Published in final edited form as:

J Pediatr Surg. 2019 June ; 54(6): 1184–1191. doi:10.1016/j.jpedsurg.2019.02.040.

Association between image-defined risk factors and neuroblastoma outcomes

Hannah M. Phelps^{a,b,*}, Josephine M. Ndolo^c, Kyle J. Van Arendonk^d, Heidi Chen^{b,e}, Hannah L. Dietrich^f, Katherine D. Watson^g, Melissa A. Hilmes^c, Dai H. Chung^d, and Harold N. Lovvorn III^{b,d}

^aSchool of Medicine, Vanderbilt University Medical Center, Nashville, TN

^bSurgical Outcomes Center for Kids, Vanderbilt University Medical Center, Nashville, TN

^cDepartment of Radiology and Radiological Sciences, Vanderbilt University Medical Center, Nashville, TN

^dDepartment of Pediatric Surgery, Vanderbilt University Medical Center, Nashville, TN

^eDepartment of Biostatistics, Vanderbilt University Medical Center, Nashville, TN

^fSchool of Nursing, Vanderbilt University Medical Center, Nashville, TN

^gDepartment of Pediatrics, Division of Pediatric Hematology-Oncology, Vanderbilt University Medical Center, Nashville, TN

Abstract

Background: The current neuroblastoma (NBL) staging system employs image-defined risk factors (IDRFs) to assess numerous anatomic features, but the impact of IDRFs on surgical and oncologic outcomes is unclear.

Methods: The Vanderbilt Cancer Registry identified children treated for NBL from 2002 to 2017. Tumor volume (TV) and IDRFs were measured radiographically at diagnosis and before resection. Perioperative and oncologic outcomes were evaluated.

Results: At diagnosis of 106 NBL, 61% were IDRF positive. MYCN-amplified and undifferentiated NBL had more IDRFs than nonamplified and more differentiated tumors ($p = 0.001$ and $p = 0.01$). Of 86 NBLs resected, 43% were IDRF positive, which associated with higher stage, risk, and TV (each $p < 0.001$). The presence of IDRF at resection was also associated with increased blood loss ($p < 0.001$), longer operating times ($p < 0.001$), greater incidence of intraoperative complications ($p = 0.03$), more frequent ICU admissions postoperatively ($p < 0.001$), and longer hospital stays ($p < 0.001$). IDRF negative and positive tumors did not have significantly different rates of gross total resection ($p = 0.2$). Five-year relapse-free and overall survival was similar for IDRF negative and positive NBL ($p = 0.9$ and $p = 0.8$).

*Corresponding author at: Vanderbilt University School of Medicine, 2209 Garland Avenue, Nashville, TN 37232-9780. hannah.m.phelps@vanderbilt.edu (H.M. Phelps).

Declarations of interest

None.

Conclusions: IDRFs at diagnosis were associated with larger, less differentiated, advanced stage, and higher risk NBL and at resection with increased operative difficulty and perioperative morbidity. However, the frequency of gross total resection and patient survival after resection were not associated with the presence of IDRFs.

Type of study: Retrospective cohort study.

Level of evidence: Level III.

Keywords

Neuroblastoma; Image-defined risk factors; Tumor volume; International neuroblastoma risk group

Neuroblastoma (NBL) is the most common extracranial solid malignancy of childhood. Patient age, tumor biology, and anatomic considerations drive multimodal treatment. Biological features known to affect disease severity include histologic category (favorable or unfavorable), degree of tumor differentiation, amplification of the MYCN protooncogene, aberrations in chromosome 11q, mutations in the *ALK* (anaplastic lymphoma kinase) gene, and DNA ploidy [1]. In 2005, Cecchetto et al. proposed a system for assessing anatomical features of NBL based on radiographic findings with the goal to stratify surgical risk for a particular tumor [2]. The International Neuroblastoma Risk Group Staging System (INRGSS) now employs image-defined risk factors (IDRFs) to stratify and stage disease [3]. Specifically, locoregional disease is classified based on the absence (stage L1) or presence (stage L2) of IDRF. Metastatic disease is classified as stage M, except when metastases are confined to the liver, skin, and/or bone marrow in a child less than 18 months old, which then categorizes stage MS.

Assessment of operative risk is an important decision point in the treatment of NBL and relies heavily on imaging studies. When feasible, complete upfront resection of locoregional disease is desirable to avoid or minimize a child's exposure to toxic chemotherapeutic agents. The role of surgical resection in more advanced, treatment-resistant disease remains controversial, and thus surgical decision-making must weigh the risks of a complex operation with uncertain therapeutic benefit [4,5]. The efficacy of current neoadjuvant therapy often can render infiltrative and vessel-encasing NBL more readily resectable. Although IDRFs are potentially useful to assess candidacy for upfront resection, to evaluate response to neoadjuvant chemotherapy, to determine an operative approach, and to anticipate intraoperative challenges, the relationship between IDRF and tumor biology, surgical outcomes, and disease prognosis remains incompletely defined especially in North America [6–10].

In European studies, the presence of IDRF has been associated consistently with operative complications and incomplete resection [2,7,11–13]. However, conflicting results have been reported on how IDRFs impact relapse-free and overall survival [11,13]. To date, no report has critically analyzed the impact of IDRFs on operative challenges and disease outcomes. This study, which is the first based on a North American institutional experience, aimed to (1) evaluate the relationship between IDRFs and tumor biology and (2) assess the overall impact of IDRFs on surgical and oncologic outcomes from NBL. We hypothesized that

IDRFs are associated with more aggressive tumor biology and are predictive of a more challenging resection and ultimately poorer oncologic outcomes.

