Prognostic Factors in Low-Stage Nonseminomatous Testicular Cancer

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Whether patients with clinical stage I nonseminomatous testicular germ-cell cancer (NSGCT) should be treated with orchiectomy and retroperitoneal lymph node dissection (RPLND) or orchiectomy and surveillance remains controversial. Proponents of the former approach cite the uncertainty and risks of monitoring young men who may harbor occult metastases, while proponents of the latter strategy contend that surgical staging overtreats 60% to 70% of men. Over the last few years, prognostic factors in the primary testicular tumor have helped clinicians make more rational decisions about whether RPLND or surveillance should follow initial orchiectomy. As of 1996, the most clinically useful prognostic factors are the percentage of embryonal carcinoma and the presence or absence of vascular invasion by tumor cells in the primary tumor. Ongoing work with flow cytometry, image analysis, proliferation markers, and oncogene and tumor-suppressor gene markers may allow us to further stratify patients as to their likelihood of occult metastases and permit rational "risk-adaptive" treatment.

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Introduction

It is estimated that in 1996, there will be 6,600 new cases of testicular cancer in the United State and 63% of these will be clinically localized at initial diagnosis.[1] If half of the new cases are nonseminomas, approximately 2,050 cases of clinical stage I nonseminomatous germ-cell tumor (NSGCT) will be diagnosed.

Major controversy persists over the care of these patients. Inexact staging does not permit true stage I disease, ie, that confined to the testis, to be distinguished from occult stage II/III retroperitoneal or distant disease.[2] Because up to 30% of men will have occult retroperitoneal disease that is not appreciated on initial staging studies, the standard of care in the United States continues to be orchiectomy followed by retroperitoneal lymph node dissection (RPLND) rather than by surveillance (observation).[3] Based on the numbers above, some might argue that over 1,400 patients (2,050 × .7) per year are being subjected to an unnecessary major abdominal procedure. In fact, this has been the major argument given by those who favor surveillance as initial treatment for clinical stage I NSGCT.[4]

Prognostic factors for testicular cancer patients have only become relevant over the last 20 years with the advent of cisplatin (Platinol)-based curative chemotherapy. Prior to this, the majority of patients died, and the value of prognostic markers was moot.

Over the last decade a number of primary tumor prognostic factors have been discovered that may be useful in stratifying clinical stage I patients as to their likelihood of harboring occult disease. Clinical use of these markers may help identify which patients are best managed by RPLND as opposed to observation or even primary chemotherapy. Use of these markers to guide therapy, termed "risk-adaptive" management, may allow for a more rational scientifically based decision in favor of RPLND or surveillance for individual patients.

The following sections will examine the value of individual histologic, clinical, and molecular and proliferative prognostic factors for stratifying the clinical stage I patient.

Histologic Factors

Vascular Invasion
The utility of vascular invasion (VI) as a prognostic marker in clinical stage I NSGCT was first recognized in a 1983 surveillance study by Peckham et al. These authors noted that 6 of 8 patients who had VI and/or lymphatic invasion (LI) relapsed, as compared with only 5 of 19 patients without these features.[5] However, not all early studies recognized the importance of VI. For example, in another study of 1,058 testicular cancer patients examined for prognostic factors in 1984, no mention of VI was made.[6]

With studies conducted in the mid-1980s, however, the potential importance of VI became more obvious.[7-10] The studies by Fugime et al[7] and Moriyama et al[8] were limited by small numbers of patients, the inclusion of all stages of disease, and the failure to perform multivariate analysis. Javadpour and Young studied 165 NSGCT patients for prognostic factors and found VI to be correlated with clinical stage I staging error and recurrence after negative RPLND.[9] The main problem with these early studies, which still plagues some studies performed recently, is the failure to clearly define what constitutes VI. Some investigators have found it difficult to differentiate blood vessel from lymph vessel invasion[7-11] or simply failed to specify exactly what was included as VI.[12]

• Multivariate and Surveillance Studies—Studies conducted in the late 1980s shed more light on the subject by carefully defining vascular and lymphatic invasion and by performing multivariate analyses on prognostic factors.[13-19] Hoskin et al were the first to carefully define VI as tumor within a luminal space lined by endothelium and containing a smooth muscle wall; LI was tumor in an endothelial-lined space but without a smooth muscle wall.[13] These investigators also were the first to carry out multivariate analysis.[13] In their multivariate prognostic factor study of clinical stage I nonseminomas, Dunphy et al even provided a photomicrograph to demonstrate the difference between VI and LI.[15]

Table 1 summarizes those factors proven by multivariate statistical testing to have prognostic importance in clinical stage I NSGCT. Both Hoskin et al[13] and Dunphy et al[15] did not find VI to be significant on multivariate analysis; however, LI was felt to be important. In the multivariate analysis of Freedman et al, both VI and LI maintained significance.[14] These three studies[13-15] used relapse on a surveillance protocol as an end point of the prognostic capability of VI. In contrast, Fung et al[16] and Moul et al[19] used pathologic stage II disease at RPLND or later recurrence as end points, and noted that VI alone, but not LI, remained significant by multivariate testing. The largest study (279 patients) by Klepp et al, which also used positive RPLND and/or recurrence after RPLND as end points, did not examine VI and LI individually. However, both variables analyzed together were predictive in their multivariate analysis.[17]

Table 2 lists surveillance studies that have commented on factors predictive of recurrence. Some studies evaluated VI and LI individually, whereas others combined VI and LI. Most of these surveillance studies did find VI to be important.

