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Review

A systematic review of molecular and biological markers in tumours of the Ewing's sarcoma family

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Abstract

The aims of this study were to perform the first systematic review of molecular and biological tumour markers in tumours of the Ewing's sarcoma family (ESFT), and evaluate the current evidence for their clinical use. A well-defined, reproducible search strategy was used to identify the relevant literature from 1966 to February 2000. Papers were independently assessed for tumour markers used in the screening, diagnosis, prognosis or monitoring of patients with ESFT. Eighty-four papers studying the use of 70 different tumour markers in ESFT's were identified. Low-quality, inconsistent reporting limited meta-analysis to that of prognostic data for 28 markers. Patients with tumours lacking S-100 protein expression have a better overall survival (OS) (hazard ratio (HR) = 0.41, 95% confidence interval (CI) 0.19, 0.89) than those with expression; patients with high levels of serum LDH had a worse OS and disease-free survival (DFS) (OS: HR = 2.92, CI 2.16, 3.94, DFS: HR = 3.38, 95% CI 2.28, 4.99); patients with localised disease and tumours expressing type 1 *EWS-FLI1* fusion transcripts had an improved DFS compared with those with other fusion transcript types (HR = 0.17, 95% CI 0.079, 0.37). The knowledge base formed should facilitate more informative future research. Improved statistical reporting and large, multicentre prospective studies are advocated.

Keywords: Ewing's Sarcoma; Tumour marker; Systematic review; Prognosis; Meta-analysis

1. Introduction

Tumours of the Ewing's sarcoma family (ESFT), which include Ewing's sarcoma of the bone, extraosseous Ewing's, peripheral primitive neuroectodermal tumours and Askin's tumour, are poorly differentiated small round cell tumours. They are most frequently found in adolescents and young adults between the ages of 10 and 20 years. The incidence of Ewing's sarcoma in the United Kingdom is 13 per million 0–24 year olds per year [1].

ESFT are histologically similar to other small round cell tumours, but they have distinct clinical behaviour and therefore require different therapeutic management. Consequently, accurate diagnosis of ESFT is essential, often requiring light and electron microscopy, and immunocytochemistry [2]. More than 90% of all ESFT exhibit specific chromosomal rearrangements between the *EWS* gene on chromosome 22 and various members of the *ETS* gene family [3–5]. These specific *EWS-ETS* gene rearrangements are considered a diagnostic feature of these tumours, and their gene products are believed to play an important role in ESFT development and biology [6].

Improved radiology and the advent of molecular markers have redefined the process of diagnosis for ESFT. Successive clinical trials suggest that dose intensification of chemotherapy regimens may improve survival [7], and the use of high-dose therapy is now formally being evaluated. Surgical advances are reflected

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in its active use, associated with selective use of radiotherapy, for local control of disease. While the benefits of modern multimodality therapy have yet to be fully realised, the consequence of treatment intensification on previously defined prognostic factors and on tumour markers also needs to be evaluated; however, this is outside the scope of this review.

At the time of diagnosis, a number of clinical features have been shown to correlate with a poor prognosis in patients with ESFT, these include large tumour volume (usually greater than 100 ml), primary pelvic tumours and the presence of metastatic disease. Response to therapy has been reported as being of prognostic value, but treatment intensification may make this less important and also change the significance of specific tumour volumes [8]. Evaluation of tumour status will occur at the time of diagnosis and subsequently to assess treatment response or for follow-up. Initial findings or changes in radiology or tumour marker status generate the need for decision-making and clinicians will seek information from multiple sources to aid the process. Whilst at times straightforward, decisions are not always clear and single factors may be important. It is in this context that tumour markers should be viewed.

The aim of this study was to conduct a systematic review of molecular and biological tumour markers described in ESFT, and to establish an evidence-based perspective on their predictive clinical power. A systematic review is the preferred means of identifying and combining existing evidence [9]. The review is systematic, and therefore reproducible, because it uses explicit and rigorous methods to identify, critically appraise, include and synthesise relevant studies. It is a particularly important tool when assessing information across small studies inevitable in rare conditions such as ESFTs. The statistical component of the systematic review is meta-analysis, which seeks to combine all the relevant results found from the literature search in a quantitative way, to produce results more precise than is possible with the individual studies [10].