1. Methods

1.1. Patient selection

The comprehensive Vanderbilt Cancer Registry was queried using ICD-O-3 morphological and topographical codes to identify pediatric (< 18 years of age at diagnosis) patients who were treated for neuroblastoma between January 1, 2002 and December 1, 2017 ($n = 134$). Patients who underwent definitive resection at an outside institution were excluded, as radiographic and perioperative data were not available consistently for these patients ($n = 27$). Radiographic assessment of IDRF was incomplete for two additional patients. One patient had the diagnostic CT scan performed at an outside institution, and the images were not available for review. Another patient on surveillance for a congenital neuroblastoma had a CT scan at diagnosis but subsequent imaging was by ultrasound; complete assessment of IDRF at resection was not reliable using this latter modality. These two patients were included in the cohort but excluded from the relevant analyses. The Vanderbilt Institutional Review Board approved this study (#100734).

1.2. Radiographic analysis & data collection

IDRFs and tumor volumes (TVs) were assessed on CT or MRI at diagnosis and on each scan obtained throughout the course of neoadjuvant therapy if administered. Two pediatric radiologists, blinded to clinical data, reviewed all images. Broadly defined, IDRFs are anatomical tumor features (e.g., vascular encasement, intraspinal tumor extension, infiltration into an adjacent organ) that might add to the complexity of a resection. The INRG defines twenty specific IDRFs which were evaluated in this cohort [3]. For the purposes of this study, IDRFs were classified as vascular, infiltrative, neurological, or extensive [7]. To estimate TV, maximum diameter was measured along each axis of the tumor in the anteroposterior (a , cm), transverse (b , cm), and craniocaudal planes (c , cm). An ellipsoid approximation was used to estimate tumor volume ($\text{cm}^3 = \text{ml}$): $\text{TV} = \frac{\pi abc}{6}$ [14]. To assess the extent of resection, TV was again measured on the first cross sectional imaging after resection, and correlated with MIBG-avidity, when available, to differentiate residual tumor from postoperative change. Gross total resection (GTR) was defined as achieving 98% extirpation relative to the last measured preoperative TV. Patient and oncologic data, including age, presenting symptoms, stage (International Neuroblastoma Staging System, INSS), Children's Oncology Group (COG) risk stratification, operative details, pathology, and outcomes, were assessed through chart review.

1.3. Statistical analysis

Data were summarized using the median and interquartile range (median [Q1, Q3]) or mean and standard error of the mean (mean $\pm$ SEM) for continuous variables and absolute number with percentage for categorical variables. The Wilcoxon rank sum test was applied to two-group continuous outcomes (Kruskal-Wallis for more than two groups) when comparing medians. Student's t-test or a paired t-test was used to compare means. Pearson's chi-

squared test was applied to categorical outcomes. Univariate and multivariate logistic regression models were assessed to further elucidate variables that predict IDRF at diagnosis. Survival was calculated from the date of diagnosis to the date of death from any cause or to the date of last known contact with the patient. Similarly, relapse-free survival was calculated from date of diagnosis to the date of death, the date that new local or metastatic disease was identified, or the date of last known contact with the patient. The distributions of RFS and OS were estimated using the Kaplan-Meier method. The log rank test was applied to test equality of survival distributions between patient groups. Plots were produced to assess TV and IDRF across the course of neoadjuvant therapy. For TV, mixed model regression was applied for time trend analysis of this continuous measurement. The natural log transformation was applied to TV to meet the assumptions of the parametric analysis. For IDRF, generalized linear mixed model regression was applied for time trend analysis of count data. Missing data were excluded from relevant analyses.

All analyses, excluding the time trend analyses, were performed using Stata 15 (StataCorp. 2017. *Stata Statistical Software: Release 15*. College Station, TX: StataCorp LLC). Time trend analyses were performed in R (R Development Core Team. 2014. *R: A language environment for statistical computing*. Vienna, Austria). All tests were 2-sided, and p -values < 0.05 were considered statistically significant.

2. Results

2.1. IDRF and tumor characteristics

Of 107 patients treated for NBL, the distribution of INRG stage was 31 L1 (29.0%), 25 L2 (23.4%), 48 M (44.9%), and 3 MS (2.8%). Imaging was available for assessment of IDRF at diagnosis in 106 of 107 patients. Imaging at resection was available for review in 86 of 87 patients having surgery. Fig. 1A–D shows representative cases highlighting IDRF at diagnosis and at resection. Among the entire cohort, a total of 173 IDRFs were detected at diagnosis, and for those receiving neoadjuvant therapy, 58 IDRFs remained at resection (Fig. 1E–F). At diagnosis, the mean number of IDRFs was 1.6 ± 0.2 , and the median TV was 142.2 ml [34, 340.9], whereas at resection, the mean number of IDRFs was 0.8 ± 0.1 ($p < 0.001$), and the median TV was 40.5 ml [12, 130.4] ($p < 0.001$; Fig. 2C). Tumor volume at both diagnosis and resection was significantly higher in IDRF positive tumors ($p < 0.001$, Fig. 2A–B).

Tumors resected upfront without neoadjuvant chemotherapy ($n = 41$) had 0.2 ± 0.1 IDRFs. NBL treated with neoadjuvant chemotherapy ($n = 46$) had a mean of 2.7 ± 0.2 IDRFs at diagnosis and 1.3 ± 0.2 IDRFs at resection ($p < 0.001$, Fig. 2D–F). The mean decrease in IDRFs with neoadjuvant chemotherapy was 1.4 ± 0.2 . Of IDRF-positive NBLs that were pretreated with neoadjuvant chemotherapy ($n = 41$), 29 tumors (70.7%) experienced a decrease in IDRF, and 12 tumors (29.3%) had complete resolution of IDRF.