• Prospective Study—From the preceding discussion, it is clear that VI is an exceedingly important prognostic factor in clinical stage I nonseminoma with regard to predicting both recurrence on surveillance and occult disease found at RPLND. A study by Pont et al prospectively utilized VI to stratify patients between surveillance and primary chemotherapy.[20] When this single prognostic factor was used, relapses occurred in only 3 (7.5%) of 40 patients, 1 in the surveillance arm and 2 in the chemotherapy arm.

These authors were very careful in defining VI as: "(1) compact aggregation of tumor cells within the lumen similar to or associated with a thrombotic occlusion and/or (2) definite endothelial destruction by tumor invasion. Isolated tumor cells or tumor cell aggregation in the vascular space without actual attachment to the wall cannot be accepted for vascular invasion, since they are possibly artifacts resulting from specimen processing. Lymphatic invasion is not evaluated since to our pathologists it appears impossible to define invasion into lymphatic spaces on a histological basis without misinterpreting artifacts."[20]

• Areas of Controversy—Despite the critical importance of VI, there is still controversy, particularly over the use of VI alone, LI alone, the combination of VI and LI, and even, as Pont et al[20] noted, the validity of diagnosing LI. Since the average small- or medium-sized hospital does not see an abundance of testicular cancer cases, and even many medical centers are hampered by a similar problem, it is essential to have an experienced reference pathologist review these cases.[21] This point is illustrated in the Testicular Cancer Intergroup Study.[22] The central laboratory detected VI in 179 (43%) of 414 specimens, whereas the local pathologist found VI in only 59 (14%) of 414 specimens. There were even more striking differences in recurrence rates comparing VI determined by local vs reference pathologists. When VI was assessed locally, recurrence rates were 35% and 19% in patients with and without VI. In contrast, these rates were 40% and 8%, respectively, when VI
was assessed by the reference pathologist.[22]

Because of differences in the definition of VI and the ability to detect it, Stephenson has pointed out that VI rates vary between 16% to 53% even among institutions that care for significant numbers of testicular cancer patients.[23] These data further emphasize the need for an experienced reference pathologist to examine all testicular cancer orchiectomy specimens for VI.

- **Neovascularization**—A related concept to VI, neovascularization, has recently been described as a prognostic marker in clinical stage I NSGCT.[24] When factor VIII staining was used to determine microvascular counts in 65 patients with clinical stage I disease, none of the patients with pathologic stage I disease had microvessel counts > 400 per high power field. Neovascularization did not remain a significant predictor of occult disease in multivariate analysis, however. Further prospective work with larger numbers of patients is needed to determine whether this prognostic marker has clinical value.

### Embryonal Carcinoma

Since the landmark studies of Friedman and Moore in 1946 and Dixon and Moore in 1952, it has been recognized that histologic cell type is an important prognostic factor in testicular tumors.[25,26] Dixon and Moore noted that the 2-year mortality of embryonal carcinoma was 59%.[26] At that time, yolk sac carcinoma was not being identified (and to this day it may be difficult for less experienced pathologists to distinguish between embryonal and yolk sac tumors). The mortality figure, therefore, represents embryonal carcinoma in a mixed tumor for the majority of their cases. Despite this, the adverse consequences of embryonal carcinoma have long been well known.

Like VI, the importance of embryonal carcinoma as a prognostic factor in low-stage NSGCT was discovered when surveillance studies were analyzed for relapse factors. In their initial surveillance report in 1982, Peckham et al established the importance of embryonal carcinoma.[27] Relapses occurred in 42.8% of patients with malignant teratoma undifferentiated (the British equivalent of embryonal carcinoma) vs only 3.4% of those with malignant teratoma intermediate (teratocarcinoma).

This same group of investigators confirmed the importance of embryonal carcinoma (malignant teratomaundifferentiated) in multivariate analysis.[13] Lymphatic invasion and embryonal carcinoma were the only two factors to remain significant on multiple regression analysis, and when both factors were present, patients had an 80% risk of relapse.[13] In the United States, Dunphy et al were the first to demonstrate the importance of embryonal carcinoma for relapse on surveillance by multivariate analysis.[15] Up to that point, the mere presence or absence of embryonal carcinoma was used as a prognostic marker.