2. Patients and methods

The systematic review followed the guidelines contained in the National Health Service (NHS) Centre for Reviews and Dissemination (1996) and had an overall philosophy to maintain breadth, synthesise the evidence qualitatively and then, only where appropriate, use quantitative methods [11].

2.1. Search strategy

Three on-line bibliographic databases of Medline, Embase and Cancerlit were chosen as a basis for identifying the relevant literature from 1966 to February 2000. The search strategy was required to obtain all the relevant literature whilst minimising the number of false-positives. An iterative procedure was used which culminated in three important sets of keywords in the strategy (Table 1). A paper was included if a word from {Ewing's Sarcoma} AND a word from {Tumour Marker} AND a word from {Clinical Area} were included anywhere in the online keywords, titles, or abstracts of the database.

The keywords in {Ewing's Sarcoma} related to the family of this disease, whereas those in {Tumour Marker} included the named markers thought *a priori* to be potentially important. The set {Clinical Area} included more specific terms for the clinical use of markers in children. The search was performed firstly in Medline, then Embase and finally Cancerlit with any duplicates being eliminated.

Two investigators independently performed the assessment of the papers. The first person read the available abstract to classify each paper; the second person, who had more background knowledge in the research area, checked the abstracts of all the accepted papers, all those initially classified as unclear and 10% of those rejected for relevance (Fig. 1).

2.2. Inclusion

For inclusion, a paper had to provide a quantitative result or give tabulated individual patient data (IPD) evaluating the use of a tumour marker in ESFT. The report had to be based on new data from humans relevant to the clinical area of screening, diagnosis, prognosis or monitoring. There was no restriction on foreign language papers or on the age of patients in the study.

The criteria for classifying the four clinical areas was that the paper had to present data in the form of summary statistics or IPD for:

- *Screening*—the use of tumour markers to screen an apparent healthy population.
- *Diagnosis*—tumour marker levels at diagnosis.
- *Prognosis*—tumour marker levels at a measured point in time with relation to the outcome of patients at the end of a specific follow-up period.
- *Monitoring*—tumour marker levels taken repeatedly during a follow-up period with relation to disease status over that period.

2.3. Exclusion

Papers that reported only laboratory work, methodology for identifying new markers or results from animal studies were excluded. Furthermore, if multiple

Table 1
Sets of keywords used in the literature search of Medline, Embase and Cancerlit

{Ewing's Sarcoma}	{Tumour Marker}	{Clinical Area}
Ewing's sarcoma	Tumour marker(s)	Patient(s)
Ewing sarcoma	Tumor marker(s)	Child
Ewings sarcoma	Marker(s)	Children
Ewing	Lactate dehydrogenase	Prognosis
Ewings	LDH	Diagnosis
Ewing's	Neuron-specific enolase	Monitoring
Askin tumour	NSE	Follow-up
Peripheral neuroectodermal tumour (PNET)	PAS	Prognostic
Primitive peripheral neuroectodermal (PPNET)	C-myc	Diagnostic
	Cytokeratin	Pediatric
	HNK-1	Paediatric
	Beta2-integrin-linked	Screening
	protein kinase	Infant(s)
	MIC-2	
	Mitotic index	
	RT-PCR	
	Translocation	
	Plasma viscocity	
	ESR	
	EWS	
	EWS-ERG	
	EWS-FLI1	
	EWS-ETS	
	Neuronal differentiation	

RT-PCR, reverse transcriptase-polymerase chain reaction. NSE, neuron-specific enolase. N.B. The terms t(11;22)(q24;q12) and t(21;22)(q22;q12) were also used in {tumour marker}, but these terms were not in an appropriate format to generate searches in these databases.

papers were written on the same or overlapping datasets then only one of these papers was included, that based on the largest number of patients, the most detailed results and the longest follow-up time. Review articles were also excluded.

2.4. Appraisal of the papers identified, data extraction and meta-analysis

Copies of the accepted papers together with those of which the relevance remained unclear after assessment by the two investigators were obtained and then read thoroughly to make a final decision as to their inclusion. Any papers rejected at this stage were independently checked by two further investigators. From the accepted papers, information was extracted on the tumour marker used, clinical area (i.e. screening, diagnosis, prognosis or monitoring), the sampling method, whether survival was overall (OS) or disease-free (DFS), and, if applicable, the marker cut-off level together with the total number of patients and deaths within each cut-off group.

