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Complex treatment of residual metastatic germ cell cancer: A single center experience

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ABSTRACT

Background: Testicular cancer is the most common solid malignancy among men aged 15–35. Radical orchiectomy and platinum-based chemotherapy (BEP) are curative in the majority of patients, including advanced, metastatic cases. According to current urooncology guidelines all non-seminoma patients harbouring post-chemotherapy residual masses of $\geq$ 1 cm should undergo salvage retroperitoneal lymph node dissection (RPLND). However, only 10% of residual tumors contain viable disease.

Objective: To assess patient outcomes and complications considering different treatment regimens and clinical characteristics.

Materials and methods: In a retrospective cross-sectional study patients (n=127) who underwent post-chemotherapy RPLND between 2007 and 2023 at our referral center were evaluated. The patients received systemic treatment at various oncology centers. The number of BEP cycles received were occasionally different from standard. Only patients with normal postchemotherapy serum tumor markers and primary testicular or extragonadal germ cell neoplasms were included. Treatment groups were established according to the number of BEP cycles received, and the extent of RPLND (bilateral or modified template). Treatment outcomes and complications were assessed.

Results: Standard 3–4 courses of BEP were received by 100 (78,7%) patients, while 11 (8,7%) patients underwent less, and 16 (12,6%) more courses than standard. On histopathologic evaluation viable germ cell tumor, teratoma, and necrosis/fibrosis was present in 26 (20,5%), 67 (52,7%) and 34 (26,8%) of specimen, respectively. In the 5–6 BEP series subgroup high rate of viable disease (37,5%) was found and significantly more nephrectomies were performed, than other chemotherapy subgroups. Extratesticular GCT, viable disease in residual mass or progression after RPLND indicated lower survival. Mild (Clavien-Dindo I-II) or no postoperative complications were reported in 93,7% of cases.

Conclusions: The study suggests no significant benefit from exceeding 3–4 courses of BEP. Timely salvage RPLND should be performed in high volume centers for optimal treatment outcomes with acceptable complication rates. Adherence to the Heidenreich criteria is advisable where practical.

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1. Introduction

Management of testicular germ cell tumors (TGCT) is one of the greatest success stories in the history of oncology, as this previously very high mortality disease became well manageable with the introduction of platinum-based chemotherapy in the late 1970 s (Higby et al., 1974). Disease specific survival is excellent in localised TGCT, and favorable even in advanced, metastatic cases (Beyer et al., 2021; Gillessen et al., 2021). Complication-free survival and fertility preservation must be the goals of care, as TGCT is the most common solid malignancy among young men (aged 15–35) (Park et al., 2018). In 5% of patients the primary GCT arises from an extragonadal region, eg. retroperitoneum, mediastinum (Oosterhuis and Looijenga, 2005).

According to current urooncology guidelines (EAU, ESMO, SWENOTECA) in cases of nonseminomatous primary tumor all post-chemotherapy retroperitoneal residual masses larger than $\geq$ 1 cm need to be removed (Hendry et al., 2002). Most common metastatic sites for TGCT include the close vicinity of the great vessels of the retroperitoneum. Therefore, it is crucial that thorough imaging studies, accurate disease classification and careful surgical planning should precede the post-chemotherapy retroperitoneal lymph node dissection (PC-RPLND).

Seminoma GCT-s are highly sensitive to radio-, and chemotherapy, with a low rate of viable disease after treatment. For pure seminoma patients with non-regressing lesions $\geq$ 3 cm, FDG-PET scan can be used with a high negative predictive value (95%) to exclude active disease (Bachner et al., 2012). Salvage RPLND can be technically demanding due to the intensive desmoplastic reaction caused by seminoma and is associated with higher complication rates (Mosharafa et al., 2003).

Residual disease may involve major retroperitoneal vessels, skeletal or visceral structures. To achieve complete resection with curative intent, some cases require extensive adjunctive surgery, associated with greater morbidity (Heidenreich et al., 2017).

