, bilaterality, multiplicity, tumor size, ETE, total harvested lymph nodes (LNs), central LNs, lateral LNs, total positive LNs, positive central LNs, positive lateral LNs, pT stage, and pN stage.

The tumor-node-metastasis (TNM) stage was classified based on the 8th edition of the American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) TNM staging system.

The 8th AJCC guideline was newly released in 2018; therefore, the staging of thyroid cancer changed from the 7th guideline. Thus, the pathologic reports of patients who underwent surgical treatment before 2018 were reviewed for new staging classifications. If ETE to the strap muscle was not indicated in the pathologic reports, staging was re-classified according to the tumor size, even for pT3 tumors. For example, extension to the perithyroidal soft tissue or minimal extrathyroidal extension was considered to be tumors below pT3a, and the pT stage was determined based only on the size of the tumor.

Patients who underwent unilateral and bilateral surgeries were grouped into the lobectomy and total thyroidectomy categories, respectively. If the staging operation was planned from the initial operation, and a completion thyroidectomy was performed, the patient was included in the total thyroidectomy category. Patients who underwent a lobectomy at a different institution and a completion thyroidectomy at our institution, owing to tumor recurrence, were excluded from the study because the surgeon who conducted the initial operation was different (13 patients).

Statistical Analysis

Statistical analysis was performed using R (R version 4.0.3 for Windows, R Core Team) (15, 16).

Clinicopathological factors of pediatric and adult PTC patients were compared. Continuous variables were expressed as mean and standard deviation (SD) values, and categorical variables were presented as numeric values and percentages. For a comparison of continuous variables, a normal distribution was first confirmed using the Shapiro–Wilk test. *T*-tests and Wilcoxon rank sum tests were then performed to compare continuous variables that were and were not normally distributed, respectively. For categorical variables, the chi-square test was performed, and Fisher's exact test was performed when errors occurred ("moonBook" package of R was used for the analysis).

In this study, differences in the prognosis of pediatric and adult patients according to the surgical method were assessed. However, this was a retrospective cohort study, and the number of pediatric patients (71) was too low. Therefore, to reduce the effects of selection bias and confounding variables, adult patients were matched with pediatric patients using propensity score matching. The number of adult patients (1,388) was greater than that of the pediatric patients; thus, one-to-many matching was performed. One-to-two matching was conducted according to previous studies that recommended one-to-one or one-to-two matching (17).

A logistic regression model was used to calculate the propensity scores of the patients. The following prognostic factors were included: sex, tumor size group, ETE, and pN.

Initially, sex, pT, and pN were evaluated to calculate the propensity scores of the patients. However, the pT stage in our data was reported using the previous staging system, AJCC 7th edition. Therefore, to avoid matching errors, the pT was recalculated based on the tumor size and ETE. The tumor size was divided into three groups based on the criteria of the new

AJCC 8th edition T staging of tumors exceeding 1 and 2 cm in size. Tumors that were greater than 4 cm in size were rare; thus, they were excluded. Therefore, the propensity score was determined based on sex, tumor size group, ETE, and pN (the "matchit" package of R was used for the analysis) (18, 19).

The Kaplan-Meier survival analysis was performed to plot survival curves, and the log-rank test was conducted to compare propensity-matched patients. A p-value < 0.05 was considered statistically significant ('survival' package and "survminer" package of R were used for this analysis).

RESULTS

Comparison of Baseline Clinicopathologic Characteristics According to Age Group, Before Propensity Score Matching

The baseline clinicopathological characteristics of 71 pediatric and 1,388 adult patients who underwent thyroid surgery for the treatment of PTC are shown in **Table 1**.

The mean age of the pediatric and adult patients was 16.6 ± 2.8 and 51.2 ± 12.4 years, respectively ($p < 0.05$). Because the prevalence of thyroid cancer in pre-pubertal and post-pubertal children is different, this should be considered. There was a significant BMI difference between pediatric and adult patients (22.4 ± 3.2, 24.9 ± 3.8, respectively, $p < 0.05$). Nineteen (26.8%) pediatric patients and 342 (24.7%) adult patients underwent a lobectomy, whereas 52 (73.2%) pediatric patients and 1042 (75.3%) adult patients underwent a total thyroidectomy. There was no significant difference in the surgical method between the two patient groups ($p = 0.803$). A recurrence of PTC was observed in 11 (15.5%) pediatric and 43 (3.1%) adult patients. The number of recurrence cases differed significantly between the two groups ($p < 0.05$). The mean follow-up durations for pediatric and adult patients were 89.9 ± 57.5 and 77.5 ± 36.5, respectively, and there was no significant difference between the two groups ($p = 0.192$). Bilaterality was observed in 4 (5.7%) pediatric and 376 (27.6%) patients, which was significantly different between the two groups ($p = 0.000$). Additionally, the number of multiplicity cases was 15 (21.1%) and 570 (41.2%) in pediatric and adult patients, respectively, which was significantly different between the two groups ($p = 0.001$). The tumor size was 2.1 ± 1.6 cm and 0.9 ± 0.8 cm in pediatric and adult patients, respectively, which was significantly different between the two groups ($p < 0.05$). Forty-two (59.2%) pediatric and 585 (42.2%) adult patients had ETE, which indicates that ETE was significantly more common in adult patients ($p = 0.007$). The number of total harvested LNs was 20.3 ± 25.8 in pediatric patients and 11.0 ± 10.2 in adult patients, which was not different between the two groups ($p = 0.801$). In contrast, the number of total positive LNs was 7.4 ± 8.0 and 2.0 ± 4.2 in pediatric and adult patients, respectively, which was significantly different between the two groups ($p < 0.05$). The distribution of pT stages in pediatric and adult patients were as follows (pediatric and adult patients, respectively): pT1a, 22 (31.0%) and 1029 (74.1%); pT1b, 19 (26.8%) and 271 (19.5%);

TABLE 1 | Clinico-pathologic characteristics of patients according to age group.

	Age < 20 (*N* = 71)	Age ≥ 20 (*N* = 1388)	*P*-value
Sex			0.060
Male	9 (12.7%)	319 (23.0%)	
Female	62 (87.3%)	1069 (77.0%)	
Age (years)	16.6 ± 2.8 (15.5–18.5)	51.2 ± 12.4 (42.0–60.0)	<0.05
Body mass index (BMI)	22.4 ± 3.2 (19.9–24.1)	24.9 ± 3.8 (22.3–26.9)	<0.05
Preoperative FNA cytology (Bethesda classification)			0.037
I	2 (3.1%)	6 (0.4%)	
II	2 (3.1%)	15 (1.1%)	
III	6 (9.2%)	70 (5.1%)	
IV	1 (1.5%)	22 (1.6%)	
V	12 (18.5%)	322 (23.3%)	
VI	42 (64.6%)	945 (68.5%)	
Surgery type			0.803
Lobectomy	19 (26.8%)	342 (24.7%)	
Right	14 (19.7%)	166 (12.0%)	
Left	5 (7.0%)	176 (12.7%)	
Total thyroidectomy	52 (73.2%)	1042 (75.3%)	
Radioactive iodine (RAI)			0.274
Yes	41 (59.4%)	687 (66.6%)	
No	28 (40.6%)	344 (33.4%)	
Recurrence			<0.05
Yes	11 (15.5%)	43 (3.1%)	
No	60 (84.5%)	1345 (96.9%)	
Follow up duration (months)	89.9 ± 57.5 (43.5–126.0)	77.5 ± 36.5 (48.0–104.0)	0.192
Bilaterality			<0.05
Yes	4 (5.7%)	376 (27.6%)	
No	66 (94.3%)	986 (72.4%)	
Multiplicity			0.001
Yes	15 (21.1%)	570 (41.2%)	
No	56 (78.9%)	813 (58.8%)	
Tumor size (cm)	2.1 ± 1.6 (0.9–2.8)	0.9 ± 0.8 (0.5–1.1)	<0.05
Tumor size in group			<0.05
Size ≤ 1cm	21 (30.0%)	1031 (74.4%)	
1 cm < Size ≤ 2 cm	20 (28.6%)	274 (19.8%)	
Size > 2 cm	29 (41.4%)	81 (5.8%)	
Extrathyroidal extension (ETE)			0.007
Yes	42 (59.2%)	585 (42.2%)	
No	29 (40.8%)	800 (57.8%)	
Total harvested LNs	20.3 ± 25.8	11.0 ± 10.2	0.801
Central LNs	9.9 ± 8.5	9.6 ± 8.1	0.856
Lateral LNs	12.6 ± 23.4	1.4 ± 6.0	<0.05
Total positive LNs	7.4 ± 8.0	2.0 ± 4.2	<0.05
Positive central LNs	5.3 ± 5.5	1.7 ± 3.5	<0.05
Positive lateral LNs	2.8 ± 5.2	0.3 ± 1.7	<0.05
pTstage			<0.05
T1a	22 (31.0%)	1029 (74.1%)	
T1b	19 (26.8%)	271 (19.5%)	
T2	21 (29.6%)	67 (4.8%)	

(Continued)

TABLE 1 | (Continued)

	Age < 20 (*N* = 71)	Age ≥ 20 (*N* = 1388)	*P*-value
T3a	7 (9.9%)	9 (0.6%)	
T3b	2 (2.8%)	8 (0.6%)	
T4a	0 (0.0%)	4 (0.3%)	
T4b	0 (0.0%)	0 (0.0%)	
pN stage			<0.05
N0	30 (42.3%)	777 (56.0%)	
N1a	27 (38.0%)	528 (38.0%)	
N1b	14 (19.7%)	83 (6.0%)	

Data are expressed as the patient number (%) or mean ± SD.

pT2, 21 (29.6%) and 67 (4.8%); pT3a, 7 (9.9%) and 9 (0.6%); pT3b, 2 (2.8%) and 8 (0.6%); pT4a, 0 (0.0%) and 4 (0.3%); pT4b, 0 (0.0%) and 0 (0.0%). There were significant differences in the pT stages between the two patient groups ($p < 0.05$). The distribution of pN stages in pediatric and adult patients were as follows (pediatric and adult patients, respectively): pN0, 30 (42.3%) and 777 (56.0%); pN1a, 27 (38.0%) and 528 (38.0%); pN1b, 14 (19.7%) and 83 (6.0%). There were significant differences in the pN stages between the two patient groups ($p < 0.05$).

Comparison of Clinicopathologic Characteristics of Propensity-Matched Patients According to Age Group

Sixty-nine pairs of pediatric and adult patients were matched in a ratio of 1:2 through propensity score matching. The clinicopathologic characteristics of the matched patients are shown in **Table 2**. The mean age of the pediatric and adult patients was 16.8 ± 2.4 and 38.4 ± 14.0 years, respectively ($p < 0.05$). Eighteen (26.1%) pediatric and 25 (18.1%) adult patients underwent a lobectomy, and 51 (73.9%) pediatric and 113 (81.9%) adult patients underwent a total thyroidectomy. There was no significant difference in the surgical method between the two patient groups ($p = 0.250$). Unlike the comparison results of the two groups before matching, the tumor size was 2.1 ± 1.6 cm and 1.8 ± 1.5 cm in pediatric and adult patients, respectively, which was not significantly different ($p = 0.185$).

Extrathyroidal extension was observed in 41 (59.4%) pediatric and 84 (60.9%) adult patients, indicating that ETE was not significantly different ($p = 0.960$). The distribution of pT stages in pediatric and adult patients were as follows (pediatric and adult patients, respectively): pT1a, 20 (29.0%) and 40 (29.0%); pT1b, 19 (27.5%) and 40 (29.0%); pT2, 21 (30.4%) and 51 (37.0%); pT3a, 7 (10.1%) and 4 (2.9%); pT3b, 2 (2.9%) and 0 (0.0%); pT4a, 0 (0.0%) and 3 (2.2%); pT4b, 0 (0.0%) and 0 (0.0%). There were no significant differences in the pT stages between the two patient groups ($p = 0.059$). The distribution of pN stages in pediatric and adult patients were as follows (pediatric and adult patients, respectively): pN0, 28 (40.6%) and 51 (37.0%); pN1a, 27 (39.1%) and 67 (48.6%); pN1b, 14 (20.3%) and 20 (14.5%). There were no significant differences in the pT stages between the two patient groups ($p = 0.386$). Recurrence was observed in 11 (15.5%) pediatric and 12 (8.7%) adult patients after surgery. The number of recurrent cases was not significantly different between the two groups ($p = 0.184$).

TABLE 2 | Clinico-pathologic characteristics of propensity-matched PTC patients according to age group.

	Age < 20 (*N* = 69)	Age ≥ 20 (*N* = 138)	*P*-value
Sex			NA
Male	9 (13.0%)	18 (13.0%)	
Female	60 (87.0%)	120 (87.0%)	
Age (years)	16.8 ± 2.4 (16–19)	38.4 ± 14.0 (29–41)	<0.05
Body Mass Index (BMI)	22.5 ± 3.3 (19.9–24.2)	24.2 ± 4.5 (20.9–27.5)	0.009
Surgery type			0.250
Lobectomy	18 (26.1%)	25 (18.1%)	
Total thyroidectomy	51 (73.9%)	113 (81.9%)	
Bilaterality			0.012
Yes	4 (5.8%)	28 (20.3%)	
No	65 (94.2%)	110 (79.7%)	
Multiplicity			0.018
Yes	14 (20.3%)	52 (37.7%)	
No	55 (79.7%)	86 (62.3%)	
Tumor size (cm)	2.1 ± 1.6	1.8 ± 1.5	0.185
Tumor size in group			NA
≤1 cm	20 (29.0%)	40 (29.0%)	
>1 cm and ≤2 cm	20 (29.0%)	40 (29.0%)	
>2 cm	29 (42.0%)	58 (42.0%)	
Extrathyroidal extension (ETE)			0.960
Yes	41 (59.4%)	84 (60.9%)	
No	28 (40.6%)	54 (39.1%)	
pT stage			0.059
T1a	20 (29.0%)	40 (29.0%)	
T1b	19 (27.5%)	40 (29.0%)	
T2	21 (30.4%)	51 (37.0%)	
T3a	7 (10.1%)	4 (2.9%)	
T3b	2 (2.9%)	0 (0.0%)	
T4a	0 (0.0%)	3 (2.2%)	
pN stage			0.368
N0	28 (40.6%)	51 (37.0%)	
N1a	27 (39.1%)	67 (48.6%)	
N1b	14 (20.3%)	20 (14.5%)	
Recurrence			0.184
Yes	11 (15.9%)	12 (8.7%)	
No	58 (84.1%)	126 (91.3%)	

Data are expressed as the patient number (%) or mean ± SD.