The associations between tumor characteristics and number of IDRF present at diagnosis and resection are summarized in Table 1. Higher stage tumors had a greater number of IDRF than lower stage tumors at both diagnosis and resection ($p < 0.001$). On histology, undifferentiated tumors typically had more IDRFs at diagnosis ($p = 0.001$), but no

significant difference was detected in the number of IDRFs present at resection according to histologic differentiation ($p = 0.695$). MYCN amplification was associated with a greater number of IDRFs at diagnosis compared to non-MYCN amplified tumors (3 IDRFs [1.5, 4] and 1 IDRF [0, 3], respectively; $p = 0.010$). However, no significant difference was observed in number of IDRFs present at resection based on MYCN amplification (0 IDRF [0, 1] and 0 IDRF [0, 1.5], respectively; $p = 0.660$). Of note, all MYCN amplified tumors also demonstrated less differentiated histology ($p = 0.001$). Multivariate logistic regression accounting for age, histology, MYCN status, and INSS stage indicated that age ≥ 12 months (OR 4.39 [95% CI 1.32–14.59]; $p = 0.016$), less differentiated histology (OR 6.01 [1.68–21.47]; $p = 0.006$), and advanced stage (OR 7.26 [95% CI 2.18–24.20]; $p = 0.001$) were significant predictors of the presence of IDRF at diagnosis. Notably, MYCN status was not a significant predictor of IDRF at diagnosis in this model (OR 0.77 [95% CI 0.14–4.35]; $p = 0.770$). This result suggests that MYCN amplification may associate with the *number* of IDRF and not the absolute *presence* of IDRF at diagnosis, given the uniformly less differentiated histology of tumors harboring this genomic alteration.

Fig. 3A–C depicts IDRF and TV response to neoadjuvant chemotherapy after each cycle. Less differentiated tumors had a better TV response to neoadjuvant chemotherapy than more differentiated tumors ($p = 0.0035$; Fig. 3A). IDRF among less differentiated tumors appeared also to regress with neoadjuvant chemotherapy more frequently, but this relationship was not statistically significant ($p = 0.514$; Fig. 3A). At diagnosis, less differentiated tumors were larger (340.9 ml [147.2, 687.2] vs. 125.4 ml [51.3, 154.0], $p = 0.013$) and had more IDRFs (3 IDRFs [2,4] vs. 1.5 IDRFs [0.25, 2.75], $p = 0.044$) than more differentiated tumors, which may contribute to the higher rate of TV and IDRF decay. Similarly, MYCN amplified tumors demonstrated a significantly greater response to neoadjuvant chemotherapy for both IDRF and TV, although amplified tumors tended also to have more IDRFs and larger TV at diagnosis (Fig. 3B). High risk tumors also regressed more sharply to neoadjuvant chemotherapy as compared with intermediate risk tumors (Fig. 3C). No low risk tumors were treated with neoadjuvant chemotherapy.

Tumor site, stage, and risk stratification correlated significantly with presence of IDRF at time of resection (Table 2). Tumors having an extraadrenal, retroperitoneal origin were more commonly IDRF positive at resection (86%, 12/14) compared with tumors originating from other sites ($p = 0.009$). Tumors that were IDRF positive at resection also tended to be higher stage and risk ($p = 0.001$ and $p < 0.001$, respectively). Conversely, tumors presenting with a paraneoplastic syndrome were histologically differentiated and more often IDRF negative at resection ($p = 0.027$).

2.2. IDRF and operative outcomes

A minimally invasive approach was more commonly employed to resect IDRF negative than IDRF positive NBL. Of 19 NBL resected with MIS, 18 (94.7%) were IDRF negative, and one was IDRF positive (5.3%; $p < 0.001$, Table 2). Analysis of perioperative outcomes suggested increased operative difficulty when resecting IDRF positive tumors. Intraoperative blood loss was increased for resection of IDRF positive, rather than negative, NBL (240 ml [75,450] and 25 ml [10,100], respectively; $p < 0.001$, Table 2). Resection of IDRF positive

tumors more commonly included an adjacent organ resection ($p = 0.042$). Operating times were longer for IDRF positive rather than negative NBL (363.5 min [279, 528] and 184 min [133.5, 290.5], respectively; $p < 0.001$, Table 2). Children with IDRF positive tumors were more commonly admitted to the ICU postoperatively (32.4%, $n = 12$) compared to children with IDRF negative tumors (0%; $p < 0.001$, Table 2). Finally, hospitalization after resection of IDRF positive tumors was 5 days [4,6] compared with 3 days [2,4] after resection of IDRF negative tumors ($p < 0.001$, Table 2).

Intraoperative complications were more common among IDRF positive tumors ($p = 0.033$, Table 2). Intraoperative complications associated with resection of IDRF negative tumors included one superior mesenteric artery injury, one thoracic duct injury, and one inferior vena cava injury. Complications occurring during resection of IDRF positive tumors included three renal vein injuries, two inferior vena cava injuries, one aortic injury, and two other vascular injuries for which the vessel was not specified. Postoperative complications included, among IDRF-negative patients, one return to OR for bleeding, one return to OR for placement of chest tube, and one cardiopulmonary complication. Among IDRF-positive patients, one had cardiopulmonary complications and one required return to OR for nephrectomy owing to kidney infarction ($p = 0.888$).

To evaluate the effect of neoadjuvant chemotherapy on operative complexity and outcomes, a subgroup analysis compared neuroblastomas having 0 or 1 IDRF that were resected upfront to tumors having 0 or 1 IDRF that were resected after neoadjuvant chemotherapy. We found that neuroblastomas resected *after* neoadjuvant chemotherapy were significantly smaller (i.e., by TV; 13.4 ml [9.3, 33] vs. 45.2 ml [16.7, 139], $p = 0.002$), yet resection in this context associated with significantly greater blood loss (100 ml [50, 250] vs. 10 ml [10, 50]; $p < 0.001$), longer operative times (281 min [195, 394] vs. 176 min [127, 251]; $p = 0.001$), and longer length of stay (4 days [3,5] vs. 3 days [2,4]; $p = 0.005$). These findings are likely attributable to the intense inflammatory and desmoplastic reaction that occurs after administration of neoadjuvant chemotherapy.

