Chapter 4

Predictors of occult metastasis in clinical stage I nonseminoma: a systematic review of 2500 patients

Abstract

Patients with clinical stage I nonseminomatous testicular germ cell tumour should ideally only receive adjuvant therapy when they are at high risk for occult metastasis. We aimed to quantify the importance of predictors for occult metastasis by performing a systematic review of the relevant literature. In addition, we reviewed published multivariable models and risk-adapted treatment policies. We identified 23 publications between 1979 and 2001, reporting in total 2587 patients. 29% (759/2587) of the patients had occult metastases, which was diagnosed either at retroperitoneal lymph node dissection (n = 193) or during follow-up (n = 566). Odds ratios (OR) were pooled using meta-analysis techniques. Vascular invasion of the primary tumour cells had the strongest effect (OR: 5.2 [95% confidence interval: 4.0 – 6.8]). MIB-1 staining of the primary tumour cells was a promising predictor (OR: 4.7 [2.0 – 11]). Intermediate effects were found for embryonal carcinoma in the primary tumour (OR: 2.9 [2.0 – 4.4]), and a high pathological stage of the tumour (OR: 2.6 [1.8 – 3.8]). Size of the primary tumour and age of the patient showed weaker though also statistically significant effects. Until now, multivariable models often included vascular invasion and embryonal carcinoma with one or two weaker predictors. None of the published risk-adapted treatment policies included MIB-1 staining. A risk-adapted treatment policy should be developed that incorporates all relevant predictors, such that adjuvant therapy is targeted better to those with occult metastases.

Introduction

Most patients with clinical stage I nonseminomatous testicular germ cell tumour (NSGCT) can be cured by orchidectomy alone. Approximately 30% have occult metastatic disease, which can be detected at retroperitoneal lymph node dissection (RPLND) or on surveillance.[1-3] Some consider RPLND as a staging procedure which might be followed by adjuvant chemotherapy, if metastases are revealed.[4] Others prefer surveillance with chemotherapy administration after detection of relapse.[5,6] Both treatment policies have excellent long-term survival rates (98% to 99%), but each has important drawbacks. RPLND and chemotherapy can induce morbidity.[7] In particular, chemotherapy may have long-term adverse effects.[8,9] Conversely, surveillance can lead to detecting relapses at a more advanced disease stage, if compliance is poor. Therefore, efforts have been made to select patients at

high risk for occult metastasis, who can be offered immediate adjuvant treatment.[10,11] Further, unnecessary treatment may be avoided in patients at low risk.

To distinguish high risk patients from low risk patients, many groups studied the associations between tumour and patient characteristics and occult metastasis in clinical stage I NSGCT.[2,5,6,12-35] This resulted in a number of well-known predictors, particularly vascular invasion and embryonal carcinoma. Several predictors have been combined in multivariable regression models[17,26] and these models underlie risk-adapted treatments. [11,36] Patients at high or intermediate risks are then offered chemotherapy (often several cycles of bleomycin, etoposide, and cis-platin [BEP]) or RPLND, while patients at low risk go on a surveillance protocol without further treatment until relapse.

Several reviews have qualitatively summarised studies on predictors. However, a systematic review is required to quantify the strength of the known predictors more precisely. From the evidence based medicine literature, systematic reviews have been promoted. Such reviews include a comprehensive search for available data and a quantitative summary.[37] In addition to a systematic review of predictors for occult metastasis, we reviewed reported multivariable models and proposed risk-adapted treatment policies.

Patients and Methods

Study identification

We searched the Medline database from 1979 to 2001 to identify all English-language studies on predictors of occult metastasis for clinical stage I NSGCT patients. Search textwords were 'testicular neoplasms' and 'neoplasm staging or neoplasm metastasis' and 'risk factors or prognosis'. Relevant references in articles were also considered. Studies were included if the association between occult metastasis and patient/tumour characteristics was quantified. The clinical staging procedure had to be described and had to contain determination of the serum tumour markers alpha-fetoprotein (AFP) and human chorionic gonadotropin (HCG) after orchidectomy and radiological examination of chest and abdomen.[38] If studies included patients from the same centre with overlapping time periods, we included the study with the largest population or with the longest follow-up period. Some overlapping studies were included both, because different characteristics were studied.