Disease Free Survival Analysis of Propensity-Matched Patients According to Age Group

A Kaplan-Meier analysis demonstrated no significant difference in disease-free survival (DFS) between the two patient groups (**Figure 2**, log-rank $P = 0.13$). The 5 Year disease free survival rate (5Y DFSR) was 83.54% (lower 95% CI: 74.62%, upper 95%

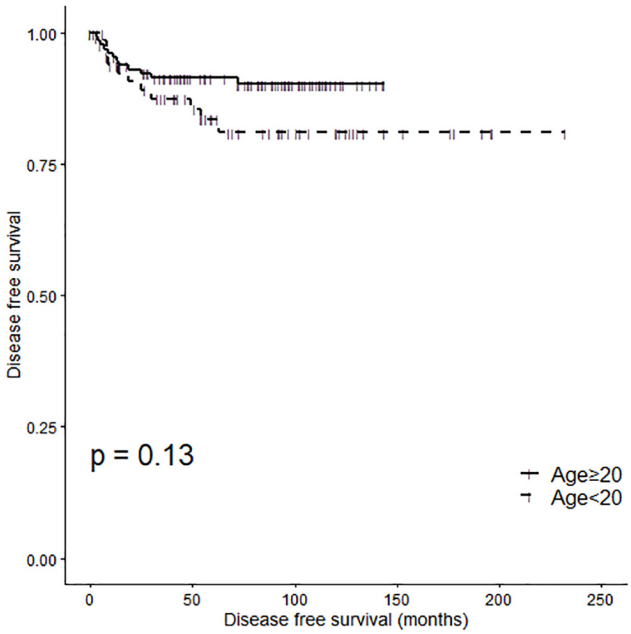

FIGURE 2 | Disease-free survival rates of subgroups according to age group (log rank $p = 0.13$).

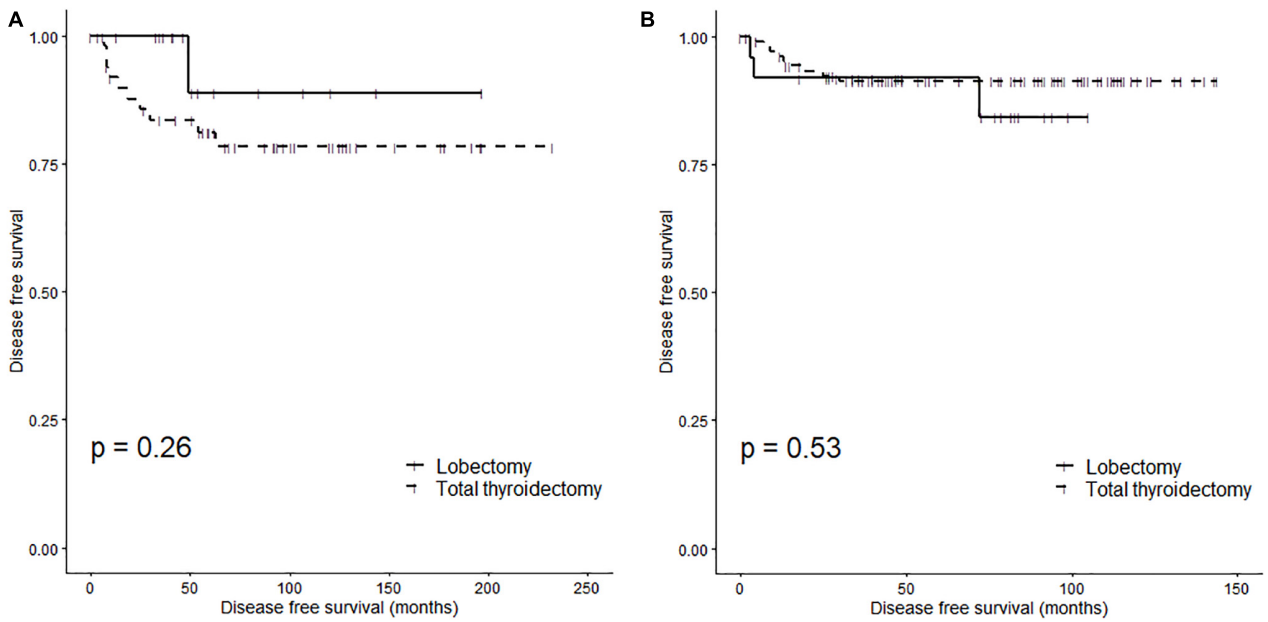

FIGURE 3 | Disease-free survival rates of subgroups according to surgery type: **(A)** pediatric patients (log rank $p = 0.26$) and **(B)** adult patients (log rank $p = 0.53$).

CI: 93.52%) and 91.46% (lower 95% CI: 86.75%, upper 95% CI: 96.42%) in pediatric and adult patients, respectively.

Figure 3 shows the Kaplan-Meier curve of the DFS according to the surgical method in pediatric (**Figure 3A**) and adult (**Figure 3B**) patients. There was no significant difference in DFS according to the surgical method in pediatric patients (log-rank $p = 0.26$). The 5Y DFSR was 88.90% (lower 95% CI: 70.60%, upper 95% CI: 100.00%) and 81.16% (lower 95% CI: 70.75%, upper 95% CI: 93.10%) in pediatric patients who underwent a lobectomy and total thyroidectomy, respectively. Similarly, there was no significant difference in DFS according to the surgical method in adult patients (log-rank $p = 0.53$). The 5Y DFSR was 92.00% (lower 95% CI: 81.96%, upper 95% CI: 100.00%) and 91.32% (lower 95% CI: 86.05%, upper 95% CI: 96.91%)

in adult patients who underwent a lobectomy and total thyroidectomy, respectively.

DISCUSSION

From the results of our study, it can be concluded that the prognosis was sufficiently good, even in pediatric patients and with standard treatment according to the stage of cancer, such as tumor size, ETE, and pN stage. The detection and diagnosis of cancer tends to be delayed in pediatric patients compared to adult patients. However, if detected early, a lobectomy can be performed, and recurrence and metastasis can be prevented and treated early through continuous follow-up. This treatment approach can considerably improve the quality of life of pediatric patients with thyroid cancer in the future. In South Korea, patients have excellent access to hospitals, owing to the universal supply of the national health insurance system. Additionally, owing to the high coverage of private insurance, it is not uncommon for young patients to undergo thyroid ultrasound examinations and fine needle aspiration examinations (20). Thus, an environment that is favorable for the early detection of thyroid cancer has been established.

A total thyroidectomy can fundamentally reduce the recurrence of thyroid cancer by removing the entire thyroid tissue; however, it can cause various complications and sequelae in pediatric patients who require long-term follow-up. A total thyroidectomy is known to have a higher risk of complications, such as hypothyroidism and recurrent laryngeal nerve damage after surgery, than a lobectomy (21). Patients may also have to take calcium supplements throughout their lives, owing to hypoparathyroidism. Moreover, patients who undergo a total thyroidectomy must take levothyroxine for the rest of their lives. In some patients, the accumulation of free T4 from levothyroxine increases the burden on the heart, which may cause heart failure (22). There are also reports that long-term use of levothyroxine may promote osteoporosis in postmenopausal women (23, 24). Therefore, it is necessary to actively recommend a lobectomy for the treatment of pediatric thyroid cancer patients who do not require a total thyroidectomy.

CONCLUSION

This study showed that the prognosis of both pediatric and adult patients who underwent a total thyroidectomy and lobectomy was not significantly different. If more pediatric patients can be considered for the less-aggressive lobectomy than a total thyroidectomy through various preoperative examinations and meticulous pre-diagnosis, it may be possible to properly determine the balance between improving long-term quality of life while providing fundamental cancer treatment.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

This retrospective study was reviewed and approved by the Institutional Review Board of the Korea University College of Medicine.

AUTHOR CONTRIBUTIONS

JY, S-WA, HB, DP, and HK: substantial contributions to the conception or design of the work, acquisition, analysis, or interpretation of data for the work. JY and S-WA: drafting the work or revising it critically for important intellectual content. GD, RT, SP, and HK: final approval of the version to be published. JY, S-WA, HK, DP, HB, SP, GD, and RT: agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Korean Thyroid Association Clinical Research Award 2020.

REFERENCES

1. Karapanou O, Tzanela M, Vlassopoulou B, Kanaka-Gantenbein C. Differentiated thyroid cancer in childhood: a literature update. *Hormones (Athens).* (2017) 16:381–7.
2. Byeon HK, Kim SB, Oh HS, Kim HK, Choi IH, Kim H, et al. Clinical analysis of pediatric thyroid cancer: a single medical institution experience of 18 years. *Ann Otol Rhinol Laryngol.* (2019) 128:1152–7.
3. Holmes L Jr., Hossain J, Opara F. Pediatric thyroid carcinoma incidence and temporal trends in the USA (1973-2007): race or shifting diagnostic paradigm? *ISRN Oncol.* (2012) 2012:906197.
4. Harness JK, Thompson NW, McLeod MK, Pasieka JL, Fukuuchi A. Differentiated thyroid carcinoma in children and adolescents. *World J Surg.* (1992) 16:547–3; discussion 53–4.
5. Park S, Jeong JS, Ryu HR, Lee CR, Park JH, Kang SW, et al. Differentiated thyroid carcinoma of children and adolescents: 27-year experience in the yonsei university health system. *J Korean Med Sci.* (2013) 28:693–9.
6. Demidchik YE, Demidchik EP, Reiners C, Biko J, Mine M, Saenko VA, et al. Comprehensive clinical assessment of 740 cases of surgically treated thyroid cancer in children of Belarus. *Ann Surg.* (2006) 243:525–32.
7. Monaco SE, Pantanowitz L, Khalbuss WE, Benkovich VA, Ozolek J, Nikiforova MN, et al. Cytomorphological and molecular genetic findings in pediatric thyroid fine-needle aspiration. *Cancer Cytopathol.* (2012) 120:342–50. doi: 10.1002/cncy.21199
8. Francis GL, Waguespack SG, Bauer AJ, Angelos P, Benvenga S, Cerutti JM, et al. Management guidelines for children with thyroid nodules and differentiated thyroid cancer. *Thyroid.* (2015) 25:716–59.

9. Haugen BR. 2015 American thyroid association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: what is new and what has changed? *Cancer.* (2017) 123:372–81.
10. Papendieck P, Gruneiro-Papendieck L, Venara M, Acha O, Maglio S, Bergadá I, et al. Differentiated thyroid carcinoma: presentation and follow-up in children and adolescents. *J Pediatr Endocrinol Metab.* (2011) 24:743–8. doi: 10.1515/jpem.2011.241
11. Alzahrani AS, Alkhafaji D, Tuli M, Al-Hindi H, Sadiq BB. Comparison of differentiated thyroid cancer in children and adolescents (</=20 years) with young adults. *Clin Endocrinol (Oxf).* (2016) 84:571–7. doi: 10.1111/cen.12845
12. Patel NA, Bly RA, Adams S, Carlin K, Parikh SR, Dahl JP, et al. A clinical pathway for the postoperative management of hypocalcemia after pediatric thyroidectomy reduces blood draws. *Int J Pediatr Otorhinolaryngol.* (2018) 105:132–7. doi: 10.1016/j.ijporl.2017.12.011
13. Kim K, Lee CR, Kang SW, Lee J, Jeong JJ, Nam KH, et al. Clinical assessment of pediatric patients with differentiated thyroid carcinoma: a 30-year experience at a single institution. *World J Surg.* (2020) 44:3383–92. doi: 10.1007/s00268-020-05598-9
14. Lee YJ, Cho YJ, Heo YJ, Chung EJ, Choi YH, Kim JI, et al. Thyroid nodules in childhood-onset Hashimoto's thyroiditis: frequency, risk factors, follow-up course, and genetic alterations of thyroid cancer. *Clin Endocrinol (Oxf).* (2021) 95:638–48. doi: 10.1111/cen.14490
15. Stiglic G, Watson R, Cilar L. R you ready? Using the R programme for statistical analysis and graphics. *Res Nurs Health.* (2019) 42:494–9. doi: 10.1002/nur.21990
16. Chan BKC. Data analysis using R programming. *Adv Exp Med Biol.* (2018) 1082:47–122.
17. Austin PC. Statistical criteria for selecting the optimal number of untreated subjects matched to each treated subject when using many-to-one matching on the propensity score. *Am J Epidemiol.* (2010) 172:1092–7. doi: 10.1093/aje/kwq224
18. Tam S, Boonsripitayanon M, Amit M, Fellman BM, Li Y, Busaidy NL, et al. Survival in differentiated thyroid cancer: comparing the AJCC cancer staging seventh and eighth editions. *Thyroid.* (2018) 28:1301–10. doi: 10.1089/thy.2017.0572
19. Verburg FA, Mader U, Luster M, Reiners C. The effects of the union for international cancer control/American joint committee on cancer tumour, node, metastasis system version 8 on staging of differentiated thyroid cancer: a comparison to version 7. *Clin Endocrinol (Oxf).* (2018) 88:950–6. doi: 10.1111/cen.13597
20. Kim S, Kwon S. The effect of extension of benefit coverage for cancer patients on health care utilization across different income groups in South Korea. *Int J Health Care Finance Econ.* (2014) 14:161–77. doi: 10.1007/s10754-014-9144-y
21. Kuba S, Yamanouchi K, Hayashida N, Maeda S, Adachi T, Sakimura C, et al. Total thyroidectomy versus thyroid lobectomy for papillary thyroid cancer: comparative analysis after propensity score matching: a multicenter study. *Int J Surg.* (2017) 38:143–8. doi: 10.1016/j.ijsu.2016.09.083
22. Mastroianno S, Di Stolfo G, Maggio A, Pacilli M, Potenza DR, Massaro R, et al. Role of subclinical iatrogenic hyperthyroidism in the setting of heart disease and arrhythmic burden. *Endocr Metab Immune Disord Drug Targets.* (2020) 20:959–62. doi: 10.2174/1871530320666191227103627
23. Mirza F, Canalis E. Management of endocrine disease: secondary osteoporosis: pathophysiology and management. *Eur J Endocrinol.* (2015) 173:R131–51.
24. Delitala AP, Scuteri A, Doria C. Thyroid hormone diseases and osteoporosis. *J Clin Med.* (2020) 9:1034. doi: 10.3390/jcm9041034