- **Percentage of Embryonal Carcinoma**—Fung et al performed a semiquantitative analysis by scoring tumors on the basis of whether they contained 50% or more or less than 50% of embryonal carcinoma.[16] Using this approach in 60 patients, they found no statistically significant correlation between embryonal carcinoma and pathologic stage II disease found by RPLND. Wishnow et al were the first to do a quantitative analysis of the percentage of embryonal carcinoma (%EMB).[28] In their study of 82 surveillance patients, more than 80% of embryonal carcinoma, VI, and preorchiectomy alpha-fetoprotein (AFP) level more than 80 ng/dL were important prognostic factors for relapse. When none of these factors was present (30 of 82 patients), no patient relapsed. When one or more factors were present (52 patients), the relapse rate was 46%. Although this was an intriguing study, it lacked multivariate statistical analysis to examine interrelationships among the prognostic factors. Allhoff et al also studied clinical stage I NSGCT patients using semiquantitative %EMB of two categories (less than 50% and 50% or more); they found 50% or more of embryonal carcinoma to be significant for relapse on surveillance by univariate analysis (P = .036).[29] Vascular invasion was not included in their analysis, however, and %EMB was not used as a continuous variable. McLeod et al and the Testicular Cancer Intergroup Study also performed a semiquantitative analysis of embryonal carcinoma (pure, mixed, and absent embryonal elements) in 352 clinical stage I patients.[30] Pure embryonal carcinoma was significant in the multivariate model, but this semiquantitative approach correctly predicted occult disease in only 73% of patients.

More recently, in a preliminary study of flow cytometric parameters as predictors of occult disease in a small cohort of 36 clinical stage I NSGCT patients, our group found that the continuous variable of %EMB accurately predicted occult disease 72% of the time.[31] Additional work by our group examined a larger cohort of clinical stage I NSGCT patients for comprehensive quantitative histology as prognostic factors for retroperitoneal disease at RPLND or later relapse.[19] In 92 patients, the
primary tumor was assessed for VI, LI, tunical invasion, %EMB, percentage of yolk sac carcinoma, percentage of teratoma, and percentage of seminoma. Univariate statistical analysis revealed that all of these factors, except percentage of seminoma, were important. However, only VI and %EMB remained significant on multivariate analysis.

We generated a model using VI and %EMB to arrive at a prediction of the risk of occult disease in the clinical stage I cohort. In this cohort, the model correctly predicted occult disease in 86% of cases. In one of the largest studies of prognostic factors in clinical stage I NSGCT (Table 1), Klepp et al did not confirm the importance of embryonal carcinoma by multivariate analysis in the strictest sense. However, careful review of this paper shows that embryonal carcinoma was, indeed, important.[17] When pure embryonal carcinoma was present, 63/211 (29.9%) of clinical stage I patients had positive nodes at RPLND, as compared with 17.9% when embryonal carcinoma was absent or 39.1% when there was pure embryonal carcinoma. Even this only qualitative assessment of embryonal carcinoma was nearly significant (P = .08) on multivariate analysis.

Klepp et al also looked at the risk of relapse after a negative RPLND but noted no correlation with embryonal carcinoma. When positive nodes at RPLND and later relapses were combined, the presence of embryonal carcinoma (41.7% relapse rate) vs its absence (25.4% relapse rate) was statistically significant by univariate analysis (P = .024) but not by multivariate testing. This study, although very well done, may be limited by problems in recognizing embryonal elements. Klepp et al[17] noted embryonal elements in 211 (76%) of 279 cases, whereas the Testicular Cancer Intergroup Study noted embryonal carcinoma in over 90% of cases upon careful review.

Pathologic assessment is critically important to all the primary tumor histologic factors. Unfortunately, quantifying cell type and VI is subject to some subjectivity and interpretation, and this affects the results of these studies (Table 1). Ideally, a cell type-specific stain or marker should be available to more objectively quantify embryonal or other elements. Although a purportedly embryonal-specific antibody called 43-9F has been identified,[32] we have not found it to be specific for embryonal carcinoma (Heidenreich A, Sesterhenn IA, Moul JW, unpublished data). Therefore, we feel that this antibody is not useful for objective embryonal carcinoma assessment.

**Volume of Embryonal Carcinoma**—Most recently, Albers et al have introduced the concept of the volume of embryonal carcinoma as a prognostic factor.[33] In 90 clinical stage I patients, an absolute volume of embryonal carcinoma less than 2 cc (along with MIB-1 immunohistochemistry; discussed below) defined a subgroup of patients (30%) at very low risk of occult disease. Unfortunately, the volume of embryonal carcinoma was estimated based on available histology slides and has not yet been confirmed by prospective whole-mount testicular tumor volume assessment.

**Summary**—In summary, we feel that embryonal carcinoma is extremely important as a prognostic marker for occult disease in clinical stage I NSGCT. An experienced reference pathologist should do a careful assessment for embryonal carcinoma (as well as VI), including percentage of embryonal elements. From our recent work, %EMB and VI can be used to provide clinically useful probabilities of occult disease (Table 3). More investigation of the volume of embryonal carcinoma as a prognostic factor is needed.

