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A systematic review and evaluation of the use of tumour markers in paediatric oncology: Ewing's sarcoma and neuroblastoma

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Contents

List of abbreviations

ABMT	autologous bone marrow transplant
CI	confidence interval
DFS	disease-free survival
ESFT	Ewing's sarcoma family of tumours
HR	hazard ratio
HVA	homovanillic acid
INSS	International Neuroblastoma Staging System
IPD	individual patient data
LDH	lactate dehydrogenase
NSE	neurone-specific enolase
OS	overall survival
PCR	polymerase chain reaction
pPNET	peripheral primitive neuroectodermal tumour
SE	standard error
VMA	vanillylmandelic acid

All abbreviations that have been used in this report are listed here unless the abbreviation is well known (e.g. NHS), or it has been used only once, or it is a non-standard abbreviation used only in figures/tables/appendices in which case the abbreviation is defined in the figure legend or at the end of the table.

Executive summary

Objectives

- To perform the first systematic review of studies of tumour markers in the Ewing's sarcoma family of tumours (ESFT) and in neuroblastomas in order to identify measures of potential clinical value for the clinical areas of screening, diagnosis, prognosis and monitoring; the review focuses particularly on the role of markers for defining prognosis.
- To facilitate the development of future research strategies, including improvement of the standard of scientific reporting and specification of deficiencies in the literature.

Methods

The databases MEDLINE, EMBASE and CANCERLIT were searched iteratively to identify the relevant literature from 1966 to February 2000. Sets of keywords relating to **tumour markers**, **ESFT** or **neuroblastoma**, and **clinical use** were developed; papers were identified if they contained a word from each of these sets.

To be included, papers had to provide a quantitative result or tabulated individual patient data (IPD) evaluating the use of a tumour marker in ESFT or neuroblastomas, based on primary research data from humans relevant to screening, diagnosis, prognosis or monitoring. Review articles and those reporting only laboratory work, methodologies for identifying new markers, or results from animal studies were thus excluded. Histological characteristics of tumours were not included in the markers reviewed.

From papers classified as 'relevant', information was extracted on the tumour marker used, the clinical area of application, the age range of patients, stage of disease, whether the outcome was overall survival (OS) or disease-free survival (DFS), and the cut-off level of the marker.

Meta-analysis was performed, where possible, for those tumour markers on which three or more papers provided data. For the meta-analysis of prognostic data, estimates of the natural log of the hazard ratio ($\log_e$(HR)) and its variance were sought. Where direct estimates were not reported, indirect estimation or IPD were used to obtain an unadjusted, or if necessary, an adjusted estimate.

The 'relevant' papers were also screened for any results from economic or psychosocial evaluations of the clinical use of tumour markers in ESFT or neuroblastomas.

Results

Tumours of the Ewing's sarcoma family

Eighty-four 'relevant' papers were identified which studied 70 different markers. Eighty-four papers related to diagnosis, 45 to prognosis and five to monitoring, but none to screening. Meta-analysis of the data from the diagnosis or monitoring papers was not possible because of the poor quality and reporting of data.

Meta-analysis of prognostic papers was possible but hindered by the extremely poor presentation of survival analyses. Of 132 attempts to obtain estimates of $\log_e$(HR) and its variance, only 83 proved successful. Only six of these 83 HRs were provided directly in a paper, ten had to be calculated indirectly and the remaining 67 were calculated using the IPD available.

High levels of serum lactate dehydrogenase and lack of S-100 protein expression in the tumour were significantly associated with a worse prognosis and an increased risk of death or disease recurrence/death. Expression of the EWS–FLI type 1 fusion transcript in tumours from patients with localised disease was associated with a more favourable outcome and reduced risk of disease recurrence/death, compared with expression of other EWS–ETS fusion transcripts. However, these results must be treated with caution given the poor reporting problems identified.

No studies reported an economic or psychosocial evaluation, which perhaps reflects the lack of certainty about which markers show enough clinical effectiveness and importance to warrant subsequent economic/psychosocial studies.

Neuroblastomas

Four hundred and twenty-eight 'relevant' papers were identified, which studied 195 different markers. The screening results demonstrated uncertainty as to whether population-based screening for neuroblastomas is clinically effective and cost-effective, and, if so, what is the optimal age at which to screen, and also the optimal screening strategy, that is, single stage or multi-stage. No meta-analysis of the data from the diagnosis or monitoring papers was performed because of the large degree of heterogeneity and inadequacy in reporting.

Thirteen tumour markers were studied in depth for their prognostic value. Of 575 occasions where levels of one of these markers were related to survival by summary statistics or IPD, only 204 successful estimates of $\log_e$(HR) and its variance were obtained because of inadequate, incomplete and inconsistent reporting. IPD were used to obtain 41 of these estimates.

Development of clinically meaningful results was difficult because of heterogeneity in the stage of disease, age of patients, marker cut-off level, outcome observed (OS or DFS), type of estimate (unadjusted or adjusted), and adjustment factors. Publication bias was also observed. Despite these problems, the following were found to be significantly associated with patients experiencing a worse outcome: amplification of the *MYC-N* gene; expression of diploid cells (a DNA index of 1) in the tumour; high expression of neurone-specific enolase in the tumour at diagnosis; high serum levels of lactate dehydrogenase and/or ferritin; high multidrug resistance gene-product expression in the tumour; deletion of chromosome 1p; low tumour expression of CD44 and/or TrkA; and a low urinary VMA:HVA ratio. Studies published since the start of our review indicate that chromosome 17q is an important prognostic marker, and so in retrospect we also reviewed the prognostic literature for this marker; gain of chromosome 17q was found to be associated with a worse OS and DFS.