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REVIEW
published: 14 July 2022
doi: 10.3389/fped.2022.852185

Multidisciplinary Treatment Strategies for Wilms Tumor: Recent Advances, Technical Innovations and Future Directions

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OPEN ACCESS

Edited by:
Luca Pio,
St. Jude Children's Research Hospital, United States

Reviewed by:
Alastair Millar, University of Cape Town, South Africa
Michael Nightingale, Royal Children's Hospital, Australia

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Specialty section:
This article was submitted to Pediatric Surgery, a section of the journal Frontiers in Pediatrics

Received: *10 January 2022*
Accepted: *27 May 2022*
Published: *14 July 2022*

Citation:
Theilen T-M, Braun Y, Bochennek K, Rolle U, Fiegel HC and Friedmacher F (2022) Multidisciplinary Treatment Strategies for Wilms Tumor: Recent Advances, Technical Innovations and Future Directions. Front. Pediatr. 10:852185. doi: 10.3389/fped.2022.852185

Significant progress has been made in the management of Wilms tumor (WT) in recent years, mostly as a result of collaborative efforts and the implementation of protocol-driven, multimodal therapy. This article offers a comprehensive overview of current multidisciplinary treatment strategies for WT, whilst also addressing recent technical innovations including nephron-sparing surgery (NSS) and minimally invasive approaches. In addition, surgical concepts for the treatment of metastatic disease, advances in tumor imaging technology and potentially prognostic biomarkers will be discussed. Current evidence suggests that, in experienced hands and selected cases, laparoscopic radical nephrectomy and laparoscopic-assisted partial nephrectomy for WT may offer the same outcome as the traditional open approach. While NSS is the standard procedure for bilateral WT, NSS has evolved as an alternative technique in patients with smaller unilateral WT and in cases with imminent renal failure. Metastatic disease of the lung or liver that is associated with WT is preferably treated with a three-drug chemotherapy and local radiation therapy. However, surgical sampling of lung nodules may be advisable in persistent nodules before whole lung irradiation is commenced. Several tumor markers such as loss of heterozygosity of chromosomes 1p/16q, 11p15 and gain of function at 1q are associated with an increased risk of recurrence or a decreased risk of overall survival in patients with WT. In summary, complete resection with tumor-free margins remains the primary surgical aim in WT, while NSS and minimally invasive approaches are only suitable in a subset of patients with smaller WT and low-risk disease. In the future, advances in tumor imaging technology may assist the surgeon in defining surgical resection margins and additional biomarkers may emerge as targets for development of new diagnostic tests and potential therapies.

Keywords: nephroblastoma, kidney neoplasm, surgical oncology, nephron sparing surgery, minimal invasive surgery, nephrectomy, therapy, biomarkers

INTRODUCTION

Renal solid tumors account for ∼5% of all childhood tumors with 8.3–15.1 cases per one million person-years worldwide (1–3). Over 90% of these renal malignancies are Wilms tumors (WT; syn. nephroblastoma) presenting at a peak incidence of 2–3 years of age (4). The majority of cases are sporadic, whereas 10–15% present in relation to genetic malformation syndromes predisposing to tumor development such as Beckwith-Wiedemann, Denys-Drash or WAGR (i.e., WT, aniridia, genitourinary anomalies and range of intellectual disabilities) (5).

The first successful nephrectomy for a WT in a 2-year-old boy by Thomas Richard Jessop in 1877 (Leeds, England), the first histopathological description of WTs by "Max" Wilms in 1899 (Leipzig, Germany) as well as the introduction of radiation therapy and cytotoxic chemotherapy in the 1950s and 1960s are the foremost origins of what has led to today's treatment standards for WT patients (6–8). Collaborative studies by the National Wilms Tumor Study Group (NWTS)/Renal Tumor Committee of the Children's Oncology Group (COG) in North America and by the International Society of Pediatric Oncology (SIOP) in Europe laid the groundwork for protocol-driven treatment plans. Today, a combination therapy of surgical resection, adjuvant and/or neoadjuvant chemotherapy and in some cases irradiation achieves an overall survival (OS) exceeding 90% for localized WT, 75% for metastasized WT and 50% in the case of WT recurrence (9).

Despite the good outcome for the majority of patients with WT, some remain at risk for poor survival or have a treatment related long-term risk of side effects (10). Risk factors for reduced event-free survival (EFS) and OS are: more than two-third blastemal cells within the tumor after induction chemotherapy (SIOP protocol, 8–9% of patients), anaplastic histology (5–10% of patients), loss of heterozygosity (LOH) at chromosomes 1p/16q (12 and 17% of patients, respectively), (non-resolving) metastasis (12–17% of patients), bilateral WT (5–8% of patients) and recurrent WT (20% of patients) (11–19). These patients need intensive chemotherapy and radiation therapy, thus putting them at risk for treatment related side effects. Overall, 25% of WT patients suffer from subsequent side effects such as renal failure, cardiac toxicity, pulmonary restrictive disease, infertility and secondary malignancies (20, 21).

Recent and ongoing clinical trials drive treatment strategies toward a more and more risk-based and individualized therapy approach. The identification of personal risk factors will eventually confine intensive therapy to smaller subgroups of patients, thus reducing chemotherapy-related and radiation-related side effects for others without reducing survival.

This article provides a comprehensive overview of current multidisciplinary treatment strategies for WT, whilst also addressing recent technical innovations including nephron-sparing surgery (NSS) and minimally invasive approaches. In addition, surgical concepts for the treatment of metastatic disease, advances in tumor imaging technology and potentially prognostic biomarkers will be discussed.

CURRENT DIAGNOSTIC WORK-UP AND STAGING

The incidental palpation of an asymptomatic abdominal mass is the most common presentation of a child with WT. In only 20% of cases, the presentation consists of malaise, pain, fever, gross hematuria or renal hypertension (22, 23). Rarely, an acute abdomen due to tumor rupture is the first presentation of a WT. In other cases, WT is diagnosed by routine ultrasonography in patients with conditions predisposing for WT such as Beckwith-Wiedemann syndrome or in patients in whom metachronous metastasis is suspected.

Abdominal ultrasonography is the first diagnostic choice to confirm a renal mass. To differentiate a WT from other renal masses (e.g., kidney malformations) or masses in close proximity to the kidney (e.g., neuroblastoma), abdominal MRI- and CT-scans are the current standards of imaging. Urine analysis for catecholamines and metaiodobenzylguanidine scintigraphy can also help to discriminate neuroblastoma from WT. Further work-up includes laboratory tests screening for tumor-associated anemia and thrombocytopenia, kidney malfunction, altered liver enzymes in case of liver metastasis and disrupted coagulation such as WT-acquired von Willebrand disease.

In the NWTS-4, patients with a diagnostic biopsy had a higher risk for local recurrence leading to an upgrading of a stage I and II tumor to a stage III tumor in later protocols (24). An upstaging is not recommended in the SIOP protocol, as studies have not confirmed local recurrence (except in case of open tumor biopsy) (25). In general, biopsies should only be considered when a tumor different to WT is suspected. This is usually the case when renal tumors present at an age older than 6 years, the mass is completely extra renal or imaging shows distinct calcifications suggesting a histology other than WT (26).

MRI, CT and ultrasound scans contribute to pretreatment staging. The contralateral kidney should be screened for synchronous bilateral disease. Aortocaval and hilar lymph nodes, lung and liver need to be screened for metastasis. CT scans (with or without contrast) have replaced plain X-ray images of the chest to detect lung metastasis (27–30). Contrast MRI (or CT) and ultrasound examinations will need to assess extension of the tumor into the renal vein and vena cava. Echocardiography is warranted to assess heart function and the venous extension of a tumor thrombus into the atrium (31–33).

Current staging systems of WT are based on imaging and surgical findings such as local tumor stage (e.g., complete vs. incomplete resection, perioperative tumor rupture or lymph node metastasis), bilateral disease and hematogenous metastasis to the lung and liver (rarely bone, brain or other sites) (**Table 1**). The histopathology of the resected tumor defines the prognostic risk group. The NWTS/COG classifies tumors in favorable and unfavorable, whereas SIOP differentiates low-, intermediate- and high-risk tumor histopathology according to the predominant cell components within the tumor (30, 34, 35) (**Table 2**).

In recent years, central review panels assist in staging, radiology interpretation, surgical decision-making and histology examinations of patients with WT [established by

TABLE 1 | Current staging systems of WT according to NWTS/COG and SIOP (post-surgery).

Stage		NWTS/COG staging	SIOP staging
I	Complete resection with negative margins	Primary tumor within renal capsule, no capsule involvement No extension to renal sinus	Tumor is confined to the kidney, no penetration of the renal capsule No tumor infiltration of the vessels at the renal sinus, the renal pelvis and the ureter
II	Complete resection with negative margins	Primary tumor penetrating renal capsule but not Gerota's fascia Lymphatic and venous involvement at renal sinus Tumor extension into renal vein/vena cava	Viable tumor is present within – soft tissue of renal sinus – the wall of renal pelvis or ureter Viable tumor is present but completely resected within – perirenal fat – blood vessels/lymphatic vessels at the renal sinus – adjacent organs (except adrenal gland) – vena cava
III	Incomplete resection, residual tumor	Macroscopic or microscopic residual disease – Preoperative biopsy of the tumor (including open and percutaneous biopsy) – Tumor rupture pre- or intraoperatively, removal of tumor tissue in fragments – Positive retroperitoneal/abdominal lymph node(s) – Peritoneal involvement – Positive resection margins at ureter, renal vein, main tumor	Viable tumor – at the resection margin – at the site of tumor rupture Viable or non-viable tumor – in abdominal lymph nodes – thrombus at the resection margin of ureter, renal vein, vena cava – implants within the abdominal cavity – penetrating through the peritoneal surface Venous tumor thrombus resected piecemeal Open tumor biopsy prior to chemotherapy
IV	Metastatic disease	Hematogenous metastasis (e.g., lung, liver, bone or brain)	Hematogenous metastasis (e.g., lung, liver, brain or bone) Lymphatic metastasis beyond abdominal and retroperitoneal lymph nodes
V	Bilateral disease	Bilateral synchronous disease (+ stage should be evaluated for each side separately)	Bilateral synchronous disease (+ stage should be evaluated for each side separately)

TABLE 2 | Prognostic risk groups for WT according NWTS/COG and SIOP relating to the histopathology of embryonal renal tumors in childhood.

NWTS/COG		SIOP	
Prognostic risk group	**Histology (pre-chemotherapy)**	**Prognostic risk group**	**Histology (post-chemotherapy)**
Mesoblastic histology	Mesoblastic nephroma*	Low-risk	Mesoblastic nephroma* Cystic partially differentiated nephroblastoma* Completely necrotic WT after neoadjuvant chemotherapy
Favorable histology	WT with – epithelial – stromal – blastemal – or mixed (triphasic) cell components	Intermediate- risk	WT with – epithelial – stromal – or mixed cell components Regressive histology features Focal anaplasia
Unfavorable histology	WT with focal and diffuse anaplasia	High-risk	WT with – blastemal cells – diffuse anaplasia Renal rhabdoid tumor* Renal clear cell sarcoma* Renal cell carcinoma*

**Non-Wilms tumors.*

the NWTS/COG (i.e., AREN03B2 umbrella study) as well as by the SIOP-Renal Tumor Study Group (RTSG) (i.e., UMBRELLA SIOP-RTSG 2016; https://fnkc.ru/docs/SIOP-RTSG2016.pdf)] (30, 34, 36). It has been shown that the COG central review adjusted the initial risk stratification in ∼20% of cases (23). Centralized review and assistance by WT study groups may eventually lead to more concise outcome data. To integrate and combine the complex sets of medical data, e-health tools have been developed such as "p-medicine" utilized by the UMBRELLA SIOP-RTSG protocol.

OVERVIEW OF CURRENT MULTIDISCIPLINARY TREATMENT STRATEGIES

The two most commonly applied treatment regimens for WT derive from the NWTS/COG and SIOP. Current treatment protocols of both groups are based on the concept of risk-adapted therapy including surgery, chemotherapy and irradiation. Throughout the multiple WT studies, patients have been identified as requiring either intensified or reduced therapy according to their individual risk factors. The NWTS/COG-based protocols have followed the concept of upfront tumor resection to plan therapy according to biological information and local stage of the tumor. In contrast, the SIOP protocol includes upfront chemotherapy before surgical resection to shrink and downstage the tumor, thus increasing the chance for complete resection and minimizing the operative risk of a tumor rupture. However, in case of a kidney tumor in a child under 6 months of age, the SIOP protocol recommends upfront tumor resection, as most kidney tumors in this age group either need no further therapy (e.g., congenital mesoblastic nephroma) or need intensified chemotherapy at the outset (e.g., malignant rhabdoid tumors) (30). Local stage and tumor biology findings are used for a risk-modified treatment. Despite these strategic treatment differences between the NWTS/COG and SIOP approach, patient outcome is nearly identical.

Metastatic Disease

About 10–12% of patients with WT present with metastasis. Lymph node, lung and liver are the most prominent metastatic sites. In recent studies, particular attention has been given to the treatment of lung metastasis in WT. Standard treatment for lung metastasis has consisted of escalated systemic therapy and whole lung radiation therapy (WLRT) regardless of lung nodule response. In the NWTS/COG AREN0533 study, patients with complete response in chest CT scans after 6 weeks of DD-4A did not receive further WLRT. However, patients with incomplete response and those with LOH at chromosomes 1p/16q received additional intensified therapy with four cycles of systemic cyclophosphamide and etoposide and WLRT according to the NWTS/COG protocol (**Box 1**). Both groups, complete and incomplete responders, had significantly improved 4-year EFS and OS estimates (85.4 and 95.6%, respectively) compared to the previous NWTS-5 (72.5 and 84.0%, respectively) (27). Similar results have been stated by SIOP (17) (**Box 2**). In uncertain lung lesions on chest CT or in so-called "slow incomplete responders" assessed by chest CT imaging, some studies encourage to biopsy (at least two) such lesions to certify metastasis before WLRT is applied (37).