Meta-analysis was performed, where possible, in order to explain between-subject heterogeneity [10], but for each clinical area only those tumour markers on which three or more papers provided data were considered. Fixed effects meta-analysis was used unless there was significant evidence of heterogeneity across studies (i.e. the Q-statistic was statistically significant at the 10% level), in which case random effects meta-analysis was used [10]. For the meta-analysis of the prognosis papers, extraction of the $\log_e$(hazard ratio) and its variance was sought from each paper by one of three methods [12]:

(i) Using the direct estimates of these quantities given.
(ii) Calculating indirect estimates using summary information available within the paper itself.
(iii) Using the IPD to calculate estimates from a Cox proportional hazards model [13,14].

A meta-regression was also performed where appropriate, to estimate the effect of the cut-off point chosen on the hazard ratio [10].

If there were sufficient data to perform a meta-analysis, then the references of relevant papers were checked; if this 'reference explosion' highlighted new papers, these were obtained and assessed as above.

3. Results

From the searches, 1089 papers were identified; 781 were first identified in Medline, then an additional 273 in Embase and then a further 35 from Cancerlit. These were then classified by the two investigators. The second investigator agreed that 75% of the first investigator's 'relevant' papers were indeed relevant or uncertain (80

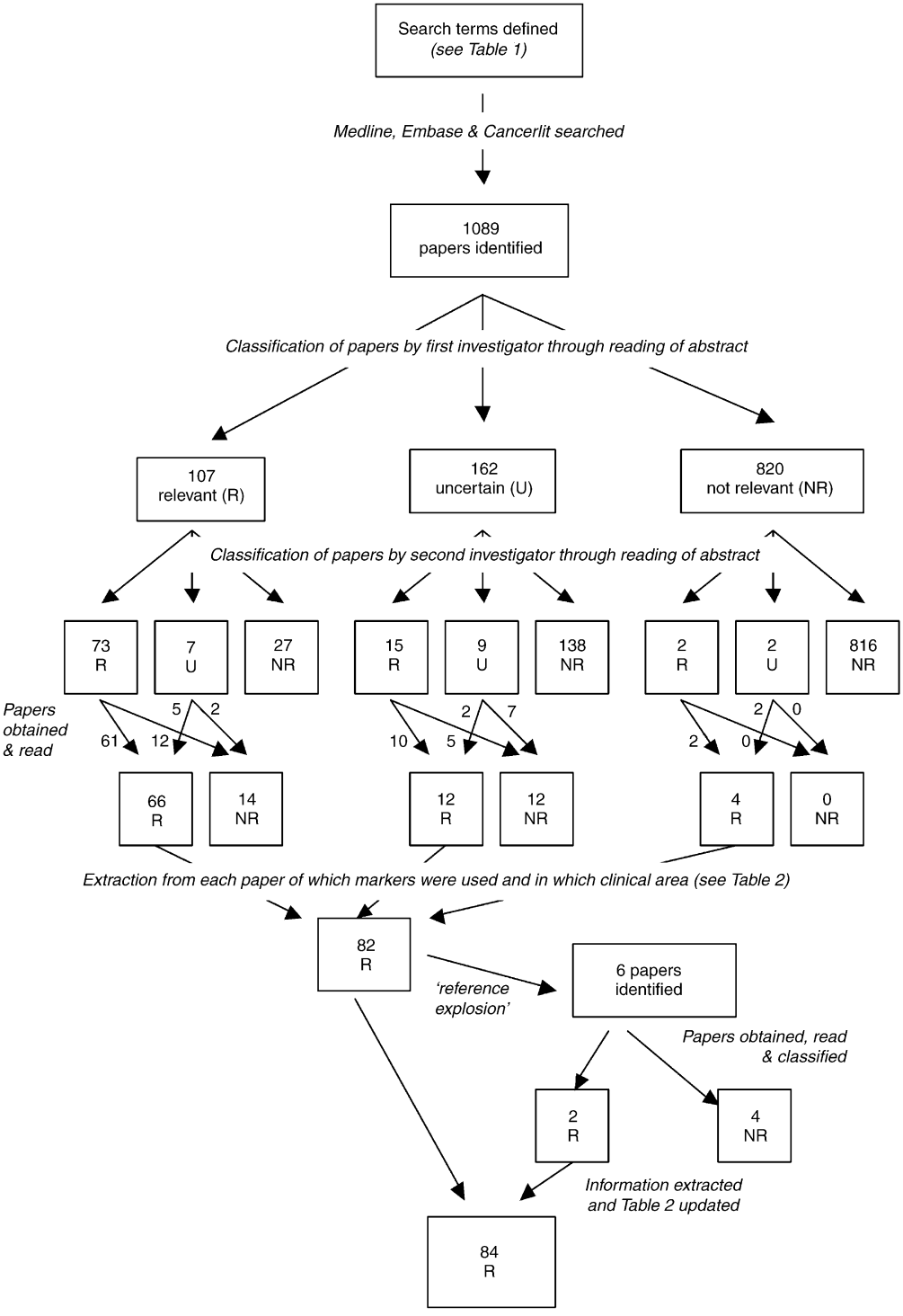

Fig. 1. Flow chart showing the strategy of the systematic review to obtain a final set of relevant papers. (A paper was classified as 'relevant' if it provided a quantitative result or gave tabulated IPD, from humans about a tumour marker in ESFT in relation to the clinical area of screening, diagnosis, prognosis or monitoring.) The results at each stage of the process are also shown. R, relevant; NR, not relevant; U, unclear; IPD, individual patient data.

out of 107), and agreed that 78 of 82 (95%) 'not relevant' papers were indeed not relevant (Fig. 1). The classification produced 82 papers of relevance, but a 'reference explosion' of important prognostic marker papers highlighted six more articles of which two were relevant (Fig. 1). This gave a final total of 84 relevant papers (Further reading references).