2.3. IDRF and oncologic outcomes

Presence of IDRF did not appear to affect oncologic outcomes appreciably. As expected, negative resection margins were more commonly achieved for IDRF negative tumors than for IDRF positive tumors ($p = 0.022$, Table 2). However, no significant difference was detected in ability to achieve GTR (i.e., 98% TV), with 80% ($n = 36$) of IDRF negative tumors having GTR and 67.7% ($n = 23$) of IDRF positive tumors having GTR ($p = 0.211$, Table 2). No significant difference was observed between local relapse for IDRF negative and positive tumors ($p = 0.676$, Table 2). Presence of IDRF at diagnosis or resection was not significantly correlated with RFS or OS (Fig. 3D–G).

2.4. Subgroup analyses based on INSS risk stratification and INRG stage

Because tumor biology drives survival outcomes from neuroblastoma, subgroup analyses were performed to assess the impact of IDRF on outcomes within assigned INSS risk groups. Neither 5-year RFS nor OS was significantly different based on the presence or absence of IDRF at diagnosis or resection for any of the INSS risk subgroups analyzed.

When comparing low and intermediate risk tumors, 5-year RFS was 0.90 (95% CI, 0.73–0.97) for tumors that were IDRF negative and 0.89 (95% CI, 0.63–0.97) for tumors that were IDRF positive *at diagnosis* ($p = 0.990$). Similarly, OS was 0.88 (95% CI, 0.67–0.96) for IDRF negative and 1.00 (95% CI, 1.00–1.00) for IDRF positive tumors *at diagnosis* ($p = 0.170$). When comparing low and intermediate risk tumors *at time of resection*, 5-year RFS was 0.90 (95% CI, 0.71–0.97) for IDRF negative and 0.79 (95% CI, 0.38–.94) for IDRF positive tumors ($p = 0.534$). OS was 0.87 (95% CI, 0.65–0.96) for IDRF negative and 1.00 (95% CI, 1.00–1.00) for IDRF positive tumors ($p = 0.298$). Among high risk tumors, 5-year RFS was 0.25 (95% CI, 0.01–0.67) for IDRF negative and 0.70 (0.49–0.83) for IDRF positive tumors *at diagnosis* ($p = 0.160$). OS was 0.40 (95% CI, 0.05–0.75) for IDRF negative and 0.59 (95% CI, 0.40–0.74) for IDRF positive tumors *at diagnosis* ($p = 0.795$). Also for high risk tumors, 5-year RFS was 0.39 (95% CI, 0.15–0.64) for IDRF negative and 0.74 (95% CI, 0.47–0.88) for IDRF positive tumors *at resection* ($p = 0.052$). OS was 0.54 (95% CI, 0.25–0.76) for IDRF negative and 0.74 (95% CI, 0.48–0.88) for IDRF positive tumors *at resection* ($p = 0.701$).

Further subgroup analysis based on IRNG stage was performed. Stage MS tumors were excluded from this analysis owing to low numbers ($n = 3$). Not surprisingly, RFS was similar for Stage L1 and L2 tumors but appeared poorer for Stage M tumors, although not statistically. OS was similar for Stage L1 and L2 tumors but was significantly poorer for metastatic disease (Fig. 4A–B). When comparing IDRF-positive tumors with and without metastatic disease, metastatic biology was associated with poorer OS, but RFS was not statistically different (Fig. 4C–D). These data collectively suggest that the principal determinant of survival from neuroblastoma is the presence of metastatic disease at diagnosis and other biologic factors more than IDRF alone.

2.5. Outcomes for patients not having resection

Among the 20 patients not having resection, 3 had resolution of NBL without intervention (2 Stage 4S; 1 prenatally diagnosed and observed); 8 patients responded to medical therapy alone; 6 patients died from disease progression or drug toxicity during neoadjuvant chemotherapy; and 3 patients were receiving additional therapy at the time of this analysis.

3. Discussion

This study foremost confirms that the presence of IDRF poses significant operative challenges to the resection of NBL. Moreover, at diagnosis, increasing number of IDRFs was associated with larger, less differentiated, MYCN-amplified, advanced stage, and higher risk NBL, suggesting an important link between tumor biology and IDRF. Our thorough evaluation of perioperative details in the context of IDRF provides additional understanding of how NBLs behave locally, encasing vital vascular structures and infiltrating adjacent organs, thereby potentially complicating resection. Importantly, the presence of IDRF did not preclude gross total resection or long-term survival from NBL, suggesting that a meticulous yet aggressive resection can be achieved despite these anatomic challenges.

In accordance with prior studies, we too observed a higher rate of operative complications, particularly vascular injury, in tumors that harbored IDRF at time of resection [12,13]. A

recent study demonstrated an increased risk of nephrectomy in the presence of specific IDRF involving the renal vessels or tumor infiltration into the kidney parenchyma [10]. Our study supports this concept to assess for potential adjacent organ resection in association with infiltrative IDRF, as we too found a correlation between IDRF and adjacent organ resection. Our analysis highlights several additional indicators of greater operative difficulty when resecting IDRF positive tumors: longer operating time, increased blood loss, greater need for ICU care postoperatively, and prolonged hospitalization. Further from our series, neoadjuvant chemotherapy reduced or eliminated IDRF. However, typically, this strategy induces a desmoplastic reaction, which itself can increase the difficulty of resection with tenacious vascular adherence, as documented in this analysis. Thus, IDRFs describe multiple factors that contribute to operative challenges.