Classic TGCT serum tumor markers (AFP, ßhCG, LDH) also provide important staging and prognostic information. Normalization of marker expression is associated with appropriate treatment response; although these markers have limited sensitivity (Barlow et al., 2010; Gilligan et al., 2010).They are also not reliable indicators of the presence of viable tumor after chemotherapy. Salvage RPLND is indicated in marker negative patients only. According to the Heidenreich criteria residual masses ipsilateral to the primary tumor and smaller than 5 cm are qualified for a modified template RPLND. In patients with lymph nodes $\geq$ 5 cm in size or on the contralateral side, bilateral dissection is indicated (Heidenreich et al., 2009).

Post-chemotherapy residual masses contain viable tumour, teratoma, and necrosis/fibrosis in 11%, 45% and 44% of the cases, respectively (Cheney et al., 2015).

The standard chemotherapy regimen in metastatic GCT (seminoma or non-seminoma) is three or four cycles of cisplatin, etoposide, bleomycin (BEP) depending on prognostic group of the disease (Beyer et al., 2021; Gillessen et al., 2021) In case of relapse of progression after standard systemic treatment salvage paclitaxel-based chemotherapy (TIP) is recommended (Kondagunta et al., 2005).

In post-pubertal testicular germ cell tumors somatic mutations in the genes KIT, KRAS, and NRAS are commonly found (Coffey et al., 2008; Hacioglu et al., 2017). Furthermore, cKIT mutations (Biermann et al., 2007), pathogenic germline CHEK2 variants (AlDubayan et al., 2019), and chromosomal alterations (such as gain of X, 7, 8, 21, and loss of Y, 1p, 11, 13, 18) are frequently observed (Oosterhuis and Looijenga, 2019). With 78 susceptibility loci confirmed, the heritability of TGCT is estimated between 37% and 49% (Pluta et al., 2021).

Meiotic recombination is driven by Spo11-catalyzed DNA double-strand breaks (DSB), are promoted by histone modifications. Mutations and epigenetic alterations interfering with post-translational histone modifications are considered to play a predominant role in cancer developement (Karányi et al., 2020, 2018; Hetey et al., 2017; Székvölgyi et al., 2006). The ectopic expression of meiotic genes is detected in a wide variety of cancers - including TGCT-, where it promotes genomic instability and tumor progression (Lingg et al., 2022). In cancer cells, the dysregulated expression and function of meiotic DSB repair genes promotes aberrant recombination causing chromosomal translocations or deletions of large areas of the genome (Bruggeman et al., 2023). Meiosis-related TEX11 gene was also discovered to play a critical role in platinum-based chemotherapy resistance in TGCT (Kitayama et al., 2022).

Our aim was to assess treatment outcomes and complications evaluating 16 years of experience with PC-RPLND cases at a single referral center.

2. Materials and methods

In a retrospective cross-sectional study, medical records of patients who underwent salvage or second-look RPLND between 2007 and 2023 in a single RPLND referral center were collected from the EMR. Patient parameters including age at surgery, primary tumor localisation and histology, prior systemic treatment, residual mass size (N stage) and histopathology, adjunctive vascular and visceral surgeries and complications as well as long term patient outcomes were analyzed. Patients with primary germ cell tumor, a history of platinum-based chemotherapy, normal post-chemotherapy serum tumor markers before PC-RPLND were enrolled.

Treatment groups were established according to the number of BEP cycles given; patients were classified into “standard” (3–4 BEP cycles), “less than standard” (1–2 BEP cycles) and “more than standard” (5–6 BEP cycles) chemotherapy groups. After completion of systemic treatment, patients were re-staged, and in non-seminoma patients with $\geq$ 1 cm residual masses salvage RPLND was performed via median laparotomy by two high volume surgeons. In pure seminoma cases stable residual masses $\geq$ 3 cm on repeat imaging were also resected. As FDG-PET CT is not supported by health insurance in Hungary in this indication, it was obtained by one seminoma patient only. Second look RPLND was performed in cases of retroperitoneal recurrence.