A total of 70 different tumour markers were studied in these 84 papers in relation to the diagnosis, prognosis or monitoring of ESFT, but no marker was used for

screening purposes (Table 2). There were 84 different papers on diagnosis, 45 on prognosis and five on monitoring; 46 of the 84 papers covered two or more clinical areas. Markers in each clinical area were investigated further if they were reported in three or more papers in a specific area. However, it was not possible to perform a meta-analysis of the data from the diagnostic papers because the results (at best) compared a quoted proportion of patients with high/low or positive/negative marker levels. Marker levels in serum or tumours from patients with ESFT were not compared with those in a sample of serum or tissue from healthy controls in any of the 84 diagnosis papers. In addition, no meta-analysis was carried out using the five monitoring papers because none of the nine markers investigated within were studied across three or more of the papers.

More consistent and detailed information was available from the prognostic papers; of the markers studied further in this area, lactate dehydrogenase (LDH), neuron-specific enolase (NSE), S-100 protein, cytokeratin, Leu-7/HNK-1/CD57, MIC-2/HBA71/CD99/12E7, EWS-FLI1/t(11;22)(q24;q12) and EWS-ERG/t(21;22)(q22;q12) provided sufficient data to perform a meta-analysis. In the papers from which it was possible to obtain the $\log_e$(hazard ratio) and its variance, the sampling method of marker levels proved to be consistent within each tumour marker. Only LDH values were measured in serum from patients with ESFT; all other markers were detected in the tumour.

Individual and overall pooled estimates of the hazard ratio (HR) are shown for all the markers in Fig. 2. Serum LDH was associated with both OS and DFS. Patients with high levels of serum LDH had an increased risk of death approximately 2.9 times greater than for those with low values (Hazard Ratio (HR) = 2.92, Confidence Interval (CI) 2.16, 3.94, $P < 0.0001$) (Fig. 2). Furthermore, patients with high LDH levels had an approximately 3.4 times greater risk of disease recurrence (HR = 3.38, 95% CI 2.28, 4.99, $P < 0.0001$). When DFS and OS results were pooled, assuming that if a patient had a recurrence then he/she would die soon after that [15], there was a statistically significant, approximately 3.2 times, increased risk of