To elucidate the overall prognostic significance of IDRF, we assessed the association between NBL tumor biology and histology with IDRF. Interestingly, we found that less differentiated NBLs were associated with a greater number of IDRFs at diagnosis, yet these tumors typically responded to neoadjuvant chemotherapy in a manner that reduced or eliminated altogether the number of IDRFs at resection. Of note, the degree of tumor differentiation was based on the pathology at diagnosis. The tumor differentiation that can occur after exposure to neoadjuvant chemotherapy might have contributed to the loss of this histologic effect on the number of IDRF between diagnosis and resection. Indeed, the proportion of undifferentiated or poorly differentiated tumors decreased significantly from 56% at diagnosis to 35% at resection. Further, the finding that more differentiated tumors had fewer IDRFs could help explain why paraneoplastic syndromes were more commonly associated with IDRF negative tumors, as paraneoplastic syndromes occur most commonly in the context of well differentiated neuroblastoma [15]. In our cohort, five of six NBLs presenting with a paraneoplastic syndrome had a histologic diagnosis of differentiating neuroblastoma or ganglioneuroblastoma. Importantly, all were IDRF negative.

In this study, MYCN-amplification, the principal biological feature of treatment resistance in NBL, was associated with a greater number of IDRF at diagnosis, yet neoadjuvant therapy appeared to eliminate this association by time of resection. Although MYCN-amplification has previously been linked with ultimate treatment failure, this result suggests that MCYN-amplified tumors do respond to initial neoadjuvant chemotherapy [16]. In the current study, MYCN tumors more commonly demonstrated less differentiated histology, consistent with the finding that in vitro retinoic acid-induced differentiation of neuroblastoma cells correlated with decreased expression of the *N-myc* protooncogene [17]. More recently, it has been shown that high MYCN levels suppress estrogen receptor alpha (ER α) and other genes necessary for nerve growth factor (NGF) signaling and neural differentiation [18,19]. High cell turnover of less differentiated, MYCN-amplified tumors may explain the observed initial response to neoadjuvant chemotherapy, with preferential survival and proliferation of MCYN-amplified cells potentially leading to ultimate treatment resistance. Of note, one recent study found that MYCN-amplified NBL also had a greater number of IDRF compared to tumors with numerical-only or segmental chromosome alterations [9]. The relationship between biologic properties, radiographic phenotype, and clinical outcomes is an area of emerging interest in oncology, and our findings further support the potential to

gain critical biologic and prognostic information from imaging studies that can guide the treatment of NBL.

Despite posing a more laborious resection, IDRFs do not appear significantly to compromise the oncologic integrity of the operation at our institution. In prior studies, IDRFs have been associated with incomplete resection of NBL [7,12]. Margin positive resections were indeed more common among IDRF positive patients in this study. However, IDRF did not appear to impact negatively the ability to achieve a strict gross total resection of more than 98%. The disparate results could in part be attributable to differences in the definition of complete resection (ranging from 90% to 98% resection) and varying methods used to measure completeness of resection (ranging from surgeon comments in the operative report to actual measurement of residual tumor volume on postoperative imaging as in this study). Indeed, our radiologists were precise in this assessment. The association of IDRF with long-term oncologic outcomes (i.e., OS and RFS) is controversial [11,13]. Surprisingly, although IDRF positive tumors tended to have more aggressive tumor biology, our study found no significant association between relapse-free or overall survival and presence of IDRF at diagnosis or at resection. It should be noted that the survival impact of GTR on patients with metastatic disease remains controversial, and the benefit of an aggressive resection for patients with high risk disease has not been proven unequivocally. Evaluation of resection extent in high risk neuroblastoma was included as a specific, prospective aim on COG Trial A3973. The investigators reported that GTR greater than or equal to 90% correlated with reduced cumulative incidence of local progression but did not appear to have an impact on EFS or OS. These results may be explained by a predominant effect of metastatic biology on outcome. The authors concluded that cure of high risk neuroblastoma requires both local and metastatic control, and GTR is effective in achieving local control and should be pursued when feasible and safe [5]. Additionally, since greater radiation doses are provided to patients with macroscopic versus microscopic residual disease, the ability to achieve GTR also impacts radiation dose [5].

The clinical utility of IDRFs extends beyond the aforementioned associations with tumor biology and intraoperative challenges. As minimally invasive surgery becomes a more popular approach for resection of solid tumors in children, IDRFs may offer a means to evaluate candidacy for a minimally invasive approach [6,20–22]. It is worth noting that IDRFs appear to have influenced resection approach in the current study, as all but one of the tumors resected with MIS were IDRF negative.

The authors acknowledge several limitations with the present study that temper interpretation. First, although our Children's Hospital is a large regional referral center for the comprehensive treatment of NBL, any retrospective analysis of a single-institution experience offers a limited sample size and impedes generalizability despite close adherence to COG protocols. Prospective data collection and assessment of IDRF would be the preferred study design to avoid biases inherent with the use of records that were not designed for the study. Further, our evaluation of surgical and oncologic outcomes using a nonrandomized study sample is subject to confounding, meaning that observed outcomes could be attributable to factors other than the presence of IDRF. Second, not all IDRFs contribute equally to the risks of a complete resection, yet in the present study we considered

absolute number of IDRF rather than differentiating between specific IDRF (i.e., vascular, infiltrative, extensive or neurological). A thorough analysis of the relative risk of each specific IDRF on operative and oncologic outcomes would require a much larger sample size. Third, treatment algorithms for NBL have varied over time and are based on risk stratification. Thus, it is difficult to account for how differences in treatment algorithms over the 15-year study period and across risk strata may have contributed to outcomes. Despite these limitations, we believe that our central conclusions remain valid and clinically relevant. Importantly, our analysis provides new information regarding the relationship between IDRF and tumor biology at diagnosis of NBL, as well as how IDRF can impact its ultimate resection.