Aspects of Surgical Intervention

Complete resection with tumor-free margins remains the primary surgical aim for cure of WT. Generally, the NWTS/COG protocol schedules surgical resection initially after diagnosis before any further treatment. Neoadjuvant chemotherapy is only given to NWTS/COG protocol-treated patients in cases, in which the tumor has ruptured preoperatively, the tumor is deemed to be irresectable (e.g., large tumors with intraoperative risk for rupture, extensive organ invasion with the risk for organ removal and venous tumor extension beyond the hepatic veins) or the patient is at risk for anesthesia-related complications due to cardiocirculatory compromise by a high tumor burden or extensive venous thrombosis. In contrast, SIOP protocol-treated patients are typically operated after four cycles of neoadjuvant chemotherapy.

General Surgical Principles

In unilateral WT, complete tumor nephroureterectomy through a transabdominal approach is the standard of surgical care for NWTS/COG- and SIOP-treated patients. The ureter should be resected along the tumor kidney and divided as close as possible to the bladder as the tumor may extend along the ureter. For local staging, hiliar and (inter-)aortocaval lymph node sampling as well as peritoneal exploration is required. Lymph node sampling should be performed even in cases of negative nodal preoperative imaging. An insufficient nodal dissection may lead to under-staging and under-treatment in cases positive lymph nodes remain undetected (24, 38). The impact of an undefined lymph node status in patients with WT has been reviewed by many studies. For instance, NWTS-4 and NWTS-5 both demonstrated an increased likelihood of finding a positive lymph node when more than seven lymph nodes were sampled (39). OS has also been shown to improve significantly with the number of resected lymph nodes (i.e., 5-year OS of 87% with no lymph nodes sampled to 95% with more than 10 lymph nodes sampled) (40). In the current treatment protocols, dissection of at least seven lymph nodes is therefore recommended (30, 34).

The general nature of WT growth is expansion (i.e., pushing organs away). In rare cases, adherence or invasion to adjacent organs such as liver, diaphragm, adrenal gland or bowel is seen. **Table 3** summarizes general surgical principles in (unilateral) WT.

Special (surgical) considerations have to be respected in case of large tumors, ruptured tumors, intravascular tumor extension, bilateral tumors, tumors in syndromic patients and tumors in a horseshoe kidney.

Large Tumors and Tumor Rupture

Preoperative tumor rupture or intraoperative tumor spill is of particular concern in WT as it creates a risk for local recurrence (24, 47). Spontaneous or traumatic tumor rupture is detected on preoperative imaging at a rate ranging between 3 and 23% (24, 48, 49). Intraoperatively, tumors larger than 12 cm in diameter are noted to be at risk for rupture (50). Patients treated according to the NWTS/COG protocol (i.e., no preoperative chemotherapy) have a risk of intraoperative tumor spillage of up to 10% (38, 50). An intraoperative spillage rate of 12% has been reported by SIOP in patients who had not received preoperative chemotherapy treatment (51). However, among SIOP-treated patients with neoadjuvant chemotherapy, the risk for intraoperative tumor spillage had decreased to around

BOX 1 | Basic treatment principles of WT as per NWTS/COG (covering most clinical situations).

Stage I (patients <2 years of age): Tumors with favorable histology and tumor kidney weight <550 g: RN without further treatment
Stage I and II: Tumors with favorable histology: RN + adjuvant chemotherapy with regimen EE-4A for 18 weeks; tumors with favorable histology and LOH 1p/16q: RN + adjuvant chemotherapy with regimen DD-4A for 24 weeks
Stage III: Tumors with favorable histology: RN + adjuvant chemotherapy with regimen DD-4A for 24 weeks + flank RT; tumors with favorable histology and LOH 1p/16q: RN + adjuvant chemotherapy with regimen M for 31 weeks + pulmonary RT
Stage IV: Tumors with favorable histology and isolated lung metastasis: RN + adjuvant chemotherapy with regimen DD-4A for 24 weeks + whole-lung RT; tumors with favorable histology and LOH 1p/16q: RN + adjuvant chemotherapy with regimen M for 31 weeks + pulmonary RT
Stage V: Neoadjuvant chemotherapy with DD-4A for 6–12 weeks, partial nephrectomy, adjuvant chemotherapy depends on path

LOH, loss of heterozygosity; RN, radical nephrectomy; RT, radiation therapy; regimen EE-4A (vincristine, dactinomycin for 18 weeks); regimen DD-4A (vincristine, dactinomycin, doxorubicin for 24 weeks), regimen M (vincristine, dactinomycin, doxorubicin, cyclophosphamide, etoposide).

BOX 2 | Basic treatment principles of WT as per SIOP (covering most clinical situations).

Stage I–III: Neoadjuvant chemotherapy (except patients <6 months of age: RN) with AV for 4 weeks followed by RN, adjuvant therapy is determined by local stage and histology:

- **Stage I:** No further treatment to patients with RN and low-risk histology, all other patients receive chemotherapy with either AV for 4 weeks (intermediate-risk tumors, TV <500 ml) or AVD for 27 weeks (high-risk tumors, TV >500 ml)
- **Stage II:** Chemotherapy with AV for 27 weeks (low- and intermediate-risk tumors, TV <500 ml) or with HR-1 for 34 weeks (high-risk tumors, TV >500 ml) + flank RT for high-risk tumors
- **Stage III:** Chemotherapy with AV for 27 weeks (low- and intermediate-risk tumors, TV <500 ml) + flank RT for intermediate-risk tumors or with HR-1 for 34 weeks + flank RT (high-risk tumors, TV >500 ml)

Stage IV: Neoadjuvant chemotherapy with AVD for 6 weeks, re-imaging of metastatic lesions before RN, continuation adjuvant therapy depending on remission of metastasis:

- Complete remission of metastasis: AVD for 27 weeks
- Incomplete response of metastasis: HR-1 for 34 weeks + RT of metastatic organ, surgical resection of metastatic lesions can be attempted when feasible without risk of organ morbidity
- High-risk histology of the primary tumor: HR-1 for 34 weeks + RT of metastatic organ

Stage V: Neoadjuvant chemotherapy with AV for 4–6 weeks (12 weeks maximum) followed by surgery with the aim of nephron-sparing surgery, adjuvant chemotherapy according to the histopathological subtype + abdominal/flank RT in appropriate cases.

AV, (actinomycin D, vincristine); AVD, (actinomycin D, vincristine, doxorubicin); HR-1, (etoposide, carboplatin, cyclophosphamide, doxorubicin); low-risk, (i.e., complete necrosis); intermediate-risk, (i.e., blastemal tumor components); high-risk, (i.e., predominant blastemal, anaplastic components); RN, radical nephrectomy; RT, radiotherapy; TV, tumor volume after neoadjuvant therapy.

3%. This is advocated as one of the major surgical advantages of the SIOP approach (51, 52). Once rupture of the tumor capsule is evident (pre- or intraoperatively), NWTS/COG stage I-II tumors are upgraded to stage III tumors. Upon preoperative surgical assessment, the NWTS/COG protocol provides the option for neoadjuvant chemotherapy when preoperative tumor rupture is evident, tumor spill is likely at surgery or the tumor deems unresectable without significant morbidity due to its size (24). The UMBRELLA SIOP-RTSG 2016 protocol also adjusts treatment to stage III when viable tumor cells are detected microscopically at the area of rupture (34). In addition to the upstaging of the tumor with intensified chemotherapy, whole abdominal irradiation for diffuse tumor spillage or an irradiation boost to the flank is applied in most cases (30, 53).

Intravascular Extension

In 4–10% of patients with WT, the tumor extends into the renal vein or inferior vena cava, whereas an extension into the right atrium or ventricle occurs in around 1–3% of cases (43, 54–57). A complete picture of a possible intravascular extension is essential for planning anesthesia and surgery. For example, an extensive tumor thrombus to the cardiac atrium may lead to cardiac deprivation and the need for cardiopulmonary bypass surgery for its resection (31, 56, 58). As the venous tumor extension is not always seen on preoperative imaging, intraoperative exploration by manual palpation and/or ultrasonography of the renal vein is mandatory. In general, excision of a tumor thrombus needs to be achieved in continuity, as dissection will upstage the tumor to stage III. The NWTS/COG protocol therefore recommends neoadjuvant treatment when intravascular tumor extends up to or above the hepatic veins (43, 59). It has been demonstrated that in 45–87% of patients with a tumor thrombus in their inferior vena cava, preoperative chemotherapy led to a size reduction improving surgical conditions (43, 60, 61). However, cardiac tumor extensions are often less responsive to neoadjuvant therapy, but in cases of good responds, cardiopulmonary bypass surgery can be avoided (43, 56).

Bilateral WT

About 6–7% of patients develop synchronous and <1% metachronous bilateral WT (15, 62). The prevalence is higher

TABLE 3 | General surgical principles in unilateral WT to achieve local control.

Organ/Structure	Surgical measure	Comment	References
Tumor kidney	Radical resection, avoid tumor spillage	Tumor spillage will lead to stage upgrading and more intensive therapy, tumor rupture is associated with relapse	(24, 41)
Ureter	Division at the most distal level (closest to the bladder)	To achieve negative margins in case of tumor involvement of the ureter	(42)
Renal vein, inferior vena cava	Palpation and/or intraoperative ultrasonography to rule out tumor extension, en-bloc excision of a venous tumor thrombus	Complete tumor removal, en-bloc resection of the tumor thrombus together with the primary kidney tumor to avoid tumor tissue dissection and upstaging of the tumor	(43)
Lymph node	Sampling of hiliar, paracaval and paraaortic lymph nodes (and suspicious mesentery lymph nodes)	Local staging, sampling of more than seven lymph nodes	(34, 41)
Ipsilateral adrenal gland	Can be left *in situ* when easily separated from the tumor kidney and when without signs of tumor involvement	Incidence of tumor invasion into the gland in <5% of cases	(44)
Diaphragm	En-bloc resection in case of adherent tumor	To avoid spillage dissecting the tumor off the diaphragm	(30)
Liver	Extensive en-bloc resection or partial hepatectomy is not recommended in case of direct spread	Minimize secondary liver complications, no benefit shown for survival in case of extensive hepatic resection	(24, 45)
Bowel	Partial resection of intestine/colon	In case of tumor infiltration	
Peritoneum	Peritoneal exploration	Local staging, sign of tumor extension	
Contralateral kidney	Exploration	Only in case of a suggested contralateral kidney lesion or enlarged contralateral lymph nodes in pre-operative imaging, exploration should be done prior to tumor nephrectomy of the primarily involved kidney to adapt the surgical approach intraoperatively	(24, 38, 46)

in patients with genetic predisposition syndromes carrying WT1 gene mutations and loss of imprinting (LOI) at 11p15 (15). As these genetic alterations are driving factors for early-disrupted embryologic differentiation of the kidney tissue, (multifocal) nephrogenic rests or nephroblastomatosis lesions are often seen in bilateral WT (63, 64). Conditions predisposing to metachronous bilateral WT are Denys-Drash syndrome, WAGR syndrome, Beckwith-Wiedemann syndrome, Fanconi anemia and familial WT. In these patients, up to 90% of WTs occur within the first 7 years of life necessitating close routine screening programs (15, 64).

Data from early studies revealed that patients with bilateral WT have a higher risk to suffer from end-stage renal disease (ESRD) within 20 years after treatment (cumulative incidence of 12% in bilateral WT vs. <1% in unilateral WT) (65). In patients with predisposing conditions, renal insufficiency rates are even higher (e.g., 83% in Denys-Drash syndrome and 43% in WAGR syndrome) (66, 67). In cases of synchronous or potentially metachronous bilateral WT, renal tissue can be preserved by NSS. Best conditions for successful NSS are low volume tumors in the kidney's periphery (67, 68). Neoadjuvant chemotherapy to confine the tumor enabling surgical resectability is therefore part of all treatment protocols. In a selected group of patients with bilateral WT, bilateral nephrectomy and kidney transplantation may be considered as a treatment option to eliminate the risk of initial or metachronous WT development (68, 69). This subgroup includes patients with Denys-Drash syndrome in whom prophylactic or secondary bilateral nephrectomy (in case of ESRD) is an acceptable procedure (64, 70, 71). In these patients, kidney transplantation is usually performed after 1–2 years of disease-free survival (64, 67).

Horseshoe and Solitary Kidneys

Wilms tumor in a horseshoe kidney is an exceptionally unique situation. There is a historic collection of 41 patients reported from the NWTS/COG (72). Surgical aims are the same as for unilateral WT with complete tumor nephroureteroectomy and lymph node sampling. However, some surgeons advocate NSS (73). The treatment of WT in solitary kidneys is guided by the same principles as for bilateral WT (30).

RECENT TECHNICAL INNOVATIONS

Nephron-Sparing Surgery

Performance of NSS is a necessity in children presenting with bilateral WT (74). In unilateral WT, NSS must be considered in syndromic patients with an increased risk of metachronous development of (contralateral) WT, in patients with a solitary kidney or an afunctional contralateral kidney as well as in patients who are at risk of kidney failure (46). In non-syndromic unilateral WT, NSS is not a standard procedure (75). Potential benefits are prevention of functional renal impairment and ESRD, whereas some long-term follow-up data suggest good renal function even after unilateral radical nephrectomy (RN) for WT (65, 76–79). However, higher rates of postoperative stage III WTs due to positive resection margins have been reported (75). This required conversion to RN and/or additional radiation therapy. Still, EFS and OS were comparable to patients with RN (75, 80). If NSS is considered in unilateral WT, careful patient selection should be performed preoperatively (80). Davidoff et al. (81) prospectively analyzed patients on NSS feasibility according to radiological imaging and concluded that 8% of patients could be treated with NSS. The SIOP-RTSG surgical panel formulated

a list of preconditions under which NSS for unilateral WT may be performed (i.e., tumor restricted to one pole, tumor volume <300 ml, no tumor rupture, no tumor in renal pelvis, no continuous organ invasion, no venous tumor thrombus, functioning kidney remnant after NSS) (30, 82).

Nephron-sparing surgery can be carried out by partial nephrectomy (i.e., tumor resection with a rim of normal renal tissue) or enucleation of the tumor (i.e., tumor resection without a rim of normal renal tissue) (30). At present, there are no available data comparing both methods. Method selection is highly dependent on tumor localization, size and the presence of multifocal lesions.

The main aim of NSS is to preserve as much healthy kidney tissue as possible. Preoperative chemotherapy may contribute to the preservation of renal tissue. The COG-AREN0534 reviewed 34 patients with predisposing conditions to bilateral WT presenting with unilateral tumors. This study showed that partial nephrectomy was feasible in 65% of patients after neoadjuvant chemotherapy, avoiding tumor nephrectomy and sparing renal tissue without compromising EFS and OS (46).