Outcome

The studies reported different definitions of occult metastasis in clinical stage I. If patients underwent RPLND, positive lymph nodes might reveal occult metastasis (pathological stage II [PS II]). Characteristics of patients with PS II were compared with characteristics of patients with negative lymph nodes (pathological stage I [PS I]). In centres with a surveillance policy, occult metastasis was defined as relapse. Characteristics of patients with relapse were compared with those of patients without relapse. Combinations of the two policies were also described.[26,39] When more than one definition of occult metastasis was reported, we used the most informative definition. For instance, PS I versus PS II with additional follow-up for PS I to find relapses instead of PS I versus PS II only.[26]

Histologic and clinical characteristics

The association with the presence of occult metastasis was studied for the following histologic and clinical characteristics: vascular invasion of tumour cells; histology of the primary tumour; pathological stage of the primary tumour; size of the primary tumour; pre-orchidectomy levels of AFP and HCG; and patients' age.

Vascular invasion was defined as the presence of tumour cells within a well-defined endothelium-lined space. Some studies distinguished venous invasion and lymphatic invasion. If those types were not explicitly mentioned, we assumed that vascular invasion included venous and lymphatic invasion. Most studies classified the tumour histology according to the World Health Organisation system: mature teratoma; immature teratoma; embryonal carcinoma; yolk sac tumour; teratocarcinoma; choriocarcinoma; and seminoma.[40] Associations with the presence of mature teratoma; embryonal carcinoma; and yolk sac were determined. A pooled estimate could also be determined for the percentage of embryonal carcinoma at a cut off value of 50%. Further, the British definition of teratoma differentiated was analysed.[41] The pathological stage of the primary tumour was considered when determined according to the TNM classification:[42] pT1 was then compared with pT2-pT4. Size of the primary tumour was dichotomised in all studies. One study used a cut-off value of 3.5 cm,[26] whereas the other studies used 3.0 cm.[16,19,20,22] Since we could not identify heterogeneity between the studies, they were pooled. Preorchidectomy levels of AFP and HCG were classified as normal or elevated. Patients' age was twice dichotomised at a cut-off value of 30 years of age.[20,43] Two other studies used a cut-off value of 29 years.[16,22] The results were pooled, since no heterogeneity was present.

Tumour proliferative activity

Tumour proliferative activity has been measured with several techniques. Some studies used single cell cytophotometry or flow cytometric analysis to show the association between the presence of proliferative activity of the primary tumour cells and occult metastasis. All studies used different parameters for proliferative activity, which complicated any analysis. Therefore, we only analysed an immunohistochemistry technique, using the MIB-1 antibody. MIB-1 binds the Ki-67 receptor, which is expressed by proliferating cells in late G_1-, S-, G_2- and M-phases.[44] Several studies reported the percentage of Ki-67 positive primary tumour cells. A high percentage of Ki-67 positive cells indicates high proliferative activity. The percentage of positive cells was reported continuously or classified with a cut-off value of 70%.

Multivariable odds ratios and risk-adapted treatments

Multivariable associations between occult metastasis and tumour or patient characteristics reported in the literature were summarised. In some papers, aggregated or individual data were reported without an explicit multivariable analysis. In these instances, we estimated multivariable coefficients with logistic regression analysis.

In addition, we searched for reports on risk-adapted treatment protocols. We were interested in protocols with only RPLND or cis-platin based chemotherapy as adjuvant treatment. Reports were included if the outcomes of the protocol were evaluated.

