Defining risk of micrometastatic disease and tumor recurrence in patients with stage I testicular germ cell tumors

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Abstract: There is controversy in the management of patients with clinical stage I non-seminomatous germ cell tumor (NSGCT). Some experts recommend surveillance for all patients regardless of risk factors while others suggest a more risk-adapted approach by using lymphovascular invasion (LVI) and the embryonal component in the primary tumor to select patients most likely to benefit from primary treatment [retroperitoneal lymph node dissection (RPLND) or chemotherapy]. With the surveillance for all strategy, only patients who relapse are treated. While this minimizes the over treatment, problem associated with the risk adapted approach, this exposes young men to the effects of full induction cisplatin-based chemotherapy when these men could have received fewer cycles of bleomycin, etoposide, and cisplatin (BEP) or a curative primary RPLND. The challenge is identifying these men who are most likely to benefit from upfront treatment more precisely. This paper explores the currently risk adapted approaches as well as promising emerging biomarkers (microRNA) that, in early data, appear to more accurately predict the presence of microscopic disease in the retroperitoneum over conventional markers.

Keywords: Testicular cancer; microRNA; retroperitoneal lymph node dissection (RPLND)

Introduction

The majority of patients with testicular cancer present with clinical stage I disease. This is defined as any primary tumor T stage, normal tumor markers [(Alpha-fetoprotein (AFP), human chorionic gonadotrophin (hCG), and lactate dehydrogenase (LDH)], and no lymph node greater than 1 cm (axial) in the retroperitoneum. Testicular cancer is typically either designated as a non-seminomatous germ cell tumor (NSGCT) or pure seminoma. Around 50–80% of patients who present with stage I NSGCT and 80–85% of seminoma patients are cured with orchiectomy alone. Risk factors that increase the chance of relapse in stage I NSGCT patients included the presence of lymphovascular invasion (LVI) and a large embryonal cell component

(>50%) (1). When NSGCT patients have either one of these components, the relapse rate approaches 50%. Patients with NSGCT are typically offered either surveillance, 1 or 2 cycles of bleomycin, etoposide, and cisplatin (BEP), or primary retroperitoneal lymph node dissection (RPLND). There are no well-defined and validated risk factors in seminoma patients that increase the percentage of relapse. Patients with stage I pure seminoma are offered surveillance, 1–2 cycles of carboplatin, or primary radiotherapy (2). Importantly, in both seminoma and NSGCT, the overall survival rate approaches 99%, regardless of which initial treatment is chosen (1).

The challenge in the management of clinical stage I testicular cancer (CS1), particularly with NSGCT, is delivering appropriate treatment but avoiding over

treatment. Currently, there are no standard biomarkers or imaging modalities that can predict, with sufficient accuracy, microscopic disease in the retroperitoneum in CS1. Ideally, one could utilize a biomarker or imaging test that could predict the presence or absence of microscopic disease in the retroperitoneum to ensure that clinicians were delivering therapy to those that need it. In this review, we will explore emerging imaging modalities and biomarkers that aim to predict the presence or absence of micrometastatic disease in patients with CS1.

Risk adapted management strategies of stage I NSGCT

The most powerful predictor of disease relapse in stage I NSGCT is the presence of LVI. Other histologic findings, such as a predominant embryonal carcinoma component, have been associated with an increased risk of recurrence, but this typically seen concomitantly with LVI and has not been well validated as being independently associated with an increased risk of relapse. When LVI is present, up to 50% of patients will relapse, most commonly in the retroperitoneum. When patients relapse, they receive a full induction course of BEP, where up to 1/3 of patients can require a post-chemotherapy RPLND (1). In an effort to avoid the toxicity associated salvage treatment, some groups have advocated for a risk adapted treatment. The largest group to evaluate this approach prospectively is the Swedish and Norwegian Testicular Cancer Project (SWENOTECA) management program. They reported on 745 patients who underwent a risk-adapted approach for clinical stage I NSGCT patients based on LVI. Patients that were LVI positive were recommended to receive adjuvant chemotherapy with either 1 or 2 cycles of BEP and LVI negative patients were recommended to undergo surveillance or adjuvant chemotherapy. At a median follow-up of 4.7 years, the relapse rate for patients with LVI positive who chose surveillance was 41.7% and LVI negative patients was 13.2%, respectively. In the LVI positive patients, 5/157 (3.2%) relapsed after 1 course of BEP and 0/70 patients relapsed after 2 cycles of BEP. In the LVI negative patients that elected for 1 cycle of BEP, 2/155 (1.3%) relapsed. Based on these data, a risk adapted approach with adjuvant treatment (1 or 2 cycles BEP) for LVI positive patients and surveillance for LVI negative patients allows for maximum treatment efficacy while minimizing the morbidity of more aggressive salvage options (3).