**Yolk Sac Carcinoma**

Although yolk sac carcinoma exists in pure form in childhood testicular cancer, it is almost invariably part of a mixed NSGCT in adulthood tumors.[21] Like other histologic prognostic factors, the clinical utility of yolk sac carcinoma may be limited by its ability to be detected in histologic sections. This cell type may exhibit multiple patterns, including hepatoid differentiation, enteric differentiation, and a solid growth pattern that may resemble seminoma.[21] Immunohistochemical staining for AFP is very useful for identifying suspected foci as yolk sac tumors.[21]

Because of these identification problems, the percentage of tumors reported to contain yolk sac carcinoma varies, as does the importance of this cell type as a prognostic marker. In the Testicular Cancer Intergroup Study, local pathologists identified yolk sac carcinoma in 12% of tumors, whereas reference pathologists found it in 73% of cases.[22]

In clinical stage I NSGCT, the presence of yolk sac tumor has been a favorable prognostic feature;[14,17-19] however, paradoxically in advanced NSGCT, the presence of this element had an adverse impact on survival in some series.[9,34] Even in studies of stage I disease, the impact of yolk sac tumor is controversial. Only Freedman et al[14] and Jacobson et al[18] found yolk sac carcinoma to be an important risk factor on multivariate analysis. In the study of Klepp et al, yolk sac tumor predicted occult nodal disease at RPLND on univariate analysis but was marginally significant on multivariate analysis (P = .09).[17] The yolk sac tumor element did not predict relapse of patients...
following a negative RPLND, and in multivariate analysis it was not a significant predictor of positive nodes at RPLND plus later relapse.[17] Moul et al found that yolk sac tumor was significant by univariate analysis for the prediction of positive nodes at RPLND and/or later relapse in clinical stage I NSGCT, but only VI and %EMB remained significant by multivariate analysis.[19] In some studies of prognostic factors in clinical stage I NSGCT (Table 1), yolk sac tumor did not emerge as important even on univariate analysis.[13,15,16] Again, this may relate to identification of this cell type. For example, Hoskin et al[13] noted yolk sac carcinoma in only one patient, Dunphy et al[15] in 54% of patients, and Fung et al[16] in 27%, and none of these studies found this element to be important. Although Hoskin et al reported yolk sac tumor in only one case, they performed tissue staining for alpha-fetoprotein (AFP), which should have detected yolk sac elements.[13]

**Summary**—Thus, it is unclear how useful yolk sac carcinoma is as a prognostic marker in clinical stage I NSGCT. It is certainly more difficult to characterize this tumor in histologic sections, and our recent careful multivariate analysis of all cell types found that it did not remain significant.[19] With the extreme clinical utility of VI and %EMB,[19] it may be unnecessary to analyze histologic specimens for this factor. On the other hand, an accurate assessment for yolk sac tumor by an experienced reference pathologist may add to the overall prognostic assessment and guide clinical decision-making.

**Teratoma**

The presence of teratoma elements in testicular germ cell tumors has been known to have a favorable impact on prognosis since the studies of Dixon and Moore.[25] In the contemporary era of prognostic factors in clinical stage I NSGCT, the presence of teratoma lessens the likelihood of occult disease. In the Testicular Cancer Intergroup Study, pure teratoma was found in 2% of patients with stage I NSGCT and teratoma elements in 87%.[22] In stage II, teratoma was found in pure form in 3% of cases and as a mixed element in 60%.[22]

Freedman et al noted a 20% relapse rate on surveillance if cartilage or bone was present vs 31% if these elements were absent (P = .08 by the log rank test).[14] These same investigators found a 17% relapse rate with muscle tissue and a 35% rate without this element (P = .004 by log rank).[14] These factors, however, did not remain significant in stepwise regression analyses.

Dunphy et al examined the value of both mature and immature teratoma in predicting relapse on surveillance.[15] Of 93 patients, 61 (66%) had mature teratoma and a similar number (62) had immature teratoma. The relapse rate in those with mature teratoma was 21%, as compared with a rate of 41% when this element was absent (P = .08 by the chi-square test). Conversely, the relapse rate in patients with immature teratoma did not differ significantly from that in patients without this element (31% vs 23%). Even the mature teratoma element did not retain its significance on multivariate analysis, however.

Fung et al noted mature teratoma in 15 (25%) of 60 cases of clinical stage I NSGCT, immature teratoma in 36 (60%) of 60, and any element of teratoma in 46 (77%) of 60.[16] When there was 50% or less of teratoma in the tumor, the chance of occult nodes at RPLND was 44%, whereas when there was more than 50% of teratoma, the occult node rate was only 11% (P = .02).[16] In this study, the percentage of teratoma did remain significant on multivariate analysis.