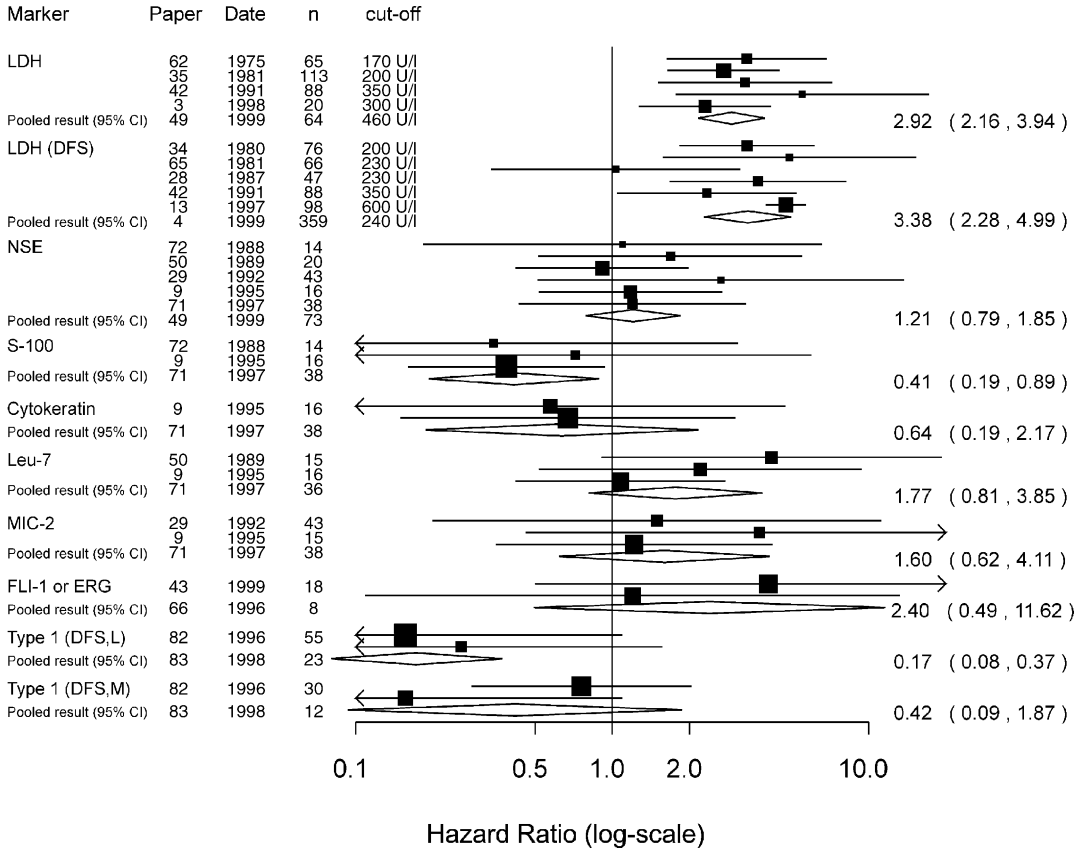

Fig. 2. Forest plot showing individual and pooled hazard ratios (HR) (A HR >1 indicates a greater instantaneous risk of death/disease recurrence for patients with high/positive marker levels or those with presence of the marker) with 95% confidence intervals (CI) for each tumour marker, with details from the primary papers (References A) of date of publication (date), number of patients (*n*) and cut-off used. The results are for OS unless DFS is stated; all disease types unless localised (L) or metastatic (M) is shown; and the area of each block is proportional to the precision of the hazard ratio. *FLI-1* or *ERG* = *EWS-FLI1* or *EWS-ERG* gene rearrangements; Type 1 = *EWS-FLI1* type 1. DFS, disease-free survival; OS, overall survival.

Table 2
Tumour markers identified by the systematic review, number of papers overall and within each clinical area[a]