Table 4.1 Characteristics of 23 studies included in the systematic review for predictors of occult metastasis in clinical stage I NSGCT (2587 patients, 759 with occult metastasis)

First author	*Year*	*Ref*	*Study period*	*N*	*Outcome*	*Metastases*	
						N	*(%)*
Hoskin	1986	16	1979-85	126	relapse	36	(29)
Freedman	1987	17	1979-83	259	relapse	70	(27)
Dunphy	1988	19	1981-86	93	relapse	28	(30)
Fung	1988	20	1979-87	60	PS II	20	(33)
Thompson	1988	22	1979-87	36	relapse	12	(33)
Costello	1989	23	1980-86	18	relapse	9	(50)
Wishnow	1989	24	1981-87	82	relapse	24	(29)
Jacobsen	1990	25	1980-84	83	relapse	23	(28)
Klepp	1990	26	1981-86	279	PS II + relapse [a]	75	(27)
Colls	1992	29	1980-90	115	relapse	34	(30)
Read	1992	43	1984-87	373	relapse	100	(27)
Sturgeon	1992	6	1981-90	105	relapse	37	(35)
Moul	1994	47	1980-93	92	PS II	38	(41)
Ondruš	1994	32	1984-93	80	relapse	29	(36)
Sosnowski	1994	48	1985-91	52	relapse	15	(29)
Albers	1995	4	1992-93	90	PS II	25	(28)
Gels	1995	33	1982-91	154	relapse	42	(27)
Nicolai	1995	50	1981-84	85	relapse	25	(29)
Albers	1997	35	1983-94	78	PS II	28	(36)
Sogani	1998	51	1979-87	105	relapse	27	(26)
Albers	1999	39	1996-99	44	relapse; or PS II [b]	15	(34)
Roeleveld	2001	52	1982-94	90	relapse	23	(26)
Alexandre	2001	53	1984-96	88	relapse	24	(27)
Total	**1986- 2001**		**1979-99**	**2587** [c]		**759**	**(29)**

[a] After RPLND, PS I patients were followed for relapse
[b] Patients were randomized for either surveillance or risk-adapted treatment
[c] Includes patients reported in multiple studies more than once

Statistical analysis

We calculated odds ratios (ORs) from the 2x2 tables as reported in the studies with patients undergoing RPLND. Surveillance studies followed the patients often for more than 2 years, indicating that few patients were censored before they could relapse. Therefore, ORs were calculated instead of hazard ratios, which would have been the most appropriate statistical measure if the patients had incomplete follow-up. The pooled OR and 95% CI were calculated with a random-effects meta-analysis method.[45] This method takes the variability between studies into account. Pooling was performed if the characteristic was reported by at least two studies.

We examined whether the predictor effects depended on differences in study characteristics.[46] The following characteristics were considered: the treatment (RPLND or surveillance), study perspective (retrospective or prospective) and study size. Study size was used to identify possible publication bias. This bias may be relevant, because statistically significant results usually have a higher chance of being published than insignificant results, leading to on average higher effect estimates in smaller studies. The heterogeneity of an effect was tested for statistical significance by fitting a weighed linear regression model of ln(study OR) on the study characteristics, where each study OR was weighed by the reciprocal of its variance. Study size was included in the model as the square root of the total number of patients. If important heterogeneity was found ($p < 0.10$), the pooled OR was calculated for each category of the study characteristic.

Results

We included 23 studies (Table 4.1) of which four included over 150 patients.[17,26,33,43] Five studies performed RPLND, 17 studies followed their patients until relapse, and one study randomised patients to either RPLND or risk-adapted treatment.[39] One of the six studies which performed RPLND monitored relapse in PS I patients, in order to find misclassified patients.[26] The reported median follow-up times in the studies with surveillance varied between 30 and 139 months. A longer follow-up time did not result in a higher proportion of identified occult metastases (Figure 4.1). This confirms that follow-up in this study was sufficient to detect occult metastasis. The studies involved 2587 patients, of whom 193 had PS II at RPLND (31.1%, 193/621) and 566 relapsed during follow-up (28.8%, 566/1966), this difference was not statistically significant ($p = 0.27$). Overall, the percentage of occult metastasis was 29.3% (759/2587).