Klepp et al also found that teratoma of any type was a significant multivariate predictor of occult nodes at RPLND, relapse after a negative RPLND, and overall occult disease in clinical stage I nonseminoma.[17] The risk of positive nodes at RPLND was 20% in the presence of teratoma and 40% in its absence.[17] Similarly, patients with teratoma had a 11% recurrence rate after a negative RPLND, whereas those without this element had a 24% recurrence rate.[17] Overall, only 45.4% of patients without teratoma elements had true pathologic stage I disease vs 71.3% of those with teratoma.[17]

Most recently, Moul et al noted that the percentage of teratoma (mature and immature) was a significant predictor of occult disease at RPLND or late recurrence by univariate testing; however, in multivariate analysis, VI and %EMB were the only factors that remained significant.[19] This study found that %EMB was so powerful that it factored out the percentage of teratoma.[19]

**Summary**—The presence of teratoma portends a favorable course for clinical stage I NSGCT. As our recent study illustrates,[19] quantification of cellular elements in NSGCT is interrelated. For example, the higher the percentage of embryonal elements, the less the percentage of yolk sac or teratoma elements that obviously can be present. Because %EMB is critically important, multivariate analysis may eliminate the interrelated factors, such as teratoma.
Other Histologic Factors

Various other histologic factors have been suggested as predictors of relapse or occult disease in clinical stage I NSGCT. However, the vast majority of these either have not been tested in multivariate analyses or have not proved significant on such analyses.

Tumor size has been analyzed most often, either by actual size or by T-stage, which may also be a factor in invasion of adjacent tissues. Pizzocaro found that T-stage predicted recurrence on surveillance.[35-37] Relapses occurred in only 3/26 (11.5%) of T1 patients, as compared with 9/20 (45%) of T2-T4 patients (P less than .05).[35] In a subsequent study by this group, T1-T2 patients had a relapse rate of 13.2% vs a 50% rate in T3-T4a cases (P less than .01).[37] Raghaven et al similarly noted relapses in 7/35 (20%) of patients with T1 disease, as opposed to 5/10 (50%) of patients with T2-T4 disease.[38] Likewise, in a study by Costello et al, 5 (41.5%) of 12 patients staged as T1 relapsed, as compared with 3 (100%) of 3 patient classified as T2-T4.[39]

Most multivariate studies of stage I NSGCT have not confirmed the importance of tumor size or T stage.[13-15,17-19] Only Fung et al found T-stage to predict occult nodal disease on multivariate analysis.[16] Among T1 patients, 6/33 (18%) had a positive RPLND, as compared with 5/12 (42%) T2 patients, 3/7 (43%) T3 patients, and 6/8 (75%) T4 patients.[16] Hoskin et al looked at actual tumor size (less than 3 cm, 3 to 5 cm, and more than 5 cm), not T-stage, and found no relationship with relapse.[13] In a study by Freedman et al, T-stage was significant by univariate but not by multivariate analysis.[14] Dunphy et al found no relationship of tumor size, multifocality, or size of largest tumor with recurrence.[15] Klepp et al assessed both size and T-stage; their univariate analysis showed that T-stage (T1 vs T2-4) was predictive of occult disease at RPLND, but tumor size (less than 3.5 vs more than 3.5 cm) was not.[17] T-stage also predicted recurrence after a negative RPLND by univariate analysis and was marginally significant (P = .084) by multivariate analysis. With regard to the prediction of positive nodes at RPLND and overall predictive value (positive RPLND or later relapse), T-stage was not significant by multivariate analysis.[17] In another multivariate analysis, tumor size was not significantly correlated with relapse on surveillance.[40]

Other factors interrelated with T-stage have been analyzed individually and have been shown to be significant or marginally important by univariate analysis. These include epididymal and/or rete testis involvement,[13,14] tunical involvement,[13,14,19] and spermatic cord involvement.[13,14]

None of these factors has maintained prognostic importance on multivariate analysis.

A wide assortment of other histologic features have been analyzed as risk factors for relapse or occult disease. One group found no relationship between tissue staining for AFP and beta-human chorionic gonadotropin (HCG) and relapse on surveillance.[13] Other investigators showed no correlation of necrosis within the primary tumor[37] or necrosis and hemorrhage[16] with occult nodes or relapse. Two groups have stated that choriocarcinoma elements in the primary tumor correlate with relapse:[14,39] however, in one of these studies,[14] this element did not remain significant in multivariate analysis. No study that has evaluated seminoma elements in NSGCT has found this factor to be important.[15-17,19,40]

Clinical Factors

Numerous clinical factors have been analyzed in clinical stage I NSGCT to determine whether they can predict either recurrence on surveillance or occult nodes or recurrence at or after RPLND. These factors have included preorchiectomy tumor marker values, scrotal violation, delay in diagnosis, patient age, side (right vs left) of the primary tumor, history of cryptorchidism, and semen quality.

Preorchiectomy Tumor Markers

The preorchiectomy tumor markers AFP and HCG have been studied most extensively as prognostic factors. In order for a testicular tumor to be defined as clinical stage I NSGCT, both HCG and AFP levels must return to normal after orchiectomy, following appropriate half-life-determined intervals. If these markers do not return to normal, the patient is not considered as having clinical stage I disease and receives different treatment.