4. Conclusions

The present study provides novel insight into the role of IDRF in treating NBL. *At diagnosis* of NBL, we demonstrated an association between IDRF and tumor biology and histology, specifically MYCN amplification and less neuroblastic differentiation. Moreover, at time of resection, we confirmed the impact of IDRF on operative approach and complexity. Indeed, a thorough analysis of IDRF *at diagnosis* can guide the NBL treatment algorithm and *at resection* can predict intraoperative difficulty and perhaps direct the operative approach. Our results suggest that IDRFs are associated with less differentiated NBL, more aggressive and treatment-resistant tumor biology, and increased morbidity with resection yet do not correlate significantly with oncologic outcomes.

Acknowledgments

The authors are indebted to the Vanderbilt Cancer Registry for providing the detailed list of patients used to conduct this study. The authors further acknowledge the support of the Surgical Outcomes Center for Kids of the Monroe Carell, Jr. Children's Hospital at Vanderbilt.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

- [1]. Cohn SL, Pearson AD, London WB, et al. The International Neuroblastoma Risk Group (INRG) classification system: an INRG Task Force report. *J Clin Oncol* 2009;27(2): 289–97. [PubMed: 19047291]
- [2]. Cecchetto G, Mosseri V, De Bernardi B, 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(33):8483–9. [PubMed: 16293878]
- [3]. Brisse HJ, McCarville MB, Granata C, et al. Guidelines for imaging and staging of neuroblastic tumors: consensus report from the International Neuroblastoma Risk Group Project. *Radiology* 2011;261(1):243–57. [PubMed: 21586679]
- [4]. Simon T, Haberle B, Hero B, et al. Role of surgery in the treatment of patients with stage 4 neuroblastoma age 18 months or older at diagnosis. *J Clin Oncol* 2013;31(6):752–8. [PubMed: 23284039]
- [5]. von Allmen D, Davidoff AM, London WB, 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(2):208–16. [PubMed: 27870572]

- [6]. Irtan S, Brisse HJ, Minard-Colin V, et al. Minimally invasive surgery of neuroblastic tumors in children: indications depend on anatomical location and image-defined risk factors. *Pediatr Blood Cancer* 2015;62(2):257–61. [PubMed: 25284263]
- [7]. Irtan S, Brisse HJ, Minard-Colin V, et al. Image-defined risk factor assessment of neurogenic tumors after neoadjuvant chemotherapy is useful for predicting intraoperative risk factors and the completeness of resection. *Pediatr Blood Cancer* 2015;62(9):1543–9. [PubMed: 25820608]
- [8]. Avanzini S, Pio L, Erminio G, et al. Image-defined risk factors in unresectable neuroblastoma: SIOPEX study on incidence, chemotherapy-induced variation, and impact on surgical outcomes. *Pediatr Blood Cancer* 2017;64(11).
- [9]. Brisse HJ, Blanc T, Schleiermacher G, et al. Radiogenomics of neuroblastomas: relationships between imaging phenotypes, tumor genomic profile and survival. *PloS one* 2017;12(9):e0185190. [PubMed: 28945781]
- [10]. Lim II, Goldman DA, Farber BA, et al. Image-defined risk factors for nephrectomy in patients undergoing neuroblastoma resection. *J Pediatr Surg* 2016;51(6): 975–80. [PubMed: 27015902]
- [11]. Monclair T, Mosseri V, Cecchetto G, et al. Influence of image-defined risk factors on the outcome of patients with localised neuroblastoma. A report from the LNESG1 study of the European International Society of Paediatric Oncology Neuroblastoma Group. *Pediatr Blood Cancer* 2015;62(9):1536–42. [PubMed: 25663103]
- [12]. Gunther P, Holland-Cunz S, Schupp CJ, et al. Significance of image-defined risk factors for surgical complications in patients with abdominal neuroblastoma. *European journal of pediatric surgery: official journal of Austrian Association of Pediatric Surgery [et al] = Zeitschrift fur Kinderchirurgie* 2011;21(5):314–7.
- [13]. Simon T, Hero B, Benz-Bohm G, et al. Review of image defined risk factors in localized neuroblastoma patients: results of the GPOH NB97 trial. *Pediatr Blood Cancer* 2008;50(5):965–9. [PubMed: 17914735]
- [14]. Lovvorn HN 3rd, Ayers D, Zhao Z, Hilmes M, Prasad P, Shinall MC Jr, et al. Defining hepatoblastoma responsiveness to induction therapy as measured by tumor volume and serum alpha-fetoprotein kinetics. *J Pediatr Surg* 2010;45(1): 121–8; [discussion 9]. [PubMed: 20105591]
- [15]. Imbach P. Neuroblastoma In: Imbach P, Kühne T, Arceci RJ, editors. *Pediatric oncology: a comprehensive guide* Berlin, Heidelberg: Springer Berlin Heidelberg; 2011 p. 113–24.
- [16]. Nicolai S, Pieraccioli M, Peschiaroli A, et al. Neuroblastoma: oncogenic mechanisms and therapeutic exploitation of necroptosis. *Cell Death Dis* 2015;6:e2010. [PubMed: 26633716]
- [17]. Thiele CJ, Reynolds CP, Israel MA Decreased expression of N-myc precedes retinoic acid-induced morphological differentiation of human neuroblastoma. *Nature* 1985; 313(6001):404–6. [PubMed: 3855502]
- [18]. Dzieran J, Rodriguez Garcia A, Westermarck UK, et al. MYCN-amplified neuroblastoma maintains an aggressive and undifferentiated phenotype by deregulation of estrogen and NGF signaling. *Proc Natl Acad Sci U S A* 2018;115(6):E1229–38. [PubMed: 29374092]
- [19]. Westermarck UK, Wilhelm M, Frenzel A, et al. The MYCN oncogene and differentiation in neuroblastoma. *Semin Cancer Biol* 2011;21(4):256–66. [PubMed: 21849159]
- [20]. Tanaka Y, Kawashima H, Mori M, et al. Contraindications and image-defined risk factors in laparoscopic resection of abdominal neuroblastoma. *Pediatr Surg Int* 2016;32(9):845–50. [PubMed: 27461435]
- [21]. Shirota C, Tainaka T, Uchida H, et al. Laparoscopic resection of neuroblastomas in low- to high-risk patients without image-defined risk factors is safe and feasible. *BMC Pediatr* 2017;17(1):71. [PubMed: 28288594]
- [22]. Phelps HM, Ayers GD, Ndolo JM, et al. Maintaining oncologic integrity with minimally invasive resection of pediatric embryonal tumors. *Surgery* 2018;164(2): 333–43. [PubMed: 29751968]