Predictors

21 studies reported the effect of vascular invasion. For the other predictors, smaller numbers of studies were available (Table 4.2). The pooled ORs of all three definitions of vascular invasion (venous, lymphatic, venous or lymphatic) were high (4.7, 5.4, and 5.2 respectively). The presence of differentiated teratoma in the primary tumour strongly decreased the risk of having occult metastasis (pooled OR=0.13), although the confidence interval was wide (0.02 - 0.85, i.e. 1.2 - 50). This predictor was present in only 6% of the patients, which

Table 4.2 Associations between predictors and occult metastasis in clinical stage I NSGCT

Predictor	*References*	*N*	*% of patients with charac-teristic*	*OR (95% CI) Occult metastasis yes versus no*	
Vascular invasion					
Venous present vs absent	17,19,22,33,43,47	1007	42	4.7	(2.5 – 8.5)
Lymphatic present vs absent	17,19,22,43,47	853	18	5.4	(3.0 – 13)
Venous or lymphatic present vs absent	6,19,20,23,25,26,29, 32,35,39,48,50-53	1364	36	5.2	(4.0 – 6.8)
Histology primary tumour					
Embryonal carcinoma present vs absent	6,17,19,26,33,43, 48,50,51	1505	85	2.9	(2.0 – 4.4)
> 50% vs ≤ 50%	20,39,49,50,52	369	51	2.8	(1.7 – 4.6)
Yolk sac present vs absent	6,17,19,26,32,33, 35,43,52,53	1599	52	0.91	(0.68 – 1.3)
Mature teratoma present vs absent	19,26,52,53	434	45	0.48	(0.27 – 0.74)
Teratoma differentiated present vs absent	17,43	595	6	0.13	(0.02 – 0.85)
Pathologic stage primary tumour					
pT2-4 vs pT1	17,29,32,33,43,50	1066	22	2.6 [a]	(1.8 – 3.8)
Size primary tumour [b]					
> 3 cm vs ≤ 3 cm	16,19,20,22,26	594	68	1.5	(0.99 – 2.3)
AFP serum level					
elevated vs normal	16,20,24,26,29,33, 52,53	990	40	0.94	(0.59 – 1.5)
HCG serum level					
elevated vs normal	16,20,22,33,52,53	550	32	1.1	(0.49 – 1.8)
Patients' age [c]					
older than 30 years vs younger	16,20,22,43,53	683	41	1.6	(1.2 – 2.4)
MIB-1 staining					
> 70% of cells vs ≤ 70%	35,39,49	212	55	4.7	(2.0 – 11)

[a] Heterogeneity of effect: surveillance studies OR=2.1 (1.4–3.4); RPLND studies OR=4.0 (2.5–7.7)
[b] > 3 cm in ref. 12, 16, 17, 19; > 3.5 cm in ref.23
[c] > 29 years in ref. 12, 19; > 30 years in ref. 17, 41

implies that it will not be very helpful in identifying the 70% patients without occult metastases. Other predictors indicating the histology of the primary tumour had more moderate effects (presence of embryonal carcinoma: OR=2.9; presence of mature teratoma: OR=0.48), just as the pathological stage of the primary tumour (OR=2.6 for pT2-4 versus pT1). A larger primary tumour, and a higher patients' age also increased the risk of occult metastasis. Further, MIB-1 staining of the primary tumour was a promising predictor (OR=4.7 for >70% stained cells versus ≤70%). No associations were found for yolk sac histology and serum levels of AFP and HCG before orchidectomy. Both positive and negative effects were found in the individual studies for these characteristics.

Some indication of heterogeneity of effect was found for the pathological stage of the tumour in relation to the definition of occult metastasis (p=0.053). The overall pooled OR was 2.4 (CI 95: 1.7 – 3.5), but the ORs were somewhat higher (4.0, CI 95: 2.5 – 7.7) in studies with RPLND treatment and lower in studies where surveillance was the standard procedure (2.1, CI 95: 1.4 – 3.4).