In patients whose tumor markers do normalize after orchiectomy, and thus, who have true clinical stage I disease, preorchiectomy absolute values of these markers have been examined as a possible predictors of occult disease. As noted in Tables 1 and 2, preorchiectomy HCG has never been found to be of prognostic significance either by univariate or multivariate analyses. However, preorchiectomy AFP has emerged as important in a number of univariate analyses[13,17,27], as well as one multivariate analysis.[17]
Hoskin et al found that 13 (48%) of 27 patients who had a normal preorchiectomy AFP relapsed on surveillance, as compared with 5 (16%) of 31 patients with an elevated initial AFP.[13] On multiple regression analysis, however, only LI and histology were significant factors. In contrast, Wishnow et al have concluded that an elevated preorchiectomy AFP (more than 80 ng/dL) was predictive of relapse on surveillance.[28] Based on a review of their raw data, however, it is difficult to justify this conclusion. Of the 82 patients, 30 (36.6%) patients had an AFP more than 80 ng/dL, and almost an equal number of these patients relapsed or did not relapse (13 vs 17).

Probably the best study of preorchiectomy markers in clinical stage I NSGCT is that of Klepp et al.[17] They found that 37 (40.2%) of 92 patients with a normal AFP had occult positive nodes at RPLND vs only 20 (18.5%) of 108 patients with an elevated AFP. Occult positive nodes were discovered in 37/87 (42.5%) of those with normal AFP and HCG levels, as compared with 21/121 (17.4%) of those with elevations of either marker and 12/55 (21.8%) of those with elevations of both. In multivariate analysis, an elevated preorchiectomy AFP was still predictive of a decreased risk of occult disease at RPLND. Combining occult nodes at RPLND and later relapse after a negative RPLND, an elevated preorchiectomy AFP conferred a reduced risk in multivariate analysis.[17]

In contrast to the study by Klepp et al, Fung et al found no association between preorchiectomy AFP and risk of occult nodes at RPLND. [16] However, the latter study was much smaller than the former. That an elevated initial AFP confers a reduced risk of occult disease makes sense because this marker signifies the presence of yolk sac elements, which, as discussed previously, also portend a lessened risk of occult disease. Conversely, embryonal carcinoma, which does not routinely contribute to marker elevation, significantly increases the risk of occult disease. When markers are not elevated, also a risk factor for occult disease, this generally signifies a higher proportion of embryonal elements in the tumor. As can been seen, the histology risk factors are interrelated with the marker risk factors. This may explain why markers or histology can be factored out in certain multivariate analyses.

• Summary—Preorchiectomy and postorchiectomy levels of tumor markers are critically important determinants of clinical stage I status. Whether the actual preorchiectomy values are important prognostic factors for occult disease in clinical stage I patients is still disputed. Whereas HCG does not appear to be important in this regard, AFP does seem to provide crucial information, although both markers are intertwined with histologic cell-type factors.

Scrotal Violation, Diagnostic Delay

Diagnostic and treatment discrepancies, such as delay in diagnosis and scrotal violation, have also been considered risk factors for recurrence on surveillance or positive RPLND. Pizzocaro et al have consistently found that transcrotal orchiectomy confers an increased risk of recurrence on surveillance.[35-37] Recurrences occurred in 13 (20%) of 65 patients who had an inguinal orchiectomy, as compared with 10 of 20 (50%) who underwent transcrotal surgery.[37] These investigators did not do multivariate analyses,[35-37] however, and no other researchers have confirmed their findings.

Hoskin et al were the first to examine a delay in diagnosis as a possible risk factor for recurrence on surveillance.[13] They compared patients who were diagnosed within 6 weeks, 7 to 13 weeks, and more than 14 weeks of symptom onset, and failed to show a correlation between diagnostic delay and relapse. Similarly, Pizzocaro et al did not find an increased risk of relapse on surveillance among patients diagnosed within 1 month of symptoms, as opposed to more than 1 month.[37] Fung et al also concluded that a delay in diagnosis (less than 1 vs 1 month or more) was not a risk factor for positive nodes at RPLND in clinical stage I NSGCT.[16] Klepp et al studied the interval between orchiectomy and RPLND ( 29or less vs more than 29 days) but found no correlation with the risk of occult nodes at RPLND.[17]

Thus, scrotal violation may not be predictive of occult disease, but a delay in diagnosis does not seem to be a risk factor.

Patient Age

The age of the patient has been examined as a potential prognostic factor. Hoskin et al compared patients less than 25 years old, 25 to 29 years old, and 30 years old or more and uncovered no difference among the three groups with regard to relapse on surveillance.[13] In contrast, Thompson et al, using the same age ranges, found a statistically significant decreased risk of recurrence on surveillance in men older than 29 years.[41] However, age was not related to relapse on surveillance in a multivariate analysis by Rorth et al,[40] nor was age (less than 30 vs 30 years or more) related to the risk of positive nodes at RPLND in a study by Fung et al.[16]
Based on these study results, age does not appear to be a prognostic factor in clinical stage I disease.