To assure a bloodless dissection and good visibility when dissecting through the kidney's parenchyma, intermittent clamping of the renal vasculature may be helpful. Some surgeons have advocated adopting operative techniques from adult kidney cancer surgery such as inducing renal hypothermia (e.g., application of ice water into the kidney bed) in the attempt to maximize the preservation of kidney tissue (62, 83, 84). As this is not yet a standard approach, future studies will need to address possible outcome benefits of this technique.

Another surgical adjunct is the use of intraoperative ultrasound in NSS. For example, ultrasound-guided mapping of the tumor nodules can aid to optimize surgical dissection lines, thus preserving healthy kidney tissue. Still, its use has to be critically reviewed as it does not guarantee tumor-free resection margins (83).

Minimally Invasive Approaches

Laparoscopic RN and laparoscopic-assisted partial nephrectomy is becoming more common for the treatment of WT as comparable outcomes have been reported. Nevertheless, the outcome analysis may be biased by the fact that laparoscopically resected tumors have lower stages of disease (75, 85–90). Additionally, robotic-assisted laparoscopy (RAL) is a developing field in pediatric surgical oncology (91–94). However, the experience of RAL in WT surgery is yet limited and its indications need to be carefully discussed in tumor and surgical reference boards (82, 94). In general, minimally invasive tumor resection is limited to tumors confined to the kidney with good exposure of the hilar vessels (85, 95). Large tumors may not leave enough operating space for laparoscopy. Therefore, patient selection is of the utmost importance (95, 96). In the UMBRELLA SIOP-RTSG 2016 protocol, the following prerequisites for laparoscopic RN including RAL have been proposed: small, central tumors with a rim of non-malignant renal tissue, extraction of the specimen in a bag without morcellation, no venous tumor thrombus, no continuous organ infiltration, no extension of the tumor beyond the ipsilateral boarder of the spine, no imminent tumor rupture (i.e., in case of no response to chemotherapy) and feasibility of lymph node sampling as well as the operating surgeon is expected to be experienced in minimally invasive nephrectomy (30, 82). In all instances, performance of minimally invasive surgery must adhere to the same general surgical principles of WT treatment such as complete lymph node sampling and thoroughness of resection (30).

ADVANCES IN TUMOR IMAGING TECHNOLOGY

Diffusion-weighted imaging (DWI) MRI technique has added a new quality to the available imaging modalities for WT. By defining the whole-tumor apparent diffusion coefficient, it provides information on the "cell density" of a tumor lesion, assisting the radiologist in defining necrotic from viable lesions after chemotherapy and small lesions in nephroblastomatosis (97–99). Imaging research studies aim at defining histological subtypes of WTs by DWI for radiologic assistance in risk stratification (100–102).

Detecting hiliar and retroperitoneal metastatic lymph node disease is critically important in WT (103, 104). Lymph node metastasis can be occult and may not necessarily be apparent by lymph node enlargement during the operation. To improve lymph node sampling in WT, first feasibility studies have been conducted to establish the concept of intraoperative sentinel lymph node detection. Two techniques have been described in the attempt to delineate the lymphatic drainage of the involved kidney: injection of a radioactive tracer (e.g., technetium-99 m phytate detected with an intraoperative gamma probe) or a fluorescence dye (e.g., indocyanine green visible under near-infra red laparoscopy) (103, 104). In this recently published study, including unilateral WTs with indication for tumor nephrectomy, sentinel lymph nodes were most frequently detected in the aorto-caval space after injection of a radioactive tracer into the normal kidney tissue adjacent to the WT (104). Further systematic studies will be needed to standardize and verify the advantages of this technique.

Surgical research on fluorescence-guided kidney surgery has also evolved. Surgical procedures delineating perfusion of the kidney including separation of the upper and lower pole, visualizing renal masses (cysts) or mapping lymphatic drainage (e.g., in lymphatic sparing varicocelectomy) have been successfully executed (105, 106). These new surgical imaging techniques may eventually have the potential to transform the surgical approach in WT treatment.

PROGNOSTIC BIOMARKERS

The number of newly identified biomarkers in WT is growing constantly. To date, more than 30 (epi-)genetic and protein biomarkers have been suggested (107). Most of these biomarkers are closely linked to tumorigenesis and predisposition of WT. Some may eventually play a role in targeted therapy. One such candidate is an antagonist of the Wnt/beta-catenin pathway, tegavivint (BC2059), which is currently under investigation by

the COG for its antitumor activity in recurrent and refractory pediatric solid tumors including WT. Currently, patients with WT-associated CTNNB1 oncogene mutations leading to Wnt/beta-catenin pathway activation can be included in this study (https://clinicaltrials.gov/ct2/show/NCT04851119) (108).

So far, only one genetic biomarker, i.e., LOH at chromosomes 1p/16q, has been integrated as a decision-making factor into the current COG treatment protocol. In the presence of LOH at chromosomes 1p/16q, patients with stage I and II favorable WT histology will be upstaged from low to standard risk receiving regimen DD-4A (i.e., vincristine, dactinomycin and doxorubicine for 24 weeks) instead of regimen EE-4A (i.e., vincristine and actinomycin D for 16 weeks) after nephrectomy. The previous NWTS-5 showed a significantly improved EFS and OS in patients with intensified therapy (27). Intensified chemotherapy is also given to patients with LOH at chromosomes 1p/16q and lung metastasis in the NWTS/COG protocol. Although it serves as a sensitive marker for risk stratification, LOH at chromosomes 1p/16q is only applicable in a small subset of patients and presently it does not offer possible treatment targets.

With 30% of favorable histology WT cases, one of the most prevalent outcome predictors is gain of function (GOF) at chromosome 1q. It is associated with significantly poorer EFS and OS, as reported by the NWTS/COG and SIOP study groups, and is currently under reinvestigation in the UMBRELLA SIOP-RTSG protocol for its prognostic value (30, 109, 110).

In general, WT is considered an embryonal tumor consisting of primordial renal cells disrupted to mature into differentiated kidney tissue. Variable proportions of blastemal (i.e., renal stem cells), epithelial and stromal cells have great influence on tumor behavior and outcome. During kidney development, organogenesis passes the stage of nephrogenic differentiation. This stage of development can persist as intra- or perilobar nephrogenic rests throughout the first few years of life and is a very likely origin for WT tumorigenesis. One important factor in driving embryonal renal differentiation is the transcription factor WT1 on chromosome 11p13. Its mutation is linked to the development of WT. WT1 mutations are reported in 10–20% of sporadic WT cases (111). Together with mutations in the CTNNB1 and AMER1 (WTX) tumor suppressor genes, it unfolds its tumorigenic role by upregulation of the Wnt/beta-catenin pathway (112). As germline alterations, WT1 mutations are common in syndromic predisposition syndromes (e.g., Denys-Drash syndrome and WAGR syndrome), bilateral WT and WT with synchronous nephrogenic rests (5, 113, 114). Another transcription factor that plays a role in bilateral as well as in predisposing syndromes is WT2 on chromosome 11p15. Mutations in this gene are much more abundant (i.e., 70% of cases) with 40% in sporadic cases and 30% in Beckwith-Wiedemann syndrome (115).

In recent research, mutations have been identified in microRNA (miRNA) processing genes in about 20% of patients with WT (116, 117). These alterations in miRNA processing are thought to conserve the embryonal stage of kidney development (116, 118). However, data concerning their influence of clinical behavior in WT have not yet been specifically reported.

When analyzing molecular markers in tumor tissues, it is important to consider that tumors consist of miscellaneous tissue parts containing different levels of marker expression. This has been shown for GOF at chromosome 1q, leaving a general uncertainty in biopsies (109, 119).

In the majority of cases, biomarkers identify WTs with treatment resistance and poor prognosis. **Table 4** provides an overview of transcription factors, oncogenes and tumor suppressor genes directing at multiple different pathways potentially involved in tumorigenesis of WT.

FUTURE DIRECTIONS

The concept of "personalized medicine" has already improved the treatment of children with WT. The future trend will increasingly be driven by two principles: (1) de-escalation of therapy with the aim to define the appropriate level of treatment to achieve the best outcome while minimizing secondary side effects, and (2) identifying tumor-related and personal risk factors justifying escalation of therapy. To address these principles, the two large WT consortia of the COG and SIOP have designed new prospective studies (i.e., AREN03B2 umbrella study within the COG registry "*Project: Every Child*" and UMBRELLA SIOP-RTSG protocol, respectively) to collect and overlay clinical and biological data on a profound scale. Within these registries, both groups have established systematic biospecimen banks with tumor and blood samples available for prospective and retrospective analysis. Due to the fact that many prognostic (bio-)markers, such as blastemal histology or LOH at chromosomes 1p/16q, are only identified in a small subset of patients, treatment centers worldwide are encouraged to enroll patients (30).

Molecular research has identified a multitude of genetic and protein biomarkers for WT that may eventually assign patients into more specific risk groups. Among the most prevalent and promising prognostic markers for adverse outcome is GOF at chromosome 1q, which is currently under investigation as part of the UMBRELLA SIOP-RTSG protocol. Other markers may potentially serve as therapeutic targets. Patients with recurrent or refractory WT and alteration of the Wnt/beta-catenin pathway can be included in a therapeutic phase I/II application study of tegavivint (BC2059). The study is open for a number of different pediatric solid tumors with refractory treatment response and mutational activation of the Wnt/beta-catenin pathway.

Genomic sequencing programs such as TARGET (Therapeutically Applicable Research to Generate Effective Treatments) in the United States and FACT (Factors Associated with Childhood Tumors) in the United Kingdom have accelerated the discovery of inherited and acquired factors possibly responsible for the development of WT (128–130). The growing knowledge on tumor biology and genetics will increasingly influence the decision-making process, and contribute to the general understanding of tumorigenesis of WT.

Another important field of research with respect to WT biomarkers is the identification of tumor DNA in blood or urine samples. In patients enrolled in SIOP and UKCCSG/CCLG

TABLE 4 | List of selected biomarkers with potential relevance for WT prognosis and/or tumorigenesis.

Biomarkers, Gene	Incidence	Relevant findings	Reference
CTNNB1	15%	– Stromal predominant histology – Upregulation of the Wnt/beta-catenin pathway – Patients with CTNNB1 mutations leading to upregulation of the Wnt/beta-catenin pathway are currently included in phase II trials as a possible treatment target for tegavivint (BC2059)	(10, 108, 111)
LOH 1p/16q	5.0% in WT with favorable histology and 9.4% in relapsed WT	– NWTS-5: LOH 1p/16q predicted inferior 4-year EFS and OS in stage III and IV tumors	(11, 107)
LOH 1p	12%	– NWTS-5: Significantly increased rate of relapse and decreased OS independent of tumor stage and histology – Predicting WT relapse (RR 2.93)	(11, 107)
LOH 16q	17%	– NWTS-5: Significantly increased rate of relapse and decreased OS independent of tumor stage and histology – Predicting WT relapse (RR 1.95)	(11, 107)
GOF 1q	30.0% overall and 18.3% in stage IV WT	– No histologic pre-dominance – NWTS-5: Inferior 4-year EFS and OS in stage I, III and IV tumors – Predicting relapse (RR 2.86) unrelated to tumor stage	(107, 109, 110)
WT1 (chr. 11p13)	10–20%	– Predominant stromal histology – Simultaneous presence of intralobar nephrogenic rests – WT1 mutations and LOH 11p15 associated with relapse – Germline mutations in Denys-Drash syndrome and WAGR syndrome – 90% of patients with Denys-Drash syndrome and 50% with WAGR syndrome develop WT	(5, 111, 112, 114, 120)
WT2 (chr. 11p15)	70%	– Germline mutation in Beckwith-Wiedemann syndrome – 4–5% of patients with Beckwith-Wiedemann syndrome develop WT – IGF2 upregulation	(115)
LOH 11p15		– IGF2 upregulation – Increased risk of recurrence – Described in anaplastic WT, relapse, and fatal cases	(107)
LOI 11p15	30–50%	– Leading to H19 and IGF2 activation and unrestrained cell growth – Predicting relapse in low-risk WT treated with surgery alone	(120, 121)
WTX (AMER1)	15–20%	– Upregulation of the Wnt/beta-catenin pathway – Combined with epigenetic 11p15 alterations	(111, 122)
miRNA processing genes	20%	– Mutations in miRNA processing genes including DROSHA (80% of miRNA mutations in WT), DGCR8 and DIS3L2 (Perlman syndrome)	(116–118)
MLLT1	4%	– High prevalence of intralobar nephrogenic rests	(123)
MYCN	<10%	– Described in treatment resistance, relapse, and fatal cases – Detected at higher proportion of pre-treated anaplastic WT (>30%), potential marker for treatment resistance	(124)
LOH 11q		– Higher detection in mixed and diffuse anaplastic WT – Associated with recurrence and fatal cases	(124)
SIX1 and SIX2	5–10%	– Blastemal predominant histology	
TRIM28	5%	– Mature epithelial histology predominant – Excellent prognosis – Frequent in bilateral and familial cases	(107, 125, 126)
TP53 (chr. 17p13)	5%	– 75% in diffuse anaplastic tumors – Found primarily in advanced tumor stages – Significantly poorer outcome in stage III and IV anaplastic tumors – Linked to increased recurrence	(105, 106)
CTR9	Described in four families and sporadic cases	– Non-syndromic WT predisposition	(127)

GOF, gain of function; LOH, loss of heterozygosity; LOI, loss of imprinting; WT, Wilms tumor.

(United Kingdom Children's Cancer Study Group/Children's Cancer and Leukemia Group), a 5–12% rate of misdiagnosis has been reported in patients without biopsy or primary surgery (131). In the near future, so-called "liquid biopsies" may aid in establishing WT diagnosis and screening patients in follow-up programs for recurrent WT (131–133).

Recent studies have focused on the global epidemiology of WT, offering a broad picture of who is at risk for the development of WT not only by world region, ethnic background, gender and age, but also by socioeconomic status and health care accessibility (1, 3, 134). The highest WT incidence rates have been reported in North America in children of African-American

descent, whereas the lowest was seen in children in East Asia (2). Differences in genetics have also been reported by ethnicity and world region. For instance, the genetic alteration of the IGF-2 gene locus playing the driving role in overgrowth syndromes and WT was less frequently seen in children with WT from Japan in comparison to Caucasian children (2, 135, 136). Future research will need to overlay epidemiology with genetic data in larger patient cohorts to identify populations at risk for WT development, treatment resistance or worse outcome.