Several studies reported the mean difference in continuous characteristics between patients with and without occult metastasis. The pooled mean difference was 42% (35% – 49%) for the percentage of primary tumour cells containing embryonal carcinoma[24,35,54] and 12% (6.8% – 18%) for the percentage of primary tumour cells staining for MIB-1.[35,54,55]

Multivariable odds ratios

Four studies reported multivariable associations with occult metastasis (Table 4.3). One study used Cox regression analysis;[17] the other multivariable estimates were based on logistic regression analyses.[33,47,53] We also estimated multivariable associations with information as reported in six other papers using logistic regression analysis.

Vascular invasion showed the strongest effect. Two studies, which possibly included partly the same patients,[47,54] reported very high estimates (OR=8.2 and 13.5). The other ORs were around 4.0 compared with a pooled univariable OR of 5.2. In eight of the 10 studies, the presence of embryonal carcinoma was used to describe the histology of the primary tumour. In two other studies absence of teratoma elements was used for this purpose. In none of the models, embryonal carcinoma and teratoma were included together.

The percentage of embryonal carcinoma was included linearly[47] in one of the four reported models (OR=1.03). A model constructed with reported data[24] containing the percentage of embryonal carcinoma and vascular invasion resulted in a similar OR for embryonal carcinoma (1.03). A model with percentage of embryonal carcinoma categorised (≤45%, 46-79%, ≥80%) together with vascular invasion showed ORs of 2.1 and 8.2 for the categories 46-79% and ≥80%.[24] In another constructed model the ORs were 7.4 and 9.0.[54]

Several studies reported that embryonal carcinoma and vascular invasion were correlated.[53] This is confirmed by the decrease in the ORs for embryonal carcinoma and vascular invasion when estimated together in a multivariable model. However, in most studies the factors remained independent predictors, which indicates that the correlation was not very high.

The contradictory multivariable estimates for serum AFP level (smaller than 1.0[16,26] and larger than 1.0[20,24,29,33,52,53]) indicated that AFP is not a valuable predictor for occult metastasis. The pooled univariable OR was also not statistically significant different from 1.0. None of the models included the three strongest predictors, vascular invasion, embryonal carcinoma and MIB-1 together, although Albers *et al.* reported that all three factors had independent effects, when combined in one model.[35]

Risk-adapted treatment

Table 4.4 lists the reported risk-adapted treatment policies and corresponding relapse rates. Oliver *et al.* reported one of the first risk-adapted treatments.[36] This treatment was based on the four variables as included in the MRC model (venous invasion; lymphatic invasion; embryonal carcinoma; and yolk sac).[17] Patients with at least two risk factors received two cycles of chemotherapy with bleomycin, etoposide, and cis-platin (BEP). The other patients went on a surveillance protocol. Only one of the 22 high risk patients (5%) relapsed after chemotherapy, whereas three of the 19 low risk patients (16%) relapsed after a median follow-up of 43 months. Cullen *et al.* considered the same four variables. Three risk factors had to be present before a patient was defined as high risk. Only two of the 114 high risk patients (2%) relapsed after chemotherapy treatment.

Overall, four of the six policies considered at least embryonal carcinoma and vascular invasion. Low risk patients went on surveillance protocol and high risk patients were offered chemotherapy, mainly two courses of BEP. Two studies also defined an intermediate risk group. Those patients underwent RPLND and subsequent chemotherapy in case of positive lymph nodes.

If we combine all the results of the treated high risk patients, irrespective of the differences in definition of 'high risk', 3% (7/273) of the patients relapsed after chemotherapy. In contrast, 16% (32/199) of the low risk patients relapsed and 13% (14/104) of the intermediate patients relapsed after PS I diagnosis, while 17% of the intermediate patients (16/104) had PS II disease.