Risk adapted management strategies of stage I seminoma

In seminoma, risk adapted management is less well defined. Although LVI has been well established as a risk factor for recurrence in NSGCT, defining a group of patients with a high risk of recurrence over 20% in seminoma is challenging. Traditional, but not validated indicators, such as rete testis invasion or primary tumor size greater than 4 cm, have been used to define seminoma patients who are thought to be at higher risk for recurrence. A group out of Spain studied a risk-adapted approach utilizing the presence of rete testis invasion. They evaluated 135 consecutive patients with stage I seminoma, where 47.4% had rete testis invasion. After orchiectomy, patients with rete testis invasion were treated with 2 cycles of carboplatin and patients without rete testis invasion underwent surveillance. After a median follow-up of 33 months, 6 patients relapsed (5 on surveillance, 1 after carboplatin). The 3-year DFS was 92% and 98.0% for patients on surveillance and after chemotherapy, respectively. Overall survival for the entire cohort was 100% (4). Although these results are excellent and 2 cycles of carboplatin is much less toxic than 3 cycles of BEP, up to 80% of patients in the rete testis group were over treated. In seminoma, a risk-adapted approach to adjuvant treatment is difficult to argue for when the relapse rate is so low and salvage treatment virtually cures all patients.

Traditional biomarkers

The management of CS1 is still based on traditional tumor markers such as AFP, hCG, and LDH. In order to be considered CS1, these markers must be normal post orchiectomy. Although around 90% of NSGCT and 30% of seminoma patients (hcG) present with an elevation in at least one of these tumor markers, and these markers are useful in prognostication, these markers are relatively unreliable in detecting recurrence during surveillance. This is particularly true for seminoma surveillance. A recent study challenged the value of routine tumor markers in stage I seminoma patients on surveillance over routine imaging. Out of the 75 patients that recurred on imaging, only 11/65 patients had elevated markers where just 1 patient had marker elevation that preceded detection on imaging. They concluded that routine serum tumor markers during surveillance in seminoma had no added benefit over cross sectional imaging (5). In NSGCT, serum tumor markers

are more accurate in first detection recurrence, with around 30% of patients initially presenting with marker only elevation during surveillance (3). Clearly, there is a need to explore new biomarkers that can more accurately predict both micrometastatic disease at presentation along with improve disease recurrence monitoring in seminoma and NSGCT patients.

Imaging

Computed tomography (CT)

CT remains the modality of choice in initial staging of the retroperitoneal lymph nodes. While CT provides excellent spatial resolution and information regarding the presence of necrosis or cystic like structures in the retroperitoneum, it is unable to discern benign from positive lymph nodes when they are less than 8–10 mm. Traditionally, the cutoff between stage I and IIA disease is 1 cm in the axial dimension. However, this is complicated as benign or reactive lymph nodes can vary widely in shape and appearance. A study by Hale *et al.* evaluated different lymph node size cut-offs by reviewing 70 patients who underwent RPLND and their preoperative imaging. They reported a sensitivity of 37% and specificity of 100% when the retroperitoneal node was 1 cm or larger (6). Lowering the positive lymph node size cut off has been shown to increase the sensitivity but decrease the specificity. When the positive lymph node cut off is 3 mm on CT in a tumor landing zone, the sensitivity and negative predictive value are over 90%, but the specificity falls to <60% (6).

Lymphotropic multiparametric resonance imaging (MRI)

Conventional MRI has been evaluated as a staging tool on stage I testicular cancer and has been found to have similar sensitivity and specificity as CT (7). However, the incorporation of traditional MRI with lymphotropic nanoparticles has yielded results that are more favorable than conventional CT. Harisinghani *et al.* reported that MRI enhanced with lymphotropic molecules demonstrated a higher sensitivity (88.2% *vs.* 70.5%) and specificity (92% *vs.* 68%) in detecting positive lymph nodes in CS1 (8). Although these results appear promising, this study was limited by small sample size, lack of confirmatory RPLND (all sentinel node biopsies), and laborious two stage imaging process that occurs over 48 hours.