Centralized review of WT imaging and pathology as well as treatment guidance has more and more been integrated in current treatment protocols. In previous studies, discrepancies in institutional and central histopathology interpretation have been reported in 20–50% of cases (9, 35). Ultimately, centralized data review may lead to more coherent data sets.

Despite significant progress in molecular biology, research defining biomarkers and identification of new treatment targets for WT, technical advances in imaging, surgery and radiation therapy will evenly be important. In the future, artificial intelligence algorithms of tumor imaging and 3-D reconstructions of WT may assist the surgeon in defining surgical resection lines, thus improving surgical outcome particularly in cases of NSS (106, 137). In addition, advances in the application of radiotherapy such as intensity-modulated radiation therapy sparing non-tumorous tissue and limiting radiation-associated toxicity will be beneficial for the WT patients (138, 139).

AUTHOR CONTRIBUTIONS

T-MT, YB, KB, UR, HF, and FF performed the literature search for the work. T-MT and YB outlined and wrote the initial manuscript draft. KB, UR, HF, and FF critically revised the initial manuscript draft for important intellectual content. All authors approved the final version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

FUNDING

This work was supported by the German Research Foundation and the Institutional Open Access Fund of the Goethe University Frankfurt within the program of Open Access Publishing.

REFERENCES

1. Cunningham ME, Klug TD, Nuchtern JG, Chintagumpala MM, Venkatramani R, Lubega J, et al. Global disparities in Wilms tumor. *J Surg Res.* (2020) 247:34–51. doi: 10.1016/j.jss.2019.10.044
2. Nakata K, Colombet M, Stiller CA, Pritchard-Jones K, Steliarova-Foucher E, IICC-3 Contributors. Incidence of childhood renal tumors: an international population-based study. *Int J Cancer.* (2020) 147:3313–27. doi: 10.1002/ijc.33147
3. Steliarova-Foucher E, Colombet M, Ries LAG, Moreno F, Dolya A, Bray F, et al. International incidence of childhood cancer, 2001–10: a population-based registry study. *Lancet Oncol.* (2017) 18:719–31. doi: 10.1016/S1470-2045(17)30186-9
4. Pastore G, Znaor A, Spreafico F, Graf N, Pritchard-Jones K, Steliarova-Foucher E. Malignant renal tumors incidence and survival in European children (1978–1997): report from the automated childhood cancer information system project. *Eur J Cancer.* (2006) 42:2103–14. doi: 10.1016/j.ejca.2006.05.010
5. Breslow NE, Beckwith JB, Perlman EJ, Reeve AE. Age distributions, birth weights, nephrogenic rests, and heterogeneity in the pathogenesis of Wilms tumor. *Pediatr Blood Cancer.* (2006) 47:260–7. doi: 10.1002/pbc.20891
6. Green DM. The evolution of treatment for Wilms tumor. *J Pediatr Surg.* (2013) 48:14–9. doi: 10.1016/j.jpedsurg.2012.10.012
7. Raffensperger J. Max Wilms and his tumor. *J Pediatr Surg.* (2015) 50:356–9. doi: 10.1016/j.jpedsurg.2014.10.054
8. Wright JC. Update in cancer chemotherapy: genitourinary tract cancer, part 2: Wilms' tumor and bladder cancer. *J Natl Med Assoc.* (1988) 80:169–81.
9. Dome JS, Mullen EA, Dix DB, Gratias EJ, Ehrlich PF, Daw NC, et al. Impact of the first generation of children's oncology group clinical trials on clinical practice for Wilms tumor. *J Natl Compr Canc Netw.* (2021) 19:978–85. doi: 10.6004/jnccn.2021.7070
10. Spreafico F, Fernandez CV, Brok J, Nakata K, Vijanic G, Geller J, et al. Wilms tumor. *Nat Rev Dis Primers.* (2021) 7:75. doi: 10.1038/s41572-021-00308-8
11. Grundy PE, Breslow NE, Li S, Perlman E, Beckwith JB, Ritchey ML, et al. Loss of heterozygosity for chromosomes 1p and 16q is an adverse prognostic factor in favorable-histology Wilms tumor: a report from the national Wilms tumor study group. *J Clin Oncol.* (2005) 23:7312–21. doi: 10.1200/JCO.2005.01.2799
12. Grundy PE, Telzerow PE, Breslow N, Moksness J, Huff V, Paterson MC. Loss of heterozygosity for chromosomes 16q and 1p in Wilms' tumors predicts an adverse outcome. *Cancer Res.* (1994) 54:2331–3.
13. Brok J, Lopez-Yurda M, Tinteren HV, Treger TD, Furtwängler R, Graf N, et al. Relapse of Wilms' tumor and detection methods: a retrospective analysis of the 2001 renal tumor study group-international society of pediatric oncology Wilms' tumor protocol database. *Lancet Oncol.* (2018) 19:1072–81. doi: 10.1016/S1470-2045(18)30293-6
14. Daw NC, Chi YY, Kalapurakal JA, Kim Y, Hoffer FA, Geller JI, et al. Activity of vincristine and irinotecan in diffuse anaplastic Wilms tumor and therapy outcomes of stage II to IV disease: results of the children's oncology group AREN0321 study. *J Clin Oncol.* (2020) 38:1558–68. doi: 10.1200/JCO.19.01265
15. Charlton J, Irtan S, Bergeron C, Pritchard-Jones K. Bilateral Wilms tumor: a review of clinical and molecular features. *Expert Rev Mol Med.* (2017) 19:e8. doi: 10.1017/erm.2017.8
16. Ehrlich P, Chi YY, Chintagumpala MM, Hoffer FA, Perlman EJ, Kalapurakal JA, et al. Results of the first prospective multi-institutional treatment study in children with bilateral Wilms tumor (AREN0534): a report from the children's oncology group. *Ann Surg.* (2017) 266:470–8. doi: 10.1097/SLA.0000000000002356
17. Verschuur A, Van Tinteren H, Graf N, Bergeron C, Sandstedt B, de Kraker J. Treatment of pulmonary metastases in children with stage IV nephroblastoma with risk-based use of pulmonary radiotherapy. *J Clin Oncol.* (2012) 30:3533–9. doi: 10.1200/JCO.2011.35.8747
18. Grundy PE, Green DM, Dirks AC, Berendt A, Breslow NE, Anderson JR, et al. Clinical significance of pulmonary nodules detected by CT and Not CXR in patients treated for favorable histology Wilms tumor on national Wilms tumor studies-4 and−5: a report from the children's oncology group. *Pediatr Blood Cancer.* (2012) 59:631–5. doi: 10.1002/pbc.24123
19. Warmann SW, Furtwangler R, Blumenstock G, Armeanu S, Nourkami N, Leuschner I, et al. Tumor biology influences the prognosis of nephroblastoma patients with primary pulmonary metastases: results from SIOP 93-01/GPOH and SIOP 2001/GPOH. *Ann Surg.* (2011) 254:155–62. doi: 10.1097/SLA.0b013e318222015e
20. Termuhlen AM, Tersak JM, Liu Q, Yasui Y, Stovall M, Weathers R, et al. Twenty-five year follow-up of childhood Wilms tumor: a report from the

childhood cancer survivor study. *Pediatr Blood Cancer.* (2011) 57:1210–6. doi: 10.1002/pbc.23090
21. Wong KF, Reulen RC, Winter DL, Guha J, Fidler MM, Kelly J, et al. Risk of adverse health and social outcomes up to 50 years after Wilms tumor: the British childhood cancer survivor study. *J Clin Oncol.* (2016) 34:1772–9. doi: 10.1200/JCO.2015.64.4344
22. Golden CB, Feusner JH. Malignant abdominal masses in children: quick guide to evaluation and diagnosis. *Pediatr Clin North Am.* (2002) 49:1369–92. doi: 10.1016/S0031-3955(02)00098-6
23. Irtan S, Ehrlich PF, Pritchard-Jones K. Wilms tumor: "state-of-the-art" update, 2016. *Semin Pediatr Surg.* (2016) 25:250–6. doi: 10.1053/j.sempedsurg.2016.09.003
24. Shamberger RC, Guthrie KA, Ritchey ML, Haase GM, Takashima J, Beckwith JB, et al. Surgery-related factors and local recurrence of Wilms tumor in national Wilms tumor study 4. *Ann Surg.* (1999) 229:292–7. doi: 10.1097/00000658-199902000-00019
25. Irtan S, Van Tinteren H, Graf N, van den Heuvel-Eibrink MM, Heij H, Bergeron C, et al. Evaluation of needle biopsy as a potential risk factor for local recurrence of Wilms tumor in the SIOP WT 2001 trial. *Eur J Cancer.* (2019) 116:13–20. doi: 10.1016/j.ejca.2019.04.027
26. Jackson TJ, Williams RD, Brok J, Chowdhury T, Ronghe M, Powis M, et al. The diagnostic accuracy and clinical utility of pediatric renal tumor biopsy: report of the UK experience in the SIOP UK WT 2001 trial. *Pediatr Blood Cancer.* (2019) 66:e27627. doi: 10.1002/pbc.27627
27. Dix DB, Seibel NL, Chi YY, Khanna G, Gratias E, Anderson JR, et al. Treatment of stage IV favorable histology Wilms tumor with lung metastases: a report from the children's oncology group AREN0533 study. *J Clin Oncol.* (2018) 36:1564–70. doi: 10.1200/JCO.2017.77.1931
28. Green DM, Fernbach DJ, Norkool P, Kollia G, D'Angio GJ. The treatment of Wilms' tumor patients with pulmonary metastases detected only with computed tomography: a report from the national Wilms' tumor study. *J Clin Oncol.* (1991) 9:1776–81. doi: 10.1200/JCO.1991.9.10.1776
29. Smets AM, van Tinteren H, Bergeron C, De Camargo B, Graf N, Pritchard-Jones K, et al. The contribution of chest CT-scan at diagnosis in children with unilateral Wilms' tumor. Results of the SIOP 2001 study. *Eur J Cancer.* (2012) 48:1060–5. doi: 10.1016/j.ejca.2011.05.025
30. van den Heuvel-Eibrink MM, Hol JA, Pritchard-Jones K, van Tinteren H, Furtwängler R, Verschuur AC, et al. Position paper: rationale for the treatment of Wilms tumor in the UMBRELLA SIOP-RTSG 2016 protocol. *Nat Rev Urol.* (2017) 14:743–52. doi: 10.1038/nrurol.2017.163
31. Fanelli MCA, Guilhen JCS, Duarte AAB, Marques de Souza FK, Dos Santos Cypriano M, Caran EMM, et al. Management of pediatric tumors with vascular extension. *Front Pediatr.* (2021) 9:753232. doi: 10.3389/fped.2021.753232
32. Khanna G, Rosen N, Anderson JR, Ehrlich PF, Dome JS, Gow KW, et al. Evaluation of diagnostic performance of CT for detection of tumor thrombus in children with Wilms tumor: a report from the children's oncology group. *Pediatr Blood Cancer.* (2012) 58:551–5. doi: 10.1002/pbc.23222
33. Brisse HJ, Smets AM, Kaste SC, Owens CM. Imaging in unilateral Wilms tumor. *Pediatr Radiol.* (2008) 38:18–29. doi: 10.1007/s00247-007-0677-9
34. Vujanic GM, Gessler M, Ooms A, Collini P, Coulomb-l'Hermine A, D'Hooghe E, et al. The UMBRELLA SIOP-RTSG 2016 Wilms tumor pathology and molecular biology protocol. *Nat Rev Urol.* (2018) 15:693–701. doi: 10.1038/s41585-018-0100-3
35. Perlman EJ. Pediatric renal tumors: practical updates for the pathologist. *Pediatr Dev Pathol.* (2005) 8:320–38. doi: 10.1007/s10024-005-1156-7
36. Hamilton TE, Barnhart D, Gow K, Ferrer F, Kandel J, Glick R, et al. Inter-rater reliability of surgical reviews for AREN03B2: a COG renal tumor committee study. *J Pediatr Surg.* (2014) 49:154–8. doi: 10.1016/j.jpedsurg.2013.09.047
37. Lopes RI, Lorenzo A. Recent advances in the management of Wilms' tumor. *F1000Res.* (2017) 6:670. doi: 10.12688/f1000research.10760.1
38. Ehrlich PF, Ritchey ML, Hamilton TE, Haase GM, Ou S, Breslow N, et al. Quality assessment for Wilms' tumor: a report from the national Wilms' tumor study-5. *J Pediatr Surg.* (2005) 40:208–13. doi: 10.1016/j.jpedsurg.2004.09.044
39. Kieran K, Anderson JR, Dome JS, Ehrlich PF, Ritchey ML, Shamberger RC, et al. Lymph node involvement in Wilms tumor: results from national Wilms tumor studies 4 and 5. *J Pediatr Surg.* (2012) 47:700–6. doi: 10.1016/j.jpedsurg.2011.08.017
40. Zhuge Y, Cheung MC, Yang R, Koniaris LG, Neville HL, Sola JE. Improved survival with lymph node sampling in Wilms tumor. *J Surg Res.* (2011) 167:e199–203. doi: 10.1016/j.jss.2010.12.026
41. Ehrlich PF, Anderson JR, Ritchey ML, Dome JS, Green DM, Grundy PE, et al. Clinicopathologic findings predictive of relapse in children with stage III favorable-histology Wilms tumor. *J Clin Oncol.* (2013) 31:1196–201. doi: 10.1200/JCO.2011.41.1165
42. Ritchey ML, Kelalis PP, Breslow N, Offord KP, Shochat SJ, D'Angio GJ. Intracaval and atrial involvement with nephroblastoma: review of national Wilms tumor study-3. *J Urol.* (1988) 140:1113–8. doi: 10.1016/S0022-5347(17)41975-6
43. Shamberger RC, Ritchey ML, Haase GM, Bergemann TL, Loechelt-Yoshioka T, Breslow NE, et al. Intravascular extension of Wilms tumor. *Ann Surg.* (2001) 234:116–21. doi: 10.1097/00000658-200107000-00017
44. Kieran K, Anderson JR, Dome JS, Ehrlich PF, Ritchey ML, Shamberger RC, et al. Is adrenalectomy necessary during unilateral nephrectomy for Wilms Tumor? A report from the children's oncology group. *J Pediatr Surg.* (2013) 48:1598–603. doi: 10.1016/j.jpedsurg.2013.04.019
45. Ehrlich PF, Ferrer FA, Ritchey ML, Anderson JR, Green DM, Grundy PE, et al. Hepatic metastasis at diagnosis in patients with Wilms tumor is not an independent adverse prognostic factor for stage IV Wilms tumor: a report from the children's oncology group/national Wilms tumor study group. *Ann Surg.* (2009) 250:642–8. doi: 10.1097/SLA.0b013e3181b76f20
46. Ehrlich PF, Chi YY, Chintagumpala MM, Hoffer FA, Perlman EJ, Kalapurakal JA, et al. Results of treatment for patients with multicentric or bilaterally predisposed unilateral Wilms tumor (AREN0534): a report from the children's oncology group. *Cancer.* (2020) 126:3516–25. doi: 10.1002/cncr.32958
47. Khanna G, Naranjo A, Hoffer F, Mullen E, Geller J, Gratias EJ, et al. Detection of pre-operative Wilms tumor rupture with CT: a report from the children's oncology group. *Radiology.* (2013) 266:610–7. doi: 10.1148/radiol.12120670
48. Brisse HJ, Schleiermacher G, Sarnacki S, Helfre S, Philippe-Chomette P, Boccon-Gibod L, et al. Pre-operative Wilms tumor rupture: a retrospective study of 57 patients. *Cancer.* (2008) 113:202–13. doi: 10.1002/cncr.23535
49. Zhang Y, Song HC, Yang YF, Sun N, Zhang WP, Huang CR. Pre-operative Wilms tumor rupture in children. *Int Urol Nephrol.* (2021) 53:619–25. doi: 10.1007/s11255-020-02706-5
50. Gow KW, Barnhart DC, Hamilton TE, Kandel JJ, Chen MK, Ferrer FA, et al. Primary nephrectomy and intraoperative tumor spill: report from the children's oncology group (COG) renal tumors committee. *J Pediatr Surg.* (2013) 48:34–8. doi: 10.1016/j.jpedsurg.2012.10.015
51. Fuchs J, Kienecker K, Furtwängler R, Warmann SW, Bürger D, Thürhoff JW, et al. Surgical aspects in the treatment of patients with unilateral Wilms tumor: a report from the SIOP 93-01/German society of pediatric oncology and hematology. *Ann Surg.* (2009) 249:666–71. doi: 10.1097/SLA.0b013e31819ed92b
52. Graf N, Tournade MF, de Kraker J. The role of pre-operative chemotherapy in the management of Wilms' tumor. The SIOP studies. International society of pediatric oncology. *Urol Clin North Am.* (2000) 27:443–54. doi: 10.1016/S0094-0143(05)70092-6
53. Kalapurakal JA, Li SM, Breslow NE, Beckwith JB, Ritchey ML, Shamberger RC, et al. Intraoperative spillage of favorable histology Wilms tumor cells: influence of irradiation and chemotherapy regimens on abdominal recurrence. A report from the national Wilms tumor study group. *Int J Radiat Oncol Biol Phys.* (2010) 76:201–6. doi: 10.1016/j.ijrobp.2009.01.046
54. Elayadi M, Hammad M, Sallam K, Ahmed G, Ahmed S, Ibrahim A, et al. Management and outcome of pediatric Wilms tumor with malignant inferior Vena cava thrombus: largest cohort of single-center experience. *Int J Clin Oncol.* (2020) 25:1425–31. doi: 10.1007/s10147-020-01667-0
55. Qureshi SS, Bhagat M, Smriti V, Murli D, Baheti A, Yadav S, et al. Intravascular extension of Wilms tumor: characteristics of tumor thrombus and their impact on outcomes. *J Pediatr Urol.* (2021) 17:69.e1–69.e8. doi: 10.1016/j.jpurol.2020.10.003
56. Cox SG, Davidson A, Thomas J, Brooks A, Hewitson J, Numanoglu A, et al. Surgical management and outcomes of 12 cases of Wilms tumor with intracardiac extension from a single center. *Pediatr Surg Int.* (2018) 34:227–35. doi: 10.1007/s00383-017-4197-x