Discussion

We systematically combined evidence on predictors for occult metastasis of NSGCT. We confirmed the strong effect of vascular invasion. The proliferative activity of the primary tumour cells showed to be a promising predictor. Intermediate effects were found for the histology of the primary tumour, and the pathologic stage of the primary tumour. Other predictors were size of the primary tumour and age. However, a risk-adapted treatment policy which takes into account at least the three strongest predictors (vascular invasion; proliferative activity of the primary tumour cells; and histology of primary tumour) was not considered yet.

Table 4.3 Multivariable Odds Ratios for risk of occult metastasis in clinical stage I NSGCT

	Reported models					*Constructed models with reported data*				
First author[ref]	*Freedman*[17]	*Moul*[47]	*Gels*[33]	*Alexandre*[53]	*Klepp*[56]	*Heidenreich*[54]	*Wishnow*[24]	*Jacobsen*[25]	*Sogani*[51]	*Leibovitch*[57]
Year	*1987*	*1994*	*1995*	*2001*	*1990*	*1997*	*1989*	*1990*	*1998*	*1995*
N	*259*	*92*	*154*	*88*	*279*	*149*	*82*	*83*	*105*	*91*
Vascular invasion	4.5 [a]	8.2 [b]	4.1 [a]	3.8 [c]	5.0 [b]	13.5 [c]	3.0 [c]	3.3 [c]	5.0 [c]	na
Embryonal carcinoma	4.1 [d]	1.03 [e]	3.7 [d]	ni	ni	7.4 [f]	1.03 [e]	3.3 [d]	2.7 [g]	4.1 [h]
Mature teratoma	ni	ni	ni	0.2 [i]	0.3 [i]	ni	na	na	ni	na
Yolk sac	0.5 [j]	ni	ni	ni	ni	ni	na	na	ni	na
AFP serum level	ni	na	ni	ni	0.5 [k]	na	2.2 [k]	na	ni	na
MIB-1 staining	na	na	na	na	na	ni	na	na	na	3.3 [l]

[a] Venous invasion, lymphatic invasion had an independent OR of 2.5
[b] Venous invasion
[c] Venous or lymphatic invasion
[d] Embryonal carcinoma present
[e] Embryonal carcinoma in % as continuous variable
[f] Categorised in three groups: 0 – 45% (reference category); 46 – 79%; ≥ 80%. OR of intermediate category
[g] Embryonal carcinoma predominant
[h] Volume of embryonal carcinoma larger than 2 ml
[i] Mature teratoma present
[j] Yolk sac present
[k] AFP level elevated
[l] More than 80% of tumour cells stained by MIB-1
na: not available
ni: not included in the model

Table 4.4 Risk-adapted treatment policies for clinical stage I NSTGC found in the literature

First author [ref]	*Year*	*Considered risk factors* [a]	*Risk groups* [b]	*Treatment*	*Relapse/n (%)*		*Median follow-up*
Oliver[36]	1992	VI, LI, EC, YS	LR: less than 2 risk factors present	Surveillance	3/19	(16)	43 months
			HR: at least 2 risk factors present	2 cycles BEP	1/22	(5)	
Pont[58]	1996	VI	LR: VI absent	Surveillance	2/25	(8)	79 months
			HR: VI present	2 cycles BEP	2/29	(7)	
Cullen[59]	1996	VI, LI, EC, YS	HR: at least 3 risk factors present	2 cycles BEP	2/114	(2)	48 months
			LR: risk factors absent	Surveillance	20/106	(19)	40 months
Klepp[56]	1997	VLI, AFP	IR: 1 risk factor present	RPLND (+chemo) [c]	13/99	(13)	
			HR: both risk factors present	3 cycles BEP	1/32	(3)	
Ondruš[60]	1998	VLI, EC	LR: risk factors absent or EC present	Surveillance	7/49	(14)	36 months
			IR: VLI present, EC absent	RPLND (+chemo) [c]	1/5	(20)	
			HR: both risk factors present	2 cycles BEP	0/18	(0)	
Böhlen[61]	1999	VLI, EC, pT	HR: at least 1 risk factor present	2 cycles PVB or BEP	1/58	(2)	93 months