Tumour marker	Total papers	Diagnosis	Prognosis	Monitoring
EWS-FLI1 or t(11;22) (or Ch11 or Ch22 relating specifically to FLI1 or t1122)	35	35	13	2
Neuron-specific enolase (NSE)	22	22	12	0
MIC-2, CD99, HBA71 or 12E7	18	18	5	0
EWS-ERG or t(21;22) (or Ch21 or Ch22 relating specifically to ERG or t2122)	16	16	8	2
Lactate dehydrogenase (LDH)	15	14	15	2
Desmin	10	10	3	0
Leucocyte or CD45	10	10	3	1
S-100 protein	10	10	4	0
Vimentin	10	10	3	0
Leu-7, HNK1 or CD57	9	9	6	0
Chromosome 8	8	8	5	0
Neurofilament	8	8	1	0
Periodic acid-schiff (PAS)	8	8	3	0
Chromosome 12	7	7	5	0
Chromosome 1 or 1q	6	6	5	0
Chromosome 2	6	6	4	0
Chromosome 3	6	6	4	0
Cytokeratin	6	6	3	0
Chromosome 21	5	5	4	0
Chromosome 16	5	5	4	0
Chromosome 18	5	5	4	0
Chromosome 7	5	5	3	0
Synaptophysin	5	5	2	0
Chromosome 10	4	4	2	0
Chromosome 14	4	4	3	0
Chromosome 17	4	4	3	0
Chromosome 20	4	4	3	0
Chromosome 4	4	4	3	0
Chromosome 5	4	4	3	0
Chromosome 6	4	4	3	0
Chromosome 9	4	4	3	0
Chromosome 13	3	3	2	0
Chromosome 15	3	3	2	0
Chromosome 19	3	3	2	0
Actin	2	2	1	0
Alkaline phosphatase	2	2	1	1
Beta actin	2	2	0	0
Chromogranin, chromogranin A or B	2	2	1	0
C-myc	2	2	1	0
Glial fibrillary acidic protein	2	2	1	0
MDM2	2	2	1	0
Muscle-specific antigen	2	2	1	0
Neural-cell adhesion molecule	2	2	1	0
PGP9.5	2	2	0	0

A further 26 markers were identified in only 1 paper which reported their use in one or more of the clinical areas—see http://www.prw.le.ac.uk/epidemio/personal/rdr3/paed.html for further details of these results.

[a] Markers have been delineated into either immunohistochemical or **cytogenetic/molecular**. The only papers describing secreted markers in ESFTs were on serum *lactate dehydrogenase*.

death for those patients with high serum LDH compared with those with low values (HR = 3.21, 95% CI 2.43, 4.26, $P < 0.0001$).

In the above analyses, the definition of high or low serum LDH varied considerably from paper to paper (Fig. 2). Plotting $\log_e$(hazard ratio) against the cut-off point weakly suggested that a higher hazard ratio was obtained when the cut-off point was between 200–350 U/l. However, a meta-regression including the cut-off point as a covariate was not significant (estimate for cut-off = −0.0010, 95% CI −0.0031, 0.0010, $P = 0.32$).

There was also significant evidence that patients with tumours that lack S-100 protein have an approximate 59% reduced risk of death compared with those that do express it (HR = 0.41, 95% CI 0.19, 0.89, $P = 0.024$). However, there was no statistical evidence that expression of NSE (HR = 1.21, 95% CI 0.79, 1.85), cytokeratin (HR = 0.64, 95% CI 0.19,

2.17), Leu-7/HNK-1/CD57 (HR = 1.77, 95% CI 0.81, 3.85)) or MIC-2/HBA71/CD99/12E7 (HR = 1.60, 95% CI 0.62, 4.11) were associated with OS. IPD for both NSE and S-100 was given in three papers [16–18]. However, when these datasets were pooled, a Cox model showed that expression of NSE in the absence of S-100 protein was not associated with OS (HR = 0.97, 95% CI 0.23, 4.11).

Individual estimates of the hazard ratio were sought from those papers looking at the prognostic impact of EWS-FLI1/t(11;22)(q24;q12) and EWS-ERG/t(21;22)(q22;q12), to assess the difference in survival for individuals with tumours containing these gene rearrangements compared with those without. Of the 14 papers studied, only two provided sufficient information for a meta-analysis [19,20]. There was evidence that the presence of the EWS-FLI1/t(11;22)(q24;q12) or EWS-ERG/t(21;22)(q22;q12) was associated with a worse OS (HR = 2.40, 95% CI 0.49–11.62]), however this was not statistically significant which may in part be a sign of the small sample size, reflected in the wide confidence intervals (Fig. 2). Of the papers studying EWS-FLI1/t(11;22)(q24;q12), two reported DFS for patients with type 1 EWS-FLI1 fusion transcripts compared with other types [21,22]. For localised disease, there was evidence that patients with tumours that expressed a type 1 EWS-FLI fusion transcript had an improved DFS than those with other types (HR = 0.17, 95% CI 0.08–0.37); for patients with metastatic disease at diagnosis, there was no statistically significant evidence of an association (HR = 0.42, 95% CI 0.09–1.87) (Fig. 2). When this DFS data was combined with that from a paper reporting OS [23], the presence of EWS-FLI1 type 1 was still statistically significantly associated with an improved outcome in patients with localised, but not metastatic disease (localised: HR = 0.22, 95% CI 0.12, 0.4; metastatic: HR = 0.51, 95% CI 0.24, 1.09). Data on 15 chromosomes other than those relating to *EWS-ETS* gene rearrangements was reported in three or more papers, although this was in the form of IPD and is not presented here.