In our study, survival analysis was conducted to assess the impact of different treatment modalities on patient outcomes. We utilized the survdiff function from the survival R package to rigorously evaluate the disparities in survival outcomes across distinct therapeutic cohorts. This function was parameterized using survival time alongside the event status.

For the purpose of this analysis, the survival time was defined as the period from the year of surgery to the end of oncological follow-up, measured in years. This approach allowed us to systematically assess the duration of survival post-treatment and to evaluate the long-term effectiveness of the therapeutic interventions. We calculated survival probabilities using 95% confidence intervals.

The survdiff function, integrated into the survival R package, utilizes the log-rank test for the statistical assessment of disparities in survival distributions. Significance was determined at $p < 0.05$. The foundational hypothesis of the log-rank test (the null hypothesis) posits that there is no statistically significant variance in the survival curves across the studied groups. Conversely, the alternative hypothesis contends that a significant discrepancy exists. The test employs the Chi-square statistic, derived from the empirical data, to evaluate the validity of the null hypothesis. The determination of statistical significance is subsequently ascertained through the computation of the p-value, providing a robust measure of the differences in survival outcomes among the groups under study. For data analysis and visualization, we utilized survminer package in the R programming environment (R version 4.2.1) (Therneau, 2023; Therneau and Grambsch, 2000; Alboukadel et al., 2021).

This study was performed in accordance with the declaration of Helsinki and was approved by the Scientific and Research Ethical Committee of the Hungarian Medical Research Council (Approval number: BM/26725–3 /2023).

3. Results

3.1. Patient characteristics

127 PC-RPLND patients were enrolled in our study, 114 salvage and 13 second look cases. Mean age at surgery was 35 years (ranged 19–69). Primary tumor was right side testicular cancer in 63, left side in 58 and retroperitoneal GCT in 6 cases. Primary GCT pathology was, non-seminoma/mixed GCT, pure seminoma or fibrosis/necrosis in 110, 13 and 4 patients, respectively. 100 patients received standard chemotherapy regimen, while 11 and 16 men underwent 1–2 or 5–6 cycles of BEP, respectively.

3.2. Systemic treatment groups and outcomes

Concerning post-chemotherapy retroperitoneal lymph node pathology, teratoma was the most prevalent in the "less than standard" and "standard" chemotherapy subgroups. The "more than standard" subgroup had the highest rate of viable tumor (37,5%), post-RPLND disease progression (43,8%), adjunctive surgeries (37,5%) and the lowest survival rate (56,3%), although these findings were not significant on statistical analysis. Patients with retroperitoneal tumors received 5–6 BEP cycles in 50% of cases.

The rate of nephrectomy was significantly higher in the "more than standard" group (p= 0,0166, Pearson's Chi-squared test). Patient demographic and clinical data considering systemic treatment groups are presented in Table 1.

3.3. GCT clinical parameters and outcomes

Residual retroperitoneal mass after systemic treatment was cN3 in 49,6%, cN2 in 40,2% and cN1 10,2% of cases. As documented in Table 2, cN3 residual tumors were the most prevalent in all GCT histology subgroups. Overall, modified template surgery was performed more frequently, than bilateral (n=84(66,1%) to n=43(33,9%)). The highest rate of bilateral template RPLND was performed in the nonseminatous primary cN3 group. A few cases of second look surgery were performed in all primary histology subgroups. Surgical complications were reported in 44 cases, most of which (n=36, 81,8%) were graded Clavien-Dindo 1–2.

Adjunctive procedures were needed in 29 cases. The aorta, inferior vena cava or a renal artery was repaired in 4, 9 and 2 cases, respectively. 3 out of 4 cases of aortic repair were needed in cN3 cases.