[a] VI: venous invasion present; LI: lymphatic invasion present; EC: embryonal carcinoma present; YS: yolk sac absent; VLI: venous or lymphatic invasion present; AFP: serum level AFP elevated; pT: pathological stage > 1

[b] LR: low risk; IR: intermediate risk; HR: high risk

[c] If RPLND revealed metastasis, the patient was offered chemotherapy

Several definitions were used for the predictors. Therefore, not all publications, though studying the same underlying phenomenon, could be combined. The meta-analysis showed that all definitions for vascular invasion (venous and lymphatic alone or together) resulted in similar estimates of effect size. This confirms that the distinction between venous and lymphatic vessels does not add information on the risk of occult metastasis. The predictor 'venous or lymphatic' is, therefore, the best one to use.

Histologic differences in the primary tumour were initially studied by the presence of embryonal carcinoma and the absence of mature teratoma or teratoma differentiated. In stepwise regression analyses, often one of the two predictors was included. Inclusion of embryonal carcinoma seems to exclude mature teratoma or teratoma differentiated and vice versa.[26,47] This was illustrated by a backward stepwise analysis[33]; among patients with complete values for all considered predictors (n=135), mature teratoma was selected together with vascular invasion and preoperative serum level of HCG. The analysis was repeated after omission of the only predictor with missing values, i.e. the maximum diameter of the primary tumour. In this analysis (n=154), embryonal carcinoma was selected instead of mature teratoma, together with vascular invasion. Since the absence of mature teratoma has a weaker association with occult metastasis than the presence of embryonal carcinoma and teratoma differentiated is not a very common definition, embryonal carcinoma is the best choice for the histology of the primary tumour.

More recently performed studies reported the percentage of embryonal carcinoma in the primary tumour often in two or three categories (≤50% and >50% or ≤45%, 46-79% and ≥80%). Sesterhenn *et al.*[30] showed the continuous relation between the percentage of embryonal carcinoma and the risk of occult metastasis with a cubic spline function. The risk linearly increased above 30%, suggesting that categorising the predictor into two categories discards much information.[62,63] The predictor could therefore better be studied as a continuous variable. Once the nature of the relation is assessed, a simple transformation (e.g. one linear term, or a number of categories with sensible cut-off points) may be defined.

In several tumours, the proliferative activity of the primary tumour is associated with metastatic behavior.[64,65] The association of proliferative activity with occult metastasis in NSGCT has been determined with several techniques like cytophotometry, flowcytometry and immunohistochemistry. The first two techniques are time-consuming and expensive, while immunohistochemistry is rather simple and cheap. Examples of immunohistochemical assessments are staining primary tumour cells for proliferating cell nuclear antigen (PCNA) or MIB-1. A high percentage of tumour cells stained for PCNA or MIB-1 was associated with a high risk of occult metastasis.[35,66] A study which evaluated both PCNA and MIB-1 however, showed only a statistically significant effect of MIB-1.[67] Combining three studies on MIB-1 showed that more than 70% positive stained tumour cells was associated with an increased risk of occult metastasis (OR=4.7).[35,39,49] However, the analysis was based on only 212 patients and the cut off value of 70% was data driven in two of the three studies. Further study on this predictor is hence necessary.

All predictors for occult metastasis were frequently found in clinical stage I patients (Table 4.2), which makes them extra valuable for identification of occult metastasis.