57. Dzhuma K, Powis M, Vujanic G, Watson T, Olsen O, Shelmerdine S, et al. Surgical management, staging, and outcomes of Wilms tumors with intravascular extension: results of the IMPORT study. *J Pediatr Surg.* (2022) 57:572–8. doi: 10.1016/j.jpedsurg.2021.08.023
58. Abdullah Y, Karpelowsky J, Davidson A, Thomas J, Brooks A, Hewitson J, et al. Management of nine cases of Wilms' tumor with intracardiac extension—a single center experience. *J Pediatr Surg.* (2013) 48:394–9. doi: 10.1016/j.jpedsurg.2012.11.024
59. Kieran K, Ehrlich PF. Current surgical standards of care in Wilms tumor. *Urol Oncol.* (2016) 34:13–23. doi: 10.1016/j.urolonc.2015.05.029
60. Xu S, Sun N, Zhang WP, Song HC, Huang CR. Management of Wilms tumor with intravenous thrombus in children: a single center experience. *World J Pediatr.* (2019) 15:476–82. doi: 10.1007/s12519-019-00272-0
61. Hadley GP, Sheik-Gafoor MH, Buckels NJ. The management of nephroblastoma with cavo-atrial disease at presentation: experience from a developing country. *Pediatr Surg Int.* (2010) 26:1169–72. doi: 10.1007/s00383-010-2667-5
62. Millar AJ, Davidson A, Rode H, Numanoglu A, Hartley PS, Daubenton JD, et al. Bilateral Wilms' tumors: a single-center experience with 19 cases. *J Pediatr Surg.* (2005) 40:1289–94. doi: 10.1016/j.jpedsurg.2005.05.013
63. Coorens THH, Treger TD, Al-Saadi R, Moore L, Tran MGB, Mitchell TJ, et al. Embryonal pre-cursors of Wilms tumor. *Science.* (2019) 366:1247–51. doi: 10.1126/science.aax1323
64. Liu EK, Suson KD. Syndromic Wilms tumor: a review of pre-disposing conditions, surveillance and treatment. *Transl Androl Urol.* (2020) 9:2370–81. doi: 10.21037/tau.2020.03.27
65. Breslow NE, Collins AJ, Ritchey ML, Grigoriev YA, Peterson SM, Green DM. End stage renal disease in patients with Wilms tumor: results from the national Wilms tumor study group and the United States renal data system. *J Urol.* (2005) 174:1972–5. doi: 10.1097/01.ju.0000176800.00994.3a
66. Lange J, Peterson SM, Takashima JR, Grigoriev Y, Ritchey ML, Shamberger RC, et al. Risk factors for end stage renal disease in non-WT1-syndromic Wilms tumor. *J Urol.* (2011) 186:378–86. doi: 10.1016/j.juro.2011.03.110
67. Cox S, Buyukunal C, Millar AJW. Surgery for the complex Wilms tumor. *Pediatr Surg Int.* (2020) 36:113–27. doi: 10.1007/s00383-019-04596-w
68. Millar AJW, Cox S, Davidson A. Management of bilateral Wilms tumors. *Pediatr Surg Int.* (2017) 33:737–45. doi: 10.1007/s00383-016-4047-2
69. Grigoriev Y, Lange J, Peterson SM, Takashima JR, Ritchey ML, Ko D, et al. Treatments and outcomes for end-stage renal disease following Wilms tumor. *Pediatr Nephrol.* (2012) 27:1325–33. doi: 10.1007/s00467-012-2140-x
70. Gariepy-Assal L, Gilbert RD, Ziaugra A, Foster BJ. Management of Denys-Drash syndrome: a case series based on an international survey. *Clin Nephrol Case Stud.* (2018) 6:36–44. doi: 10.5414/CNCS109515
71. Auber F, Jeanpierre C, Denamur E, Jaubert F, Schleiermacher G, Patte C, et al. Management of Wilms tumors in Drash and Frasier syndromes. *Pediatr Blood Cancer*. (2009) 52:55–9. doi: 10.1002/pbc.21759
72. Neville H, Ritchey ML, Shamberger RC, Haase G, Perlman S. Yoshioka T. The occurrence of Wilms tumor in horseshoe kidneys: a report from the national Wilms tumor study group (NWTSG). *J Pediatr Surg.* (2002) 37:1134–7. doi: 10.1053/jpsu.2002.34458
73. Sutthatarn P, Gomez Quevedo O, Gleason J, Davidoff AM, Murphy AJ. Management of intravascular thrombus in cases of bilateral Wilms tumor or horseshoe kidney. *J Pediatr Surg.* (2021) 5:S0022–3468. doi: 10.1016/j.jpedsurg.2021.07.025
74. Murphy AJ, Davidoff AM. Bilateral Wilms tumor: a surgical perspective. *Children.* (2018) 5:134. doi: 10.3390/children5100134
75. Wilde JC, Aronson DC, Sznajder B, Van Tinteren H, Powis M, Okoye B, et al. Nephron sparing surgery (NSS) for unilateral Wilms tumor (UWT): the SIOP 2001 experience. *Pediatr Blood Cancer.* (2014) 61:2175–9. doi: 10.1002/pbc.25185
76. Cost NG, Sawicz-Birkowska K, Kajbafzadeh AM, Tourchi A, Parigi GB, Guillen G, et al. A comparison of renal function outcomes after nephron-sparing surgery and radical nephrectomy for non-syndromic unilateral Wilms tumor. *Urology.* (2014) 83:1388–93. doi: 10.1016/j.urology.2014.01.051
77. Cozzi DA, Ceccanti S, Frediani S, Mele E, Cozzi F. Renal function adaptation up to the fifth decade after treatment of children with unilateral renal tumor: a cross-sectional and longitudinal study. *Pediatr Blood Cancer.* (2013) 60:1534–8. doi: 10.1002/pbc.24545
78. Neu MA, Russo A, Wingerter A, Alt F, Theruvath J, El Malki K, et al. Prospective analysis of long-term renal function in survivors of childhood Wilms tumor. *Pediatr Nephrol.* (2017) 32:1915–25. doi: 10.1007/s00467-017-3673-9
79. Romao RL, Lorenzo AJ. Renal function in patients with Wilms tumor. *Urol Oncol.* (2016) 34:33–41. doi: 10.1016/j.urolonc.2015.07.002
80. Vanden Berg RN, Bierman EN, Noord MV, Rice HE, Routh JC. Nephron-sparing surgery for Wilms tumor: a systematic review. *Urol Oncol.* (2016) 34:24–32. doi: 10.1016/j.urolonc.2015.07.003
81. Davidoff AM, Giel DW, Jones DP, Jenkins JJ, Krasin MJ, Hoffer FA, et al. The feasibility and outcome of nephron-sparing surgery for children with bilateral Wilms tumor. The St Jude children's research hospital experience: 1999–2006. *Cancer.* (2008) 112:2060–70. doi: 10.1002/cncr.23406
82. Fuchs J, Schmidt A, Ellerkamp V, Paulsen F, Melchior P, Timmermann B, et al. New aspects and innovations in the local treatment of renal and urogenital pediatric tumors. *Semin Pediatr Surg.* (2021) 30:151081. doi: 10.1016/j.sempedsurg.2021.151081
83. Aldrink JH, Cost NG, McLeod DJ, Bates DG, Stanek JR, Smith EA, et al. Technical considerations for nephron-sparing surgery in children: what is needed to preserve renal units? *J Surg Res.* (2018) 232:614–20. doi: 10.1016/j.jss.2018.07.022
84. Millar AJ, Davidson A, Rode H, Numanoglu A, Hartley PS, Desai F. Nephron-sparing surgery for bilateral Wilms' tumors: a single-center experience with 23 cases. *Afr J Paediatr Surg.* (2011) 8:49–56. doi: 10.4103/0189-6725.78669
85. Duarte RJ, Cristofani LM, Odone Filho V, Srougi M, Denes FT. Videolaparoscopic radical nephrectomy after chemotherapy in the treatment of Wilms' tumor: long-term results of a pioneer group. *J Pediatr Urol.* (2017) 13:50.e1–50.e5. doi: 10.1016/j.jpurol.2016.09.004
86. Ezekian B, Englum BR, Gulack BC, Rialon KL, Kim J, Talbot LJ, et al. Comparing oncologic outcomes after minimally invasive and open surgery for pediatric neuroblastoma and Wilms tumor. *Pediatr Blood Cancer.* (2018) 65:e26755. doi: 10.1002/pbc.26755
87. Warmann SW, Godzinski J, van Tinteren H, Heij H, Powis M, Sandstedt B, et al. Minimally invasive nephrectomy for Wilms tumors in children - data from SIOP 2001. *J Pediatr Surg.* (2014) 49:1544–8. doi: 10.1016/j.jpedsurg.2014.06.005
88. Lopes RI, Ming J, Koyle MA, Grant R, Fonseca A, Lorenzo AJ. "Zero-Ischemia" laparoscopic-assisted partial nephrectomy for the management of selected children with Wilms tumor following neoadjuvant chemotherapy. *Urology.* (2017) 100:103–10. doi: 10.1016/j.urology.2016.08.051
89. Davidoff AM, Interiano RB, Wynn L, Delos Santos N, Dome JS, Green DM, et al. Overall survival and renal function of patients with synchronous bilateral Wilms tumor undergoing surgery at a single institution. *Ann Surg.* (2015) 262:570–6. doi: 10.1097/SLA.0000000000001451
90. Bouty A, Blanc T, Leclair MD, Lavrand F, Faure A, Binet A, et al. Minimally invasive surgery for unilateral Wilms tumors: multicenter retrospective analysis of 50 transperitoneal laparoscopic total nephrectomies. *Pediatr Blood Cancer.* (2020) 67:e28212. doi: 10.1002/pbc.28212
91. Blanc T, Meignan P, Vinit N, Ballouhey Q, Pio L, Capito C, et al. Robotic surgery in pediatric oncology: lessons learned from the first 100 tumors-a nationwide experience. *Ann Surg Oncol.* (2022) 29:1315–26. doi: 10.1245/s10434-021-10777-6
92. Yadav P, Mahajan A, Kandpal DK, Chowdhary SK. Nephron-sparing surgery for syndromic Wilms' tumor: robotic approach. *Urology.* (2018) 116:172–5. doi: 10.1016/j.urology.2018.03.003
93. Meignan P, Ballouhey Q, Lejeune J, Braik K, Longis B, Cook AR, et al. Robotic-assisted laparoscopic surgery for pediatric tumors: a bicenter experience. *J Robot Surg.* (2018) 12:501–8. doi: 10.1007/s11701-017-0773-2
94. Blanc T, Pio L, Clermidi P, Muller C, Orbach D, Minard-Colin V, et al. Robotic-assisted laparoscopic management of renal tumors in children: preliminary results. *Pediatr Blood Cancer.* (2019) 66:3.e27867. doi: 10.1002/pbc.27867
95. Schmidt A, Warmann SW, Urla C, Schaefer J, Fideler F, Fuchs J. Patient selection and technical aspects for laparoscopic nephrectomy in Wilms tumor. *Surg Oncol.* (2019) 29:14–9. doi: 10.1016/j.suronc.2019.02.007