Nephrectomy was done in 6 cN2–3 nonseminoma patients. Ureteral injury was confirmed postoperatively on 5 occasions, and in such cases a double-J stent was placed. Ureteral resection and end-to-end ureteroureteral anastomosis were performed in 2 cases.

Table 1
Demographic and clinical parameters considering chemotherapy regimen groups.

	Less than standard 1–2 BEP cycles (n = 11)	Standard 3–4 BEP cycles (n = 100)	More than standard 5–6 BEP cycles (n = 16)
Mean age (y)	34	35	36
Primary GCT origin			
Right testis	7 (63,6%)	48 (48%)	8 (50%)
Left testis	4 (36,4%)	49 (49%)	5 (31,25%)
Retroperitoneum	0	3 (3%)	3 (18,75%)
Primary histology			
Nonseminoma (110)	9 (81,8%)	86 (86%)	15 (93,75%)
Seminoma (13)	2 (18,2%)	10 (10%)	1 (6,25%)
Fibrosis, necrosis (4)	0	4 (4%)	0
N stage			
cN1 (13)	1 (9,1%)	12 (12%)	0
cN2 (53)	4 (36,4%)	40 (40%)	9 (56,25%)
cN3 (61)	6 (54,5%)	48 (48%)	7 (43,75%)
Prognostic group			
Good (70)	9 (81,8%)	55 (55%)	6 (37,5%)
Intermediate (43)	2 (18,2%)	32 (32%)	9 (56,25%)
Poor (13)	0	12 (12%)	1 (6,25%)
Residual mass histology			
Viable tumor (26)	2 (18,2%)	18 (18%)	6 (37,5%)
Teratoma (67)	6 (54,5%)	56 (56%)	5 (31,25%)
Fibrosis/necrosis (34)	3 (27,3%)	26 (26%)	5 (31,25%)
Adjunctive surgery (29)	1 (9,1%)	22 (22%)	6 (37,5%)
Nephrectomy (6)	0	3 (3%)	3 (18,75%)
Progression after RPLND	4 (36,4%)	25 (25%)	7 (43,8%)
Alive to date	8 (72,7%)	85 (85%)	9 (56,3%)

3.4. Survival analysis of patient subgroups

Survival was lower for patients with GCT arising from the retroperitoneum and in cases of viable tumor in the resected retroperitoneal mass (Fig. 1). Disease specific survival was higher for patients with teratoma on lymph node histopathology, despite teratomas being largely resistant to radiotherapy and chemotherapy (Fig. 1). On follow up, cancer progression after salvage RPLND was found in 37 (29,13%) cases (relevant table provided in Supplements). In case of progression after salvage RPLND cancer-specific survival was significantly lower (Fig. 1). These findings were significant on biostatistical analysis.

12 (75%) of lung, 2 (66,6%) of mediastinal and all patients with liver, spinal, brain, pelvic or intestinal progression died during follow up.

Clinical parameters and outcomes were compared between retroperitoneal progression and no progression groups. Higher rate of primary retroperitoneal GCT, cN3 stage disease, viable residual tumor and adjunctive surgery, higher mean age and lower rate of IGCCCG good prognostic group patients and DSS was found in the retroperitoneal progression group, although these findings were not significant. 18 (90%) of cases of retroperitoneal progression occurred inside the former surgical field, while 2 (10%) happened outside the surgical field but still within RPLND template (relevant table provided in Supplements).

3.5. Second look RPLND outcomes

When examining patients undergoing either salvage or second look RPLND, rate of retroperitoneal origin, cN3 stage, adjunctive procedure, poor IGCCG prognostic parameters and cancer specific mortality were higher in the second look group. Residual mass pathology was comparable between groups. These findings were not significant on analysis.