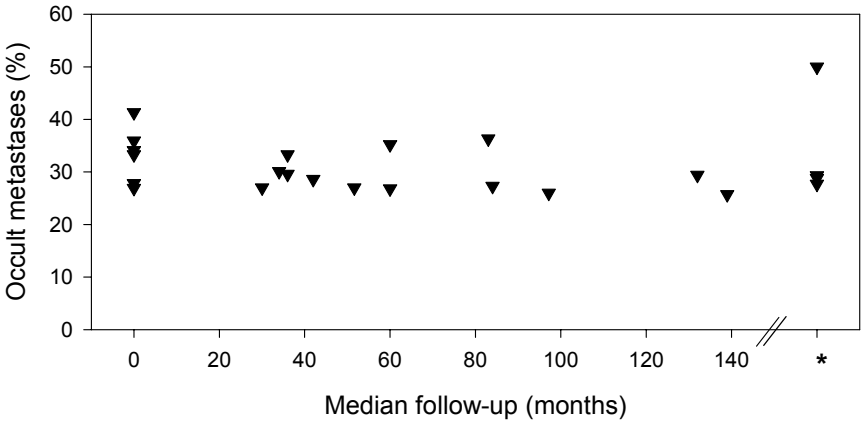

Figure 4.1 Association between length of follow-up in surveillance studies and proportion of identified occult metastases. RPLND studies were set at 0 months of follow-up. For three surveillance studies, the median follow-up time was not reported (*).

Recently, a prospective study showed that the combination of vascular invasion, > 70% MIB-1 stained cells, and ≥ 50% embryonal carcinoma cells in the primary tumour represented 29% of the patients with clinical stage I disease.[68]

We found four multivariable models predicting the risk of occult metastasis in the literature. All included histology of the primary tumour and vascular invasion. Characteristics such as serum level of AFP[56] and yolk sac in the primary tumour[17,36] were considered in several models, while their effects are not important according to our review. These chance findings can be explained by a limited sample size.

We used the reported data of Leibovitch *et al.*[57] to estimate the combined effects of embryonal carcinoma and MIB-1 staining. MIB-1, categorised as > 80% staining versus ≤ 80%, was a strong independent predictor (OR=3.3). Inclusion of vascular invasion likely diminishes this effect to some extend. We therefore anticipate that a model with vascular invasion, embryonal carcinoma, and MIB-1 will have ORs around 3 in multivariable logistic regression analysis. This implies an OR of 27 (3*3*3) for the patients with all three factors positive against those with all negative. Exploring a model with the three strong risk factors is therefore clearly worthwhile. This may enable a better identification of patients at either high or low risks than presently possible. The risk factors pathological stage of the tumour, size of the tumour and patients' age might be able to further improve the model, though correlation between risk factors may exist. A substantial number of patients will be needed to develop an accurate model with such a multivariable analysis.

A model that better discriminates between high and low risk patients could result in redefining the high, intermediate and low risk groups. Based on the risk-adapted treatment policies shown in Table 4.4, chemotherapy might be reserved for patients at high risk and those with metastases at RPLND. Patients at intermediate risk might undergo RPLND with adjuvant chemotherapy if metastases are identified, and patients at low risk might go on surveillance. For patients at very low risk a simplified surveillance protocol might be followed.[53] For instance, CT scans may be performed only twice during the first year and than once a year during the following 2 years.

A limitation of the presented meta-analysis is that we combined studies with different definitions for the outcome variable 'occult metastasis' (relapse after surveillance and pathological stage II after RPLND). Moreover, we used the OR as measure of association

instead of the statistically more appropriate hazard ratio. Our rationale was that only few occult metastases would have been missed in the surveillance studies, since the follow-up time was often long enough to identify occult metastasis (Figure 4.1). Further, heterogeneity of effect in relation to the definition of occult metastasis was only found for the risk factor pathological stage of the tumour. These results suggest that for all risk factors but the pathological stage of the tumour the effect sizes were similar for the two outcome definitions.

In conclusion, present models can define a high risk group with around 50% risk of occult metastasis and low risk groups with around 16% risk. A model with more predictors, that also considers continuous variables (percentage of embryonal carcinoma and MIB-1), may be able to identify patients at higher and lower risks. Such an evidence-based model can then be the basis for improved risk-adapted treatment policies.

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