96. Burnand K, Roberts A, Bouty A, Nightingale M, Campbell M, Heloury Y. Laparoscopic nephrectomy for Wilms' tumor: can we expand on the current SIOP criteria? *J Pediatr Urol.* (2018) 14:253.e1–253.e8. doi: 10.1016/j.jpurol.2018.01.005
97. Hales PW, Olsen OE, Sebire NJ, Pritchard-Jones K, Clark CA. A multi-Gaussian model for apparent diffusion coefficient histogram analysis of Wilms' tumor subtype and response to chemotherapy. *NMR Biomed.* (2015) 28:948–57. doi: 10.1002/nbm.3337
98. Littooij AS, Nikkels PG, Hulsbergen-van de Kaa CA, van de Ven CP, van den Heuvel-Eibrink MM, Olsen ØE. Apparent diffusion coefficient as it relates to histopathology findings in post-chemotherapy nephroblastoma: a feasibility study. *Pediatr Radiol.* (2017) 47:1608–14. doi: 10.1007/s00247-017-3931-9
99. Watson T, Oostveen M, Rogers H, Pritchard-Jones K, Olsen Ø. The role of imaging in the initial investigation of pediatric renal tumors. *Lancet Child Adolesc Health.* (2020) 4:232–41. doi: 10.1016/S2352-4642(19)30340-2
100. Hötker AM, Lollert A, Mazaheri Y, Müller S, Schenk JP, Mildenberger PC, et al. Diffusion-weighted MRI in the assessment of nephroblastoma: results of a multi-center trial. *Abdom Radiol.* (2020) 45:3202–12. doi: 10.1007/s00261-020-02475-w
101. Littooij AS, Sebire NJ, Olsen ØE. Whole-tumor apparent diffusion coefficient measurements in nephroblastoma: can it identify blastemal pre-dominance? *J Magn Reson Imaging.* (2017) 45:1316–24. doi: 10.1002/jmri.25506
102. Rogers HJ, Verhagen MV, Shelmerdine SC, Clark CA, Hales PW. An alternative approach to contrast-enhanced imaging: diffusion-weighted imaging and T_1-weighted imaging identifies and quantifies necrosis in Wilms tumor. *Eur Radiol.* (2019) 29:4141–9. doi: 10.1007/s00330-018-5907-z
103. Pachl MJ. Fluorescent guided lymph node harvest in laparoscopic Wilms nephroureterectomy. *Urology.* (2021) 158:189–92. doi: 10.1016/j.urology.2021.09.015
104. Sadeghi R, Shojaeian R, Hiradfar M, Mohammadipour A, Azadmand A, Mashhadi MP. Sentinel lymph node biopsy in pediatric Wilms tumor. *J Pediatr Surg.* (2022) 10:S0022–3468. doi: 10.1016/j.jpedsurg.2021.12.037
105. Esposito C, Coppola V, Del Conte F, Cerulo M, Esposito G, Farina A, et al. Near-Infrared fluorescence imaging using indocyanine green (ICG): emerging applications in pediatric urology. *J Pediatr Urol.* (2020) 16:700–7. doi: 10.1016/j.jpurol.2020.07.008
106. Privitera L, Paraboschi I, Cross K, Giuliani S. Above and beyond robotic surgery and 3D modeling in pediatric cancer surgery. *Front Pediatr.* (2021) 9:777840. doi: 10.3389/fped.2021.777840
107. Cone EB, Dalton SS, Van Noord M, Tracy ET, Rice HE, Routh JC. Biomarkers for Wilms tumor: a systematic review. *J Urol.* (2016) 196:1530–5. doi: 10.1016/j.juro.2016.05.100
108. Perotti D, Hohenstein P, Bongarzone I, Maschietto M, Weeks M, Radice P, et al. Is Wilms tumor a candidate neoplasia for treatment with WNT/beta-catenin pathway modulators?-A report from the renal tumors biology-driven drug development workshop. *Mol Cancer Ther.* (2013) 12:2619–27. doi: 10.1158/1535-7163.MCT-13-0335
109. Chagtai T, Zill C, Dainese L, Wegert J, Savola S, Popov S, et al. Gain of 1q as a prognostic biomarker in Wilms tumors (WTs) treated with pre-operative chemotherapy in the international society of pediatric oncology (SIOP) WT 2001 trial: a SIOP renal tumors biology consortium study. *J Clin Oncol.* (2016) 34:3195–203. doi: 10.1200/JCO.2015.66.0001
110. Gratias EJ, Dome JS, Jennings LJ, Chi YY, Tian J, Anderson J, et al. Association of chromosome 1q gain with inferior survival in favorable-histology Wilms tumor: a report from the children's oncology group. *J Clin Oncol.* (2016) 34:3189–94. doi: 10.1200/JCO.2015.66.1140
111. Scott RH, Murray A, Baskcomb L, Turnbull C, Loveday C, Al-Saadi R, et al. Stratification of Wilms tumor by genetic and epigenetic analysis. *Oncotarget.* (2012) 3:327–35. doi: 10.18632/oncotarget.468
112. Ruteshouser EC, Robinson SM, Huff V. Wilms tumor genetics: mutations in WT1, WTX, and CTNNB1 account for only about one-third of tumors. *Genes Chromosomes Cancer.* (2008) 47:461–70. doi: 10.1002/gcc.20553
113. Breslow NE, Norris R, Norkool PA, Kang T, Beckwith JB, Perlman EJ, et al. Characteristics and outcomes of children with the Wilms tumor-Aniridia syndrome: a report from the national Wilms tumor study group. *J Clin Oncol.* (2003) 21:4579–85. doi: 10.1200/JCO.2003.06.096
114. Pelletier J, Bruening W, Kashtan CE, Mauer SM, Manivel JC, Striegel JE, et al. Germline mutations in the Wilms' tumor suppressor gene are associated with abnormal urogenital development in Denys-Drash syndrome. *Cell.* (1991) 67:437–47. doi: 10.1016/0092-8674(91)90194-4
115. Brok J, Treger TD, Gooskens SL, van den Heuvel-Eibrink MM, Pritchard-Jones K. Biology and treatment of renal tumors in childhood. *Eur J Cancer.* (2016) 68:179–95. doi: 10.1016/j.ejca.2016.09.005
116. Gadd S, Huff V, Walz AL, Ooms A, Armstrong AE, Gerhard DS, et al. A children's oncology group and TARGET initiative exploring the genetic landscape of Wilms tumor. *Nat Genet.* (2017) 49:1487–94. doi: 10.1038/ng.3940
117. Wegert J, Ishaque N, Vardapour R, Georg C, Gu Z, Bieg M, et al. Mutations in the SIX1/2 pathway and the DROSHA/DGCR8 miRNA microprocessor complex underlie high-risk blastemal type Wilms tumors. *Cancer Cell.* (2015) 27:298–311. doi: 10.1016/j.ccell.2015.01.002
118. Walz AL, Ooms A, Gadd S, Gerhard DS, Smith MA, Guidry Auvil JM, et al. Recurrent DGCR8, DROSHA, and SIX homeodomain mutations in favorable histology Wilms tumors. *Cancer Cell.* (2015) 27:286–97. doi: 10.1016/j.ccell.2015.01.003
119. Cresswell GD, Apps JR, Chagtai T, Mifsud B, Bentley CC, Maschietto M, et al. Intra-tumor genetic heterogeneity in Wilms tumor: clonal evolution and clinical implications. *EBioMedicine.* (2016) 9:120–9. doi: 10.1016/j.ebiom.2016.05.029
120. Perlman EJ, Grundy PE, Anderson JR, Jennings LJ, Green DM, Dome JS, et al. WT1 mutation and 11P15 loss of heterozygosity predict relapse in very low-risk Wilms tumors treated with surgery alone: a children's oncology group study. *J Clin Oncol.* (2011) 29:698–703. doi: 10.1200/JCO.2010.31.5192
121. Bjornsson HT, Brown LJ, Fallin MD, Rongione MA, Bibikova M, Wickham E, et al. Epigenetic specificity of loss of imprinting of the IGF2 gene in Wilms tumors. *J Natl Cancer Inst.* (2007) 99:1270–3. doi: 10.1093/jnci/djm069
122. Wegert J, Wittmann S, Leuschner I, Geissinger E, Graf N, Gessler M, et al. inactivation is a frequent, but late event in Wilms tumors without apparent clinical impact. *Genes Chromosomes Cancer.* (2009) 48:1102–11. doi: 10.1002/gcc.20712
123. Perlman EJ, Gadd S, Arold ST, Radhakrishnan A, Gerhard DS, Jennings L, et al. MLLT1 YEATS domain mutations in clinically distinctive favorable histology Wilms tumors. *Nat Commun.* (2015) 6:10013. doi: 10.1038/ncomms10013
124. Williams RD, Chagtai T, Alcaide-German M, Apps J, Wegert J, Popov S, et al. Multiple mechanisms of MYCN dysregulation in Wilms tumor. *Oncotarget.* (2015) 6:7232–43. doi: 10.18632/oncotarget.3377
125. Armstrong AE, Gadd S, Huff V, Gerhard DS, Dome JS, Perlman EJ, et al. unique subset of low-risk Wilms tumors is characterized by loss of function of TRIM28 (KAP1), a gene critical in early renal development: a children's oncology group study. *PLoS ONE.* (2018) 13:e0208936. doi: 10.1371/journal.pone.0208936
126. Ooms AH, Gadd S, Gerhard DS, Smith MA, Guidry Auvil JM, Meerzaman D, et al. Significance of TP53 mutation in Wilms tumors with diffuse anaplasia: a report from the children's oncology group. *Clin Cancer Res.* (2016) 22:5582–91. doi: 10.1158/1078-0432.CCR-16-0985
127. Hanks S, Perdeaux ER, Seal S, Ruark E, Mahamdallie SS, Murray A, et al. Germline mutations in the PAF1 complex gene CTR9 pre-dispose to Wilms tumor. *Nat Commun.* (2014) 5:4398. doi: 10.1038/ncomms5398
128. Capasso M, Montella A, Tirelli M, Maiorino T, Cantalupo S, Iolascon A. Genetic pre-disposition to solid pediatric cancers. *Front Oncol.* (2020) 10:590033. doi: 10.3389/fonc.2020.590033
129. Kratz CP, Jongmans MC, Cave H, Wimmer K, Behjati S, Guerrini-Rousseau L, et al. Pre-disposition to cancer in children and adolescents. *Lancet Child Adolesc Health.* (2021) 5:142–54. doi: 10.1016/S2352-4642(20)30275-3
130. Mahamdallie S, Yost S, Poyastro-Pearson E, Holt E, Zachariou A, Seal S, et al. Identification of new Wilms tumor pre-disposition genes: an exome sequencing study. *Lancet Child Adolesc Health.* (2019) 3:322–31. doi: 10.1016/S2352-4642(19)30018-5
131. Vujanic GM, Kelsey A, Mitchell C, Shannon RS, Gornall P. The role of biopsy in the diagnosis of renal tumors of childhood: results of the UKCCSG Wilms tumor study 3. *Med Pediatr Oncol.* (2003) 40:18–22. doi: 10.1002/mpo.10216
132. Treger TD, Chagtai T, Butcher R, Cresswell GD, Al-Saadi R, Brok J, et al. Somatic TP53 mutations are detectable in circulating tumor DNA from

children with anaplastic Wilms tumors. *Transl Oncol.* (2018) 11:1301–6. doi: 10.1016/j.tranon.2018.08.006

133. Weiser DA, West-Szymanski DC, Fraint E, Weiner S, Rivas MA, Zhao CWT, et al. Progress toward liquid biopsies in pediatric solid tumors. *Cancer Metastasis Rev.* (2019) 38:553–71. doi: 10.1007/s10555-019-09825-1
134. Apple A, Lovvorn HN 3rd. Wilms tumor in Sub-Saharan Africa: molecular and social determinants of a global pediatric health disparity. *Front Oncol.* (2020) 10:606380. doi: 10.3389/fonc.2020.606380
135. Fukuzawa R, Breslow NE, Morison IM, Dwyer P, Kusafuka T, Kobayashi Y, et al. Epigenetic differences between Wilms' tumors in white and east-Asian children. *Lancet.* (2004) 363:446–51. doi: 10.1016/S0140-6736(04)15491-3
136. Haruta M, Arai Y, Watanabe N, Fujiwara Y, Honda S, Ohshima J, et al. Different incidences of epigenetic but not genetic abnormalities between Wilms tumors in Japanese and Caucasian children. *Cancer Sci.* (2012) 103:1129–35. doi: 10.1111/j.1349-7006.2012.02269.x
137. Girón-Vallejo Ó, García-Calderón D, Ruiz-Pruneda R, Cabello-Laureano R, Doménech-Abellán E, Fuster-Soler JL, et al. Three-dimensional printed model of bilateral Wilms tumor: a useful tool for planning nephron sparing surgery. *Pediatr Blood Cancer.* (2018) 65:e26894. doi: 10.1002/pbc.26894
138. Kalapurakal JA, Lee B, Bautista J, Rigsby C, Helenowski I. Gopalakrishnan M. Cardiac-sparing whole lung intensity modulated radiation therapy in children with Wilms tumor: final report on technique and abdominal field matching to maximize normal tissue protection. *Pract Radiat Oncol.* (2019) 9:e62–73. doi: 10.1016/j.prro.2018.07.005
139. Vogel J, Lin H, Both S, Tochner Z, Balis F, Hill-Kayser C. Pencil beam scanning proton therapy for treatment of the retroperitoneum after nephrectomy for Wilms tumor: a dosimetric comparison study. *Pediatr Blood Cancer.* (2017) 64:39–45. doi: 10.1002/pbc.26176

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