4. Discussion

This is the first systematic review of tumour markers that has been undertaken in ESFT and forms a knowledge base, pooling information from different studies to obtain overall measures of potential clinical value. We identified 84 papers, which showed diversity in primary interest, methodology, analysis of data and quality of reporting. Seventy different tumour markers were studied, reflecting the continual search for clinically useful markers in ESFTs. This novel review should facilitate the development of future research strategies and improved scientific reporting.

During the systematic review we classified 1095 papers overall. The search strategy used is likely to have identified the majority of the available literature, targeting in particular the databases specialising in scientific and clinical reporting. We acknowledge the review may not be fully comprehensive, although we have used 'reference explosion' to increase the power of our assessment. Although we did not evaluate the references from all the 84 relevant papers, we studied those references from the 23 papers that contributed data to the meta-analyses and so are confident we will have collated the majority of available data for the markers analysed. We also recognise that there may be the common problem of publication bias, in particular results that do not generate formal statistically significant or clinically valuable findings may not be in the public literature. However, the hazard ratios for NSE, S-100, cytokeratin, MIC-2 and Leu-7 were not statistically significant in 16 out of 17 primary studies included, suggesting this may not be a major problem.

This systematic review has evaluated tumour markers in ESFT for which there is currently a literature database, with the primary aim to establish the most important prognostic markers for clinical practice. Of concern is how difficult it has been to draw clinically relevant conclusions from a large number of the studies reviewed, and indeed the results obtained from the meta-analyses. Small sample sizes and poorly reported summary statistics were common problems, and across studies there was heterogeneity of many important clinical factors, such as the age of patients, initial stage of disease and type of treatment received. Such problems prevent us from making clear recommendations for clinical practice. For example, although the majority of patients were less than 18 years, most studies included a few adult patients, making clinical application of the results difficult.

The most frequently researched marker was serum LDH measured at diagnosis. LDH is a cytoplasmic cellular enzyme present in all major organ systems and its presence in the extracellular space may reflect disturbance of cellular integrity induced by pathological conditions. Serum LDH activity is abnormal in a large number of disorders such as myocardial infarction and haemolytic anaemia and is also used to monitor other malignancies including ovarian dysgerminoma, testicular germ cell tumour, Hodgkin's disease and non-Hodgkin's lymphoma [24]. Despite the lack of specificity, LDH is clearly of prognostic value in selected patient populations. It is also the best-studied tumour marker in ESFT and, consequently, forms a baseline to which new, more specific markers could usefully be compared. However, uncertainty on the optimal clinically significant cut-off value for serum LDH was reflected in the range of values used across the results extracted (170–600 U/l), which may explain in part why

this marker is not routinely used in ESFTs. For the meta-analysis of LDH data, the cut-off point was taken as constant across the studies, which was reasonable as the choice of cut-off did not appear to be associated with the hazard ratio. Availability of full IPD containing exact marker levels would facilitate using a common cut-off point [25], an approach that has proved beneficial elsewhere [26].

The presence of *EWS-ETS* gene rearrangements is increasingly used to define ESFTs, although a small proportion of this pathologically defined tumour group do not express these gene rearrangements. Whether these tumours contain novel *EWS* gene re-arrangements yet to be defined, or represent a sub-set of tumours that do not contain such rearrangements is a critical question. At the molecular level, *EWS-FLI1*/t(11;22)(q24;q12) rearrangements show great diversity, different combinations of exons from *EWS* and *FLI1* encoding in-frame fusion transcripts that may have functional significance. The presence of *EWS-FLI1* type 1 fusion transcripts (reflecting translocation of *EWS* exon 6 and *FLI1* exon 7) in patients with localised disease appears to be prognostic for improved DFS [21,22] and OS compared with patients with tumours containing other *EWS-ETS* gene rearrangements [23]. Further studies to evaluate the clinical significance of different fusion transcript types are being carried out as part of the current pan-European, multicentre prospective clinical outcome study European Ewing tumour Working Initiative of National Groups 99 (EuroE.W.I.N.G. 99). In addition, diagnostic values of serum LDH will be collected; however, this is not a main objective of the study emphasising the uncertainty as to which prognostic makers are important for ESFTs. From the results of our systematic review, we recommend that future studies include measurement of serum LDH, both at diagnosis and follow-up, so that its independent prognostic significance can be evaluated and compared with newer markers. Only in this way can firm recommendations be made for clinical use.