**Other Factors**

Raghaven et al found that recurrence on surveillance was much more common in patients with right-sided tumors (11/26 [42%]) than in those with left-sided tumors (2/20 [10%]).[38] No other investigator has studied this factor in surveillance. Although Fung et al did find that patients with right-sided tumors had a greater chance of having occult nodes at RPLND than those with left-sided tumors (37% vs 27%), this difference was not statistically significant.[16] Further study of this factor is needed.

Fung et al found cryptorchidism to be a risk factor for occult disease at RPLND.[16] No other author has discussed this factor.

Two groups have independently examined semen quality around the time of orchiectomy as a prognostic marker for recurrence on surveillance, with dissimilar results. Ondrus et al found that a sperm count lower than 10 million/mL predicted relapse,[42] whereas Horwich et al found that the same quantity of sperm was of no value in predicting relapse.[43]

**Molecular and Proliferative Factors**

The use of DNA and molecular genetic markers as prognostic factors in clinical stage I NSGCT is still in its infancy. This section will focus on work that has been done with flow cytometric and other proliferation and DNA content analyses, as well as very recent early work with molecular genetic markers.

**Cell Proliferation Markers**

- **DNA Index**—Cellular proliferation as a clinically useful marker in testicular cancer is not a new concept. Numerous flow cytometry and image analysis studies to gauge DNA content and proliferation in clinical stage I NSGCT have been reported over the last few years.[31,44-50] These studies have suffered from small size, lack of multivariate testing, and conflicting results.[31,45-50] Sledge et al were the first to find flow cytometric proliferative index to be a prognostic marker in advanced NSGCT by univariate analysis.[44] In contrast, Fossa et al found no correlation between DNA index and final pathologic stage in a group of 68 patients who underwent RPLND for clinical stage I NSGCT.[46] In a subanalysis of 40 patients using S-phase fraction, no significant difference between pathologic stage I and II was observed.[46]

Using image analysis, Alhoff et al[45,47] and Burger et al[48] found hyperpentaploidy to be a risk factor for occult metastatic disease. De Riese et showed that DNA index, determined by flow cytometry, was equivalent for pathologic stage I vs II; however, on univariate analysis, the S- and G2M-phases of the aneuploid cell population correlated well with pathologic stage.[49] In a small study of 23 patients, Austenfield et al[50] found that all patients who were upstaged by RPLND or who relapsed while on surveillance had aneuploid tumors, whereas no surveillance patients with diploid tumors (N = 3) had a recurrence at a minimum 15 month follow-up despite adverse histologic parameters.

In a study of 36 clinical stage I NSGCT patients, Moul et al found an equivalent mean DNA index in pathologic stage I and II cases; however, the percentage of the aneuploid cell population in S-phase and the proliferative index were significant risk factors for occult disease by univariate analysis.[31] In a multivariate analysis that included VI and %EMB, the flow cytometry proliferative parameters were not significant.[31]

Most recently, DeRiese et al studied 102 clinical stage I NSGCT patients by flow cytometry and single-cell cytophotometry.[51] Aside from patients with pure (100%) embryonal carcinoma, the percentage of aneuploid tumor cells in S-phase by flow cytometry was most predictive of pathologic stage. Multivariate analysis confirmed the importance of this flow cytometry parameter. The investigators correctly predicted 91% of pathologic stage II patients and 77% of pathologic stage I patients, for an overall accuracy of 82%. Prospective testing of this factor is warranted.

- **Proliferating cell nuclear antigen (PCNA)**—is another cellular proliferation marker that could be useful in clinical stage I NSGCT. This nuclear protein is intricately involved in the processes of DNA replication and nucleic acid excision and repair. Its expression is cell cycle-dependent, being maximal during the S-phase, and its use as a marker of cell proliferation has been shown to have prognostic significance in various tumors. Of particular advantage is the existence of several
antibodies that bind PCNA in formalin-fixed, paraffin-embedded tissue.[52] With respect to testicular cancer, Ulbright et al examined 50 archival NSGCT primary tumors by flow cytometric S-phase, PCNA expression, and p53 protein expression.[53] Mean nuclear PCNA expression was 88.4% in p53-positive cases vs 73.7% in p53-negative cases. Similarly, flow cytometric S-phase was higher in p53-positive cases than in p53 negative cases (29.6% vs 18.5%). In a more recent study, this group found that PCNA was not useful for separating pathologic stage I from stage II NSGCT.[54]

Fernandez and associates have also performed a comprehensive multivariate analysis of PCNA and histologic factors in clinical stage I NSGCT.[52] In 89 clinical stage I patients, PCNA expression in total tumor tissue and PCNA expression in the embryonal carcinoma cellular component were statistically higher in the patients who were upstaged at RPLND or whose disease later recurred. However, in multivariate analysis, the PCNA markers were factored out by the more powerful predictors of occult disease—VI and %EMB. It appears that PCNA does not provide additional staging information in clinical stage I NSGCT.