Fig. 1. IDRf at diagnosis and resection.

(A–D) Two representative cases highlight multiple vascular and infiltrative IDRf at diagnosis of NBL in a 14-month-old (A, B) and a 34-month-old (C, D). At diagnosis (A, C), each tumor was poorly differentiated and had MYCN amplification. Both tumors responded dramatically to neoadjuvant chemotherapy (B, D), which eliminated or reduced IDRf and rendered gross total resection possible. (E, F) Bar charts demonstrating the types and numbers of IDRf detected across the cohort, both at diagnosis and at resection, and the classification of IDRf present.

Fig. 2. Changes in IDRF and TV with neoadjuvant chemotherapy.

(A, B) Box plots demonstrating the relationship between TV and presence or absence of IDRF at diagnosis (A) and resection (B). Box shows interquartile range (IQR) and the horizontal line in box represents the median. Whiskers span to 1.5*IQR, and dots represent data points that lie outside of 1.5*IQR. (C, D) Bar charts demonstrate mean number of IDRF present at diagnosis and at resection for all patients (C) and for patients undergoing neoadjuvant chemotherapy only (D). (E, F) Additional bar charts demonstrating differences in mean number of IDRF at diagnosis (E) and at resection (F) for patients undergoing upfront resection versus those treated with neoadjuvant chemotherapy. Whiskers on bar charts represent standard error of the mean. P-values were generated from Wilcoxon rank sums test (Wilcoxon matched-pairs signed rank test) for medians and from Student's t test (paired t test) for means.

Fig. 3. Changes in IDRf and tumor volume (TV) across neoadjuvant chemotherapy and survival outcomes based on IDRf.

(A–C) IDRf and TV response to neoadjuvant chemotherapy based on histology (A), MYCN amplification (B), and risk stratification (C). (A) Less differentiated tumors (black curve) had more IDRfs at diagnosis than more differentiated tumors (red curve; 3 IDRf [2,4] vs. 1.5 [0.25, 2.75], $p = 0.044$). IDRfs appeared to decrease more dramatically in less differentiated tumors, but the slopes were not significantly different ($p = 0.514$). Less differentiated tumors (black plots) were larger at diagnosis compared with more differentiated tumors (red plots; 340.9 ml [147.2, 687.2] vs. 125.4 ml [51.3, 154.0], $p = 0.013$), and TV decreased more dramatically across neoadjuvant chemotherapy ($p = 0.0035$). (B) Chemotherapy appeared more effective to eliminate IDRf among MYCN-amplified tumors (black curve; $p = 0.0062$). MYCN amplified tumors (black plots) were larger than non-MYCN amplified tumors at diagnosis (red plots; 687.2 ml [329.7, 993.6] vs. 161.8 [86.0, 367.2], $p = 0.03$). TV decreased more dramatically for MYCN-amplified tumors compared to nonamplified tumors ($p < 0.0001$). (C) The IDRf and TV response to chemotherapy was more pronounced in high risk tumors (black curve) than intermediate risk tumors (red curve; $p < 0.0001$ and $p = 0.0108$, respectively). High risk tumors were larger (349.5 ml [190.2, 863.7] vs. 84.3 ml [26.7, 150.4], $p < 0.001$) and showed a greater number of IDRf at diagnosis than intermediate risk tumors (3 IDRf [2,4] vs. 2 IDRf [1, 2.5], $p = 0.03$). (D, E) Relapse-free survival and overall survival based on IDRf at diagnosis were not significantly different. (F, G) Relapse-free survival and overall survival based on IDRf at resection also were not significantly different. P-values were generated using the log rank test for equality of survival distributions. Tick marks represent censored events.

Fig. 4. Impact of metastases on neuroblastoma survival.

(A,B) 5-year survival from neuroblastoma categorized according to INRG stage (MS stage was excluded; $n = 3$). IDRF-negative (L1) and -positive (L2) tumors *without* metastases had similar relapse-free and overall survival. Five-year RFS was 0.89 (95% CI, 0.71–0.97) for Stage L1 and 0.86 (96% CI, 0.63–0.95) for Stage L2 ($p = 0.618$). Five-year OS was 0.91 (95% CI, 0.69–0.98) for Stage L1 and 0.90 (95% CI, 0.65–0.97) for Stage L2 ($p = 0.849$). Metastases at diagnosis reduced 5-year overall survival by ~30% (0.60 [95% CI, 0.42–0.74] for Stage M). (C, D) When comparing survival among only IDRF-positive (L2) neuroblastoma patients having metastases at diagnosis or not, disseminated disease significantly reduced overall survival. Tick marks represent censored events.

