Table 3. BM infiltration in Ewing sarcoma at the time of diagnosis - results of different groups

Source	Patients		Localized sarcoma		Metastatic sarcoma	
	No+/total	% positive	No+/total	% positive	No+/total	% positive
Fagnou 1998	14/43	33%	6/28	21%	8/15	53%
Zoubek 1998	16/35	46%	7/23	30%	9/12	75%
Pfleiderer 1995	6/16	38%	1/9	11%	5/7	71%
West 1997*	5/22	23%	3/16	19%	2/6	33%
Athale 2001**	7/26	27%	0/11	0%	7/15	47%
Our results	8/22	36%	5/16	31%	3/6	50%

* t(21;22) not evaluated, ** including rhabdomyosarcomas and desmoplastic small-round-cell tumours

with non-metastatic disease were RT-PCR positive for the marker mRNA in BM, a result in consent with observations (approx. 20%–30%) reported by others (Pfleiderer et al., 1995; West et al., 1997; Fagnou et al., 1998; Zoubek et al., 1998; Athale et al., 2001). Table 3 gives a summary of results published by different investigating groups to date.

Six patients in our study presented distant metastases, three in the lungs solely, one in the lymph nodes and two had lung and multiple bone metastases. We found minimal BM infiltration in only three of six patients with metastatic disease. The tumour tissue sample was available in five cases with advanced disease. In a 15-year-old girl (case 13) with systemically relapsed soft tissue ESFT arising in her neck, we were unable to confirm neither t(11;22), nor t(21;22) in the primary tumour. This particular patient should therefore be excluded from our final analysis. The failure to detect EWS/FLI-1 or EWS/ERG mRNA in this case can be explained in several ways. It may contain another rare alternative EWS/ETS rearrangement not tested in our study – (t(7;22), t(17;22) or t(2;22)), or previous chemotherapy and local radiotherapy led to neural differentiation and absence of detectable *EWS/ETS* gene expression (West et al., 1997; Knezevich et al., 1998). In the four remaining cases, two had *EWS/ERG* rearrangements documented in the tumour tissue, and two patients had multiple bone metastases without BM involvement in light microscopy evaluation. The presence of tumour cells with t(21;22) in BM had been documented in one case with RT-PCR. The BM of the second patient (case 19) was RT-PCR negative. This finding is in contrast to the results published by Zoubek et al. (1998), who reported RT-PCR positivity in BM for all five patients with bone metastases and for 50% of patients with lung metastases. West et al. (1997) found two of six patients with metastatic disease positive in BM and 5 of 10 positive in peripheral blood, which he interpreted as 50% presence of micrometastases in their group of patients with advanced disease. Unfortunately, no information on metastatic sites was given, so that comparison between the results is impossible like in other groups.

Despite the given findings, it should be noted that there are many factors which can affect the RT-PCR analysis and the correct interpretation of results. Possible factors affecting analysis are: sampling errors due to inappropriate anticoagulants used, excessive BM dilution by blood, under-sampling due to the focal BM involvement in ESFT, and others (Kovar, 1998).

During every evaluation, strict precautions were taken to avoid cross-contamination, pre- and post-amplification steps were separated from each other, negative and positive controls were included in reaction steps and all positivities were reproducible. Moreover, amplified products corresponded to those resulting from tumour tissue if available.

In five of eight positive cases, we detected tumour cells only in some samples taken at the same time from different sites (one out of two samples four times and two out of three samples in one patient). This demonstrates the importance of collecting several BM samples for more precise staging and better MRD detection.

Our results confirmed that more than 1/4 of the patients with presumed localized ESFT have minimal BM infiltration. The presence of a low number of cancer cells in BM, detected by sensitive molecular biology techniques including RT-PCR, is not a reason for applying more intensive therapy at the present time (in patients with localized disease), and the clinical significance of minimal BM infiltration at the time of disease diagnosis is unknown. In the future, some new therapeutic protocols might be designed with targeted therapy for patients with proved minimal BM infiltration.

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