- **Ki-67**—Most recently, another immunohistochemical proliferation marker, Ki-67 (MIB-1), has been analyzed as a prognostic marker in clinical stage I NSGCT. The group from Indiana University has shown that MIB-1 staining is superior to PCNA, p53, and neovascularity as a prognostic marker and that it remains significant in multivariate analysis.[54] Furthermore, they have proposed a risk model based on MIB-1 and the volume of embryonal carcinoma that selects a subset of patients at low risk for occult disease who may be suitable for surveillance.[51] In contrast, our group has not found MIB-1 staining to be clinical useful. In a series of 89 clinical stage I NSGCT patients, MIB-1 staining score did not remain significant as a prognostic marker in multivariate analysis.[55]

The Indiana and Walter Reed groups should standardize methodology for MIB-1 and embryonal assessment and conduct a joint prospective study to settle the issue of whether or not MIB-1 has value as a prognostic marker.

### Molecular Genetic Markers

The use of molecular genetic markers for staging and prognosis in urologic oncology and specifically in testicular cancer is just beginning.[56,57]

- **Oncogenes**—Regarding oncogene markers, little work has been performed. The ras oncogene family is commonly mutated in certain human tumors, particularly in cancers associated with carcinogens. However, there is a low incidence of ras mutation in testicular tumors, and no clinical correlations are evident.[58] The hst-1 oncogene, a gene that encodes a fibroblast growth factor, has been found to be overexpressed in NSGCT.[59] Interestingly, patients with metastatic disease have a greater likelihood of overexpressing hst-1. No work has been done in clinical stage I patients, however. The myc family of oncogenes, which encode nuclear cell growth and differentiation proteins, have been implicated in testicular cancer, but no work on clinical stage I disease has been performed.[56,57]

Most recently, the bcl-2 oncogene, which is a suppressor of apoptosis, has been implicated in testicular carcinogenesis.[60] A study to determine the value of bcl-2 immunohistochemical staining as a molecular prognostic marker in clinical stage I NSGCT is under way at our institution. Oncogene markers will be an exciting area for future study.

- **Tumor-Suppressor Genes**—Tumor-suppressor gene molecular markers are an equally exciting subject for research. The retinoblastoma gene, the cause of retinoblastoma and the prototypic tumor-suppressor gene, is underexpressed in testicular tumors, but work specific to stage I NSGCT has yet to be reported.[61] The other well-known current tumor-suppressor gene, p53, has become one of the most widely studied genetic markers of human cancer over the last few years.[62] Mutations in the highly conserved exons of the p53 gene have been commonly observed in a variety of human tumors. The wild-type, or normal, p53 gene product is virtually undetectable by immunohistochemical methods due to both its short half-life and the low amount of p53 present. However, mutation or other alteration of p53 results in stabilization of the protein with a prolonged half-life, and thus, levels of p53 detectable by immunohistochemistry may indicate the expression of an aberrant form of p53.[62] DeRiese et al performed a study to determine whether p53 protein expression in the primary tumor of clinical stage I NSGCT could be a marker for occult disease.[63] In 84 patients (28 of whom had stage I and 56, stage II disease), p53 expression was similar for both stages. They thus concluded...
that p53 was not useful for determining occult disease. These authors assessed p53 expression in the overall neoplastic cellular components.

Lewis et al also has studied p53 protein expression by immunohistochemistry to determine whether it is a clinically useful marker in clinical stage I NSGCT.[62] Expression of p53 in the embryonal carcinoma cellular component was statistically higher in the patients upstaged by RPLND. However, in careful multivariate analysis, VI and %EMB remained significant risk factors but p53 expression did not.

Of interest, testicular tumors are relatively unique in that overexpression of the p53 protein occurs in the absence of p53 gene mutations. Work by our group and other investigators has shown that most testicular tumors overexpress p53 protein but mutations are rare.[64,65] Study is under way to determine the cause of this.

As with oncogene markers, much more work is necessary with tumor-suppressor gene markers, many of which have yet to be discovered. These include a possible testis cancer-specific tumor-suppressor gene, that is thought to reside on the long arm of chromosome 12.

**Summary**

One outgrowth of the controversy over the role of surveillance vs primary RPLND in patients with clinical stage I nonseminomatous testicular cancer has been the accumulation of a wealth of knowledge about risk factors that may predict recurrence or occult nodal disease. Of the primary tumor histologic, clinical, and proliferative and molecular markers examined to date, VI and embryonal carcinoma appear to provide the most clinical utility. Regarding VI, blood vessel invasion appears to be most important. With respect to embryonal carcinoma, its mere presence or absence is not sufficient. Rather, quantitative assessment of the percentage and/or volume of embryonal component in the primary tumor is of critical importance. Clinically useful probability models for the likelihood of occult disease using both VI and %EMB are now available.

Of the clinical factors, only preorchiectomy AFP has been shown to be a useful predictor of occult disease by multivariate testing.

More work is necessary to determine whether proliferative or molecular markers will aid in the care of clinical stage I NSGCT patients. Prospective multicenter trials of these markers are urgently needed.

**References:**


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