Positron emission tomography (PET)

A glucose analog, 2-deoxy-2-[18F]fluoro-D-glucose (FDG), is a radiotracer that is commonly used in oncology in cancers with high glycolytic activity. FDG is injected and preferentially taken up by metabolically active cancer cells (9). PET can be combined with CT or MRI to produce fused images that can provide great anatomic and functional detail. PET is routinely used to help guide decision making in post-chemotherapy seminoma masses (>3 cm), but has limited value in NSGCT (10). FDG PET has been evaluated in stage I NSGCT in an effort to guide surveillance strategies, where FDG PET/CT negative patients underwent surveillance. This trial was stopped early, as the FDG PET/CT negative patients had a 37.9% relapse rate at 1 year (11). In a German multicenter trial, FDG PET/CT was attempted to determine the predictive values of FDG PET in primary staging in NSGCT. This trial failed to meet accrual, but did report that FDG-PET yielded similar results to CT alone. Ultimately, FDG-PET has role currently as a staging tool in stage I testicular cancer (12).

Emerging biomarkers

MicroRNA (miRNA)

MicroRNA was first described in the early 1990s and their emergence and importance in cell and cancer function has been growing. MiRNAs are non-coding small RNA molecules that act by direct interaction with messenger RNA and regulate post- transcriptional gene expression. Importantly, miRNAs are deregulated in malignancies and can be accurately quantified and measured in the serum through quantitative polymerase chain reaction (13). MiRNAs were first evaluated in testicular cancer in 2011, were miRNA of the clusers miR-371-3 and miR-302/367 were suggested as being possible important biomarkers of disease in testicular cancer (14). These markers were externally validated in several independent pilot studies (15,16). In a 2017 study, serum levels of miR-371a-3p, miR-372-3p, miR-373-3p, and miR-367-3p were collected before and active treatment in 166 consecutive patients with testicular cancer. The goal of the study was to determine the sensitivity and specificity of a 4 miRNA panel (miR-371a-3p, miR-372-3p, miR-373-3p, and miR-367-3p) against that of classical markers by monitoring miRNA levels before and after treatment.

Of the 4 miRNAs, miR-371a-3p was found to have the

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highest diagnostic sensitivity for active disease of 88.7% [95% confidence interval (CI), 82.5–93.3%] and specificity of 93.4% (95% CI, 86.9–97.3%), with an area under the curve of 0.945 (95% CI, 0.916–0.974)], easily surpassing the prognostic performance of AFP, hCG, and LDH (17). More than 86% of GCT patients express this novel marker. The expression is most valuable in seminoma, in which <20% of patients express HCG (18). Furthermore, in patients with CS1, miRNA levels nadir to undetectable, mirroring a disease free state. In addition, patients with clinical stage II disease, had decreasing levels with treatment, indicating a favorable response to chemotherapy. A major limitation of the utility of miR-371a-3p as a complete testicular cancer biomarker is its lack of expression in teratoma (17).

This work was explored further in a prospective multicenter trial by comparing the serum miRNA of 616 (359 seminoma and 257 NSGCT) patients with GCT compared to 258 controls. This cohort included patients with CS I disease, relapsing patients, and those with metastatic disease. The miRNA 371a-3p had a sensitivity of 90% and specificity of 94%, with a positive predictive value of 97.2% for detecting active disease. This was compared to more traditional tumor makers (LDH, AFP, HCG), which had a sensitivity of less than 50%. The miRNA levels not only were predictive for active disease but also declining levels correlated with response to treatment (19). Similar to prior studies, the main limitation of miRNA 371a-3p is that this does not express in teratoma. New biomarkers will need to be evaluated in teratoma to differentiate between necrosis and teratoma in the post chemotherapy setting to select those who need a RPLND.

Clearly, these novel miRNA markers are promising in providing a more accurate assessment of the active disease state in patients with testicular cancer. These markers appear especially well suited for use in guiding therapy decisions in clinical stage I patients, monitoring response to chemotherapy, and use in post chemotherapy seminoma and NSGCT. However, additional work needs to be done in predicting the presence or absence of teratoma in the retroperitoneum, as miRNA does not appear to be secreted by teratoma.

Conclusions

There has been little change in the management of CS1 in the past several decades. Modern imaging techniques do not provide additional value over conventional imaging in predicting micrometastatic disease. There is tremendous

enthusiasm for miRNA being explored further as a viable biomarker in CS1.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are 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.

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