4. Discussion

In our retrospective study 127 PC-RPLND patient's demographic, treatment, perioperative and outcome data were analyzed. 114 salvage RPLND and 13 second look RPLND cases were enrolled, primary RPLND cases were excluded. The primary germ cell tumor gave rise from the right or left testicle in 121 (95.3%) and the retroperitoneum in 6 (4.7%) cases. Primary tumor pathology was mostly nonseminatous GCT (n=110, 86.7%), pure seminoma (n=13, 10.2%) and fibrosis/necrosis (n=4, 3.1%) the rest of the cases. All our patients were under 55, except a 69 year old man with mixed GCT testicular tumor. According to literature, rate of IGCCG good, intermediate and poor risk patients are 60%, 26% and 14%, respectively (Adra et al., 2016). Our patients were classified 76,3% good, 19% intermediate and 4,7% poor prognostic group.

11 (8,7%) of the enrolled patients had their BEP chemotherapy regimen interrupted by adverse effects and received only 1–2 cycles of BEP (Fig. 2). They were placed in the „Less than standard" systemic treatment group. 72,7% of patients in the „Less than standard" group

Table 2
Surgical complications by primary tumor histology and cN stage groups.

	Seminoma			Non-seminoma			Necrosis/fibrosis		
	n=13			n=110			n=4		
	N1	N2	N3	N1	N2	N3	N1	N2	N3
n=	2	4	7	11	45	54	-	2	2
%	14,3%	35,7%	50%	10,0%	40,9%	49,1%	-	50%	50%
Extent of RPLND									
Bilateral	-	1	1	4	13	24	-	-	-
Modified	2	3	6	7	32	30	-	2	2
Type of RPLND									
Salvage	2	4	5	11	43	46	-	1	2
Second look	-	-	2	-	2	1	-	1	-
Nephrectomy	-	-	-	-	1	5	-	-	-
Ureter end-to-end anastomosis	1	-	-	-	-	1	-	-	-
Ureteral injury	-	-	-	-	1	4	-	-	-
Aortic injury	-	-	-	-	1	2	-	-	1
IVC injury	-	-	1		4	3	-	-	1
Renal artery injury	-	-	-	-	1	1	-	-	-