Table 1

Tumor characteristics and number of IDRF.

	Diagnosis			Resection		
	<i>n</i>	Median IDRF [IQR]	<i>p</i> -value	<i>n</i>	Median IDRF [IQR]	<i>p</i> -value
All patients	106	1 [0, 3]	n/a	86	0[0,1]	n/a
Histology						
Neuroblastoma, not specified	12	2 [0.5, 2]	0.001	9	1 [0,1]	0.695
Undifferentiated neuroblastoma	5	2 [0, 2]		3	0 [0, 1]	
Poorly differentiated neuroblastoma	54	2 [0, 3]		41	0 [0, 2]	
Differentiating neuroblastoma	19	0 [0, 1]		17	0 [0, 1]	
Ganglioneuroblastoma	11	0 [0, 1]		11	0 [0, 1]	
Ganglioneuroma	5	0 [0, 1]		5	0 [0, 1]	
Primary site						
Neck	2	2.5 [2, 3]	0.347	1	0 [0, 0]	0.011
Thorax	21	1 [0, 3]		18	0 [0, 1]	
Adrenal	59	0 [0, 3]		49	0 [0, 1]	
Abdomen, nonadrenal	20	2 [1, 3]		14	1 [1,2]	
Pelvis	4	1.5 [0, 3]		4	1 [0, 2.5]	
Ploidy						
Diploid	17	0 [0, 3]	0.920	13	0 [0, 0]	0.495
Aneuploid	32	1 [0, 3]		28	0 [0, 1]	
MYCN amplification						
Nonamplified	67	1 [0, 3]	0.010	56	0 [0, 1.5]	0.660
Amplified	15	3 [1.5, 4]		12	0 [0, 1]	
Timing of resection						
Upfront	41	0 [0, 0]	< 0.001	40	0 [0, 0]	< 0.001
After neoadjuvant	45	3 [2, 3]		46	1 [0, 2]	
Stage (INSS)						
Stage 1/2	31	0 [0, 1]	< 0.001	28	0 [0, 0]	< 0.001
Stage 3/4	65	2 [1, 3]		49	1 [0, 2]	
COG Risk Stratification						
Low	33	0 [0, 0]	< 0.001	28	0 [0, 0]	< 0.001
Intermediate	20	2 [0, 3]		14	1 [0, 2]	
High	47	3 [2, 3]		38	1 [0, 2]	

IDRF, image-defined risk factor; IQR, interquartile range; INSS, International Neuroblastoma Staging System; COG, Children's Oncology Group.

Table 2

NBL characteristics and operative outcomes based on presence or absence of IDRF at resection.

	<i>n</i>	IDRF negative <i>n</i> = 49	IDRF positive <i>n</i> = 37	<i>p</i> -value
Age (months)	86	19 [6, 61]	34 [18, 48]	0.276
Body surface area (m ²)	83	0.52 [0.40, 0.73]	0.62 [0.50, 0.68]	0.088
TV at diagnosis (ml)	85	46.8 [16.7, 210.7]	257.8 [136.7, 606.0]	< 0.001
TV at resection (ml)	86	23.9 [6.3, 63.2]	71.3 [36.6, 220]	< 0.001
COG risk stratification				
Low	80	53.3% (24)	11.4% (4)	< 0.001
Intermediate		13.3% (6)	22.9% (8)	
High		33.3% (15)	65.7% (23)	
Stage (INSS)				
Stage 1/2	77	52.4% (22)	17.1% (6)	0.001
Stage 3/4		47.6% (20)	82.9% (29)	
MYCN				
Nonamplified	68	82.1% (32)	82.8% (24)	0.940
Amplified		18.0% (7)	17.2% (5)	
Site				
Neck	86	2.0% (1)	(0)	0.009
Thoracic		22.5% (11)	18.9% (7)	
Adrenal		67.4% (33)	43.2% (16)	
Abdomen, nonadrenal		4.1% (2)	32.4% (12)	
Pelvis		4.1% (2)	5.4% (2)	
Paraneoplastic Syndrome				
Yes	86	12.2% (6)	(0)	0.027
No		87.8% (43)	100% (37)	
Resection approach				
MIS	86	36.7% (18)	2.7% (1)	< 0.001
Open		63.3% (31)	97.3% (36)	
Margins				
Negative	73	46.2% (18)	20.6% (7)	0.022
Positive		53.9% (21)	79.4% (27)	
Estimated blood loss (ml)	76	25 [10, 100]	240 [75,450]	< 0.001
Operating time (min)	74	184 [133.5, 290.5]	363.5 [279, 528]	< 0.001
Length of stay (days)	86	3 [2, 4]	5 [4, 6]	< 0.001
Gross total resection				
98% resection	79	80% (36)	67.7% (23)	0.211
<98% resection		20% (9)	32.4% (11)	
Adjacent organ resection				
No	86	100% (49)	91.9% (34)	0.042
Yes		(0)	8.1% (3)	
Intraoperative complications				

	<i>n</i>	IDRF negative <i>n</i> = 49	IDRF positive <i>n</i> = 37	<i>p</i> -value
No	86	93.9% (46)	78.4% (29)	0.033
Yes		6.1% (3)	21.6% (8)	
ICU				
No	86	100% (49)	67.6% (25)	< 0.001
Yes		(0)	32.4% (12)	
Local Relapse				
No	86	91.8% (45)	89.2% (33)	0.676
Yes		8.2% (4)	10.8% (4)	

IDRF, image-defined risk factor; TV, tumor volume; COG, Children's Oncology Group; INSS, International Neuroblastoma Staging System; MIS, minimally invasive surgery; ICU, intensive care unit.

Values are median [Q1, Q3] or % (No.)