The clinical implications of the results must also be considered together with psychosocial and economic aspects of tumour markers. Our search found no published evidence on either the psychosocial consequences for children and their families of using tumour markers clinically in ESFT, or the economic evaluation of tumour markers. This reflects the obvious gap in the literature for the use of tumour markers to monitor patients with ESFT. Given the evidence pointing to the psychological vulnerability of patients who survive bone tumours [30] and the potential for regular follow-up monitoring of tumour markers to generate anxiety, future research on the use of tumour markers for monitoring in paediatric oncology should also include an assessment of the influence of both psychosocial and economic outcomes. We also recommend work to investigate how the use of markers should be best communicated to children with ESFT and their families.

The present drive to improve the long-term prognosis allied with the advances in molecular understanding emphasise the need for large, multicentre trials to critically evaluate both more established markers and new molecular markers. In particular, this needs to be carried out in relation to modern therapeutic treatment strategies, so that the results of such studies can be implemented in clinical practice, for example to enable the stratification of patients for the most appropriate therapy or the identification of patients for whom current therapy would not be beneficial. While large multicentre studies are complex to organise and run, smaller studies may overcome some of their limitations by better study design, collaboration of research groups and clearer reporting of tumour marker results. In particular, for those researchers studying prognostic tumour markers, Altman and Lyman have proposed important guidelines for both conducting and evaluating prognostic marker studies that should be considered [27]. These include criteria for the purpose and design of prognostic marker studies, together with general recommendations for analysing and reporting results.

Weakness of reporting, analysis and presentation of results was frequently apparent throughout the evaluation of the 84 selected papers. The presentation of survival analyses was particularly poor, emphasising the problems addressed in the recommendations of Altman and colleagues [28]. For example, for the purposes of the meta-analyses, 132 attempts were made to obtain estimates of the hazard ratio and its variance from the data/results provided, but only 83 of these proved successful. Furthermore, only six of these 83 hazard ratios were provided directly in a paper, 10 had to be calculated indirectly and the remaining 67 were calculated using the raw individual patient data available. The hazard ratio and its confidence interval provide an important estimate of the difference in risk of death/disease recurrence between two groups of patients, but this was often not acknowledged in the selected papers, which often quoted only an inexact P value. It is clearly important that the quality of statistical reporting improves if clear conclusions are to be formed about tumour markers. Results for all tumour markers considered, even those found not to be (statistically) significant, should be presented. We recommend that the hazard ratio (or $\log_e$(hazard ratio)), its standard error and 95% confidence interval always be reported when comparing the time to death or disease recurrence between groups of patients (defined explicitly by a specific marker level or status), together with the group sizes and the number of events in each patient group. If just P values are presented their exact values should be given.

Presentation of full IPD, either in the paper or on the Internet, is also highly desirable and would greatly assist

those undertaking meta-analysis and evidence-based evaluations [25,29]. Where possible, the IPD should include all exact initial marker levels, time of any disease recurrence, overall follow-up time and final disease status so that the hazard ratio, or other statistics, may be calculated if required. Presentation of other patient information, such as age and type of treatment received, is also highly desirable to facilitate the study of markers in subgroups of patients. Availability of IPD would also allow a better evaluation of combinations of markers, which may enable more specific and accurate prognostic assessments.

This systematic review emphasises the uncertainty on the clinical utility of many of the studied tumour markers in ESFT, reflecting the small size of many studies and poor statistical reporting. This underlines the need for large, multicentre quality controlled studies which would enable the potential of individual markers in prognosis, monitoring and possibly diagnosis to be evaluated. The value of markers both individually and in combination should be prospectively evaluated. Comparison of marker levels in serum from patients with ESFT with those in a healthy population is critical. Psychosocial and economic issues should also be measured.

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