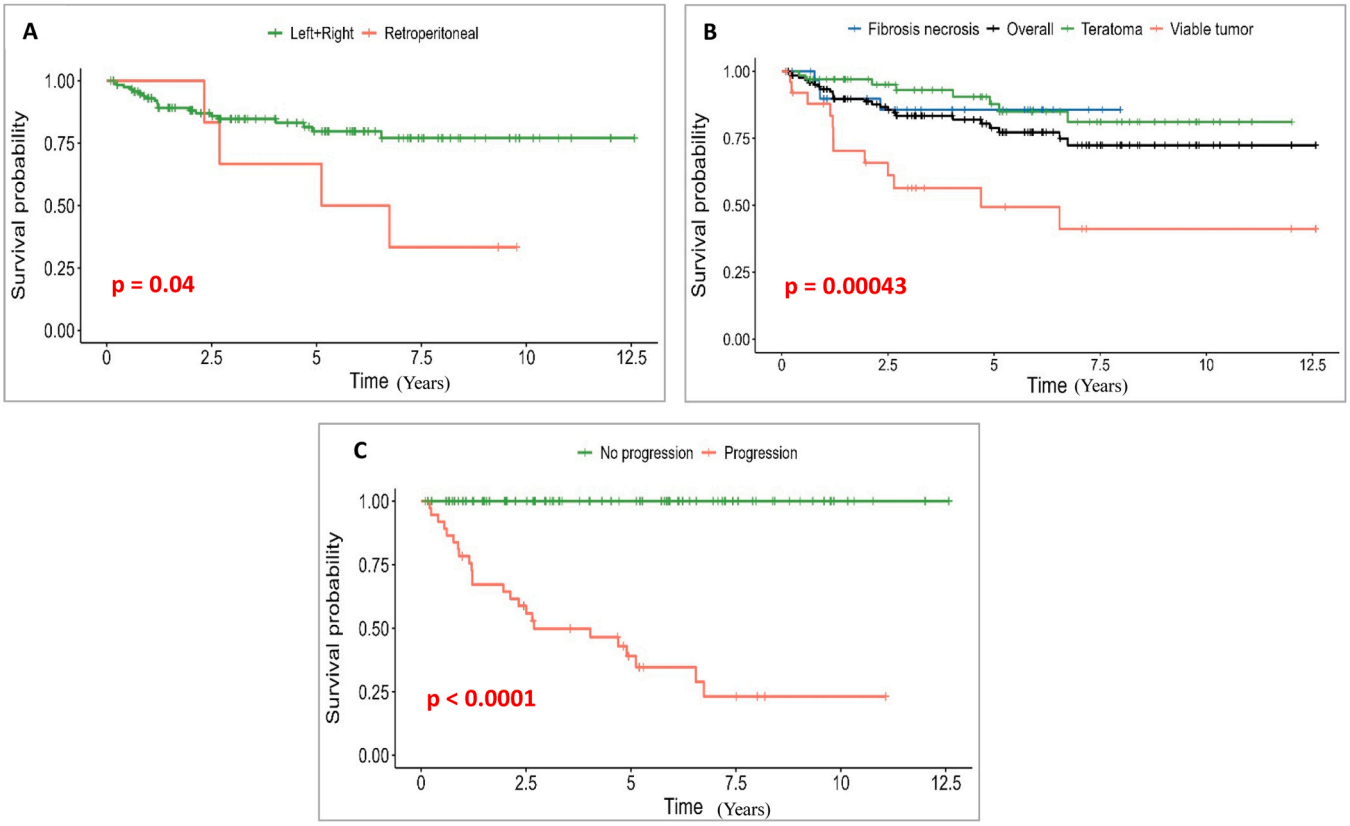

Fig. 1. A) Survival considering primary tumor origin. **Green: left or right testicular GCT. Orange: retroperitoneal GCT.** B) Survival considering residual mass histopathology. **Black: overall survival. Green: teratoma. Blue: fibrosis, necrosis. Orange: viable tumor.** C) Survival considering progression after salvage RPLND. **Green: no progression after RPLND. Orange: disease progression after RPLND.**

had viable tumor or teratoma in the residual lymph node specimen (Fig. 2). For these patients PC-RPLND was the only potentially curative therapeutic option. Progression after RPLND was higher (36,4% vs 25%), survival was lower (72,7% vs 85%) than in the "Standard" treatment group.

In the "More than standard" group 16 (12,6%) patients received 5–6 cycles of BEP (Fig. 2). The majority (62,5%) was in the poor or intermediate IGCCCG prognostic group. Postchemotherapy N stage was comperable to the "Standard" treatment group, with higher incidence of viable tumor on LN pathology (37,5%), progression after RPLND (43,8%) and low CSS (56,3%). The rate of adjuvant surgery and particularly nefrectomy was also higher in the "More than standard" group. As residual mass stage, pathology and surgical complications were unchanged and moreover, the long term toxicity of platinum-based chemotherapy is well known (Hellesnes et al., 2021), these patients would have benefited from earlier surgery.

Metastatic germ cell tumors require complex treatment in specialized high volume centers for optimal outcomes. RPLND is a technically demanding surgery performed on young cancer patients, hence it is to be done in high volume referral centers. The appropriate number of RPLND per year for high volume centers is 10 according to Heidenreich et al. (Heidenreich et al., 2003) and 8 according to SEMS Trial criteria

Table 3
Comparing clinical and outcome data in second look and salvage RPLND. Patients. *Postchemotherapy.

	Second look (n=13)	Salvage RPLND (n = 114)
Mean age (y)	39	34
Primary GCT origin		
Right testis	6 (46,2%)	57 (50%)
Left testis	4 (30,7%)	54 (47,4%)
Retroperitoneum	3 (23,1%)	3 (2,6%)
Primary histology		
Nonseminoma	9 (69,2%)	101 (88,6%)
Seminoma	2 (15,4%)	11 (9,6%)
Fibrosis, necrosis	2 (15,4%)	2 (1,8%)
N stage*		
cN1	0	13 (11,4%)
cN2	2 (15,4%)	49 (43%)
cN3	11 (84,6%)	52 (45,6%)
Prognostic group		
Good	5 (38,5%)	65 (57%)
Intermediate	5 (38,5%)	39 (34,2%)
Poor	3 (23%)	10 (8,8%)
Residual mass histology		
Viable tumor	3 (23,1%)	23 (20,2%)
Teratoma	6 (46,2%)	61 (53,5%)
Fibrosis/necrosis	4 (30,7%)	30 (26,3%)
Adjunctive surgery	4 (30,7%)	25 (21,9%)
Alive to date	8 (61,5%)	94 (82,5%)

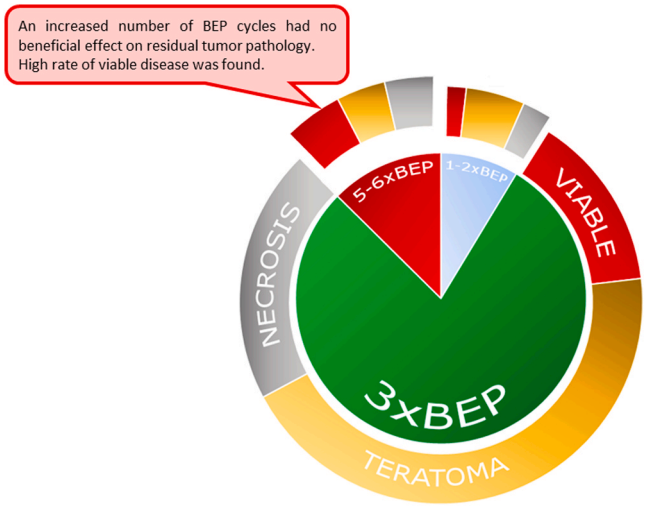

Fig. 2. Residual lymph node histopathology comparing systemic treatment groups. Inner circle: systemic treatment groups. **Green: standard BEP chemotherapy regimen (3–4x BEP). Blue: less BEP cycles than standard (1–2x BEP). Red: more BEP cycles than standard (5–6x BEP).** Outer ring: residual LN histology. **Red: viable tumor. Yellow: teratoma. Gray: fibrosis, necrosis.**

(Daneshmand et al., 2021). 9 RPLND/year were performed at our referral center in the previous 14 years.

In our cohort 29 patients (22,8%) needed adjunctive surgeries (e.g. nephrectomy, major vessel or ureter reconstruction). The tissues of veins are much more vulnerable than arteries, hence vena cava lacerations were more common than aortic injuries (n=4 (3.15%) vs n=9 (7.1%). Caval injuries occurred in all primary tumor histology subgroups. Approximately 7% of patients undergoing PC-RPLND for cN2–3 disease will need vena caval resection, according to earlier reports (Ehrlich et al., 2009; Beck et al., 1998).

In 6 cases the tumor infiltrated the renal vessels in such an aggressive manner, that the kidney was unsalvagable. Therefore, in these 6 patients, all of which were bulky (cN2–3) nonseminoma cases, nephrectomy was performed to achieve complete tumor resection. The two renal artery repair procedures of the cohort also happened in nonseminoma cases. Larger residual tumors needed more complex surgery; 3/4 cases of aortic repair, also 5/6 cases of nephrectomies were performed in cN3 nonseminoma cases. Heidenreich et al. found that postoperative complications occurred more often in the group of complex RPLND (41.7% versus 7.2%, P = 0.02) with the majority representing Clavien-Dindo grade I–IIa. In their study 79.1% of patients, that required complex surgery, had bulky disease, while only 26.58% of those undergoing standard surgery had extensive residual mass (Heidenreich et al., 2017). In our cohort 93,7% of patients faced no or minor (Clavien-Dindo I-II) surgical complications.

Ureteral injury was confirmed postoperatively in 5 nonseminoma patients, and in such cases a double-J stent was placed to stop urine leakage. The ureteral stent was removed after 4–6 weeks.

Ureteral resection and reconstruction were performed in 2 cases of tumor infiltrated ureter.

In one pure seminoma patient postchemotherapy staging CT confirmed a 4 cm long left side proximal ureteral stricture with small (cN1) left paraaortic residual lymph node. In another patient (nonseminomatous primary GCT, bulky disease) the tumor infiltrated the left ureter causing left side hydronephrosis. Both patients underwent modified template RPLND with left ureteral resecion and uretero-ureteral end-to-end anastomosis and ureteral stenting was performed. Resection of the ureter is reported in 2–4% of cases in literature. (Heidenreich et al., 2017)

The inferior mesenteric artery (IMA) was ligated in most cases, where left paraaortic lymph node dissection was performed. The ligation of the IMA was not counted as adjunctive intervention, as it is part of the normal template and often unavoidable. Colonic ischemia as a result of AMI ligation is unlikely in these young patients (Haldipur et al., 2011).

Retroperitoneal progression after salvage RPLND was found in 20 cases (15,7%).

On the one hand, progression can be explained with the biological parameters of the tumor; 60% of cases were cN3 stage, 20% were retroperitoneal GCT, 75% were intermediate or poor IGCCCG prognostic group. Teratoma and viable tumor were found in 55% and 35% of cases, respectively.

On the other hand, surgical errors must also be considered in case of progression inside the operative field or the surgical template. For instance, in 5 out of 12 cN3 cases, modified template RPLND was performed instead of bilateral (Heidenreich et al., 2009). According to surgery records, unresectable disease was found in 3 cases, thus operating on the contralateral, clinically negative field was unnecessary. In the other 2 cases the procedure was ended due to major blood loss.

9 (45%) of our patients with retroperitoneal progression were alive at the end of this study.

This study has several potential limitations. Retrospective research entails the analysis of data originally collected for purposes other than research, such as medical charts and diagnostic testing reports, which may result in some missing information. Additionally, our study was conducted to a single center, which may restrict the generalizability of our findings to the broader population. However, it is worth noting that this single center manages the highest number of salvage RPLND cases in Hungary, whereas most other hospitals handle very few cases each year (Böszörményi-Nagy and Molnár, 2023; Böszörményi-Nagy, 2022). It is recommended that a minimum number of 8 RPLND cases per year per center be performed to ensure sufficient experience in the managing procedure (Daneshmand et al., 2021).

5. Conclusions

Survival was significantly lower in cases of extratesticular GCT, viable disease in residual mass or progression after RPLND. An increased number of BEP cycles in metastatic GCT appears to have no beneficial effect on residual lymph node pathology, surgical outcome or survival. Patients should be referred to high volume centers for oncologic and

surgical treatment to achieve optimal results and ideally low rate of complications. Whenever feasible, Heidenreich criteria should be used to determine surgical template.

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CRediT authorship contribution statement

István Buzogány: Writing – review & editing, Supervision, Resources. **Farkas Süközd:** Writing – review & editing, Supervision, Resources. **Krisztina Biró:** Writing – review & editing, Supervision, Data curation, Conceptualization. **Zoltán Gábor Páhi:** Visualization, Investigation, Formal analysis. **Fruzsina Fazekas:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Zsuzsanna Ujfaludi:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Tamás Beöthe:** Writing – review & editing, Visualization, Supervision, Resources, Methodology, Formal analysis, Conceptualization. **Tibor Pankotai:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jbiotec.2024.04.018.

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