No papers reported a psychosocial or an economic evaluation; two papers reported cost data in relation to screening but the information was of limited value. Once a tumour marker has been identified as clinically effective, the decision to use the marker in practice (e.g. for screening or monitoring) also involves the cost of its implementation and the psychological impact it has on patients; hence, it was disappointing to identify such large gaps in the literature, but this perhaps reflects the uncertainty as to which markers are indeed clinically effective.

Conclusions

Implications for clinicians

- There is currently insufficient evidence to judge the clinical role of tumour markers in the treatment of the two childhood malignancies we studied. A large number of markers have been studied in the literature but the majority of studies are so poorly designed and reported that strong clinical conclusions cannot be made from this systematic review. However, we did manage to identify markers that showed possible prognostic importance.
- For ESFT, the following were found to be **potentially** important prognostic tools and associated with a worse outcome: high levels of serum lactate dehydrogenase, lack of S-100 protein expression in the tumour, and lack of expression of the EWS–FLI type 1 fusion transcript in the tumour.
- For neuroblastomas, the following were found to be **potentially** important tools and associated with a worse outcome: amplification of the *MYC-N* gene; expression of diploid cells (a DNA index of 1) in the tumour; high expression of neurone-specific enolase in the tumour at diagnosis; high serum levels of lactate dehydrogenase and/or ferritin; high multidrug resistance gene-product expression in the tumour; gain of chromosome 17q; deletion of chromosome 1p; low tumour expression of CD44 and/or TrkA; and a low urinary VMA:HVA ratio.
- Clinical interpretation of the above findings is very difficult because of poor and heterogeneous reporting in the literature identified. The benefits of using these prognostic markers in practice needs to be properly studied in large, multicentre studies.
- The current rapid development of genetic epidemiology may quickly provide new genetic markers and genetic sequences that supersede many of the markers we have identified as important.

Implications for those conducting and reporting primary studies

- **Reporting of results needs to be improved**. Results of all the marker analyses should be presented – both significant and non-significant results – further details are described in the main report. In particular, individual patient data should be made available, including exact

initial marker level, method of measurement, time of disease recurrence, follow-up time, final disease status and treatment received for all tumour markers considered.

- **A move toward evidence-based use of tumour markers is needed**. Investigation of potentially new and clinically better markers should not be at the expense of establishing how existing markers can be most effectively used in practice. For this, **collaboration of research groups is required** to assess clinical application of markers in studies with much greater patient numbers and to achieve consistency in reporting, for example for cut-off level, outcome assessed (OS or DFS), and adjustment factors.
- **Central repositories for IPD required**. To help collate and manage IPD, central repositories are necessary for each disease area.
- **Future genetic studies to follow our guidelines of reporting and facilitate access to IPD**. With the growth in genetic epidemiology potentially leading to identification of genetic markers that could supersede the important markers currently in use, it is very important that those studies are reported properly and make available IPD. Again, central repositories are required to collate and manage such IPD.
- Large, multicentre well-controlled studies are required to assess levels of multiple markers.
- **Economic and psychosocial evaluation of markers is required**. Once a marker has been identified as clinically effective, the decision to use the marker in practice (e.g. for screening or monitoring) also involves the cost of its implementation and the psychological impact it has on patients. Hence, economic and psychosocial evaluations are necessary.

Implications for meta-analysts

- Sensitivity and multifactorial analyses are needed to explore and adjust for effects of different cut-offs, stages of disease, outcomes (OS or DFS), ages and adjustment factors.
- Results from IPD (the 'gold standard' in the reporting of data) need to be compared with those from indirect methods to assess the reliability, validity and bias of use of the latter.

Implications for those conducting future systematic reviews

- Those considering future systematic reviews of tumour markers should seek to obtain individual patient data, as this is likely to be the most productive approach.

Chapter 1
Background

Aims and objectives of the project

- To perform the first systematic review (and meta-analyses) of studies of tumour markers in the Ewing's sarcoma family of tumours (ESFT) and in neuroblastomas in order to identify measures of potential clinical value.
- To facilitate the development of future research strategies, including improvement of the standard of scientific reporting and specification of deficiencies in the literature.

Epidemiology of paediatric tumours

Ewing's sarcoma

Ewing's sarcoma was first clarified as a distinct primary bone tumour by James Ewing in various reports published between 1921 and 1939.[1–3] Tumours in the ESFT group, (incorporating Ewing's sarcoma, Askin's tumour and peripheral primitive neuroectodermal tumours (pPNETs) most frequently occur in children and young adults, with a peak incidence in the second decade of life. ESFT accounts for 10–15% of all primary malignant bone tumours, [4,5] and the mean annual incidence is estimated at 0.6 per million of the total population.[6]

This type of tumour is highly malignant, arising in bone or soft tissue, and is thought to be of primitive neural cell origin. Despite improvements in treatment and outcome, only 60% of patients with Ewing's sarcoma are long-term survivors.[7] Recent studies have shown an unfavourable outcome for patients with metastatic disease (23%) or pelvic primaries (41%); however, a number of patients with more favourable prognostic indicators develop metastatic disease and die. Ewing's sarcoma rarely occurs under the age of 5 years or over the age of 30 years.[8] Furthermore, the cancer is rare in black children in Africa[9,10] and in Chinese[11] populations. The most common symptoms of the disease are increasing persistent pain and swelling of the affected area. These symptoms may increase rapidly over a short period, or remain constant for months. Slight to moderate fever is also common, especially in patients with advanced disease, and is reported in about one-third of all patients.[12] The distribution of Ewing's sarcoma in the body of a cohort of 300 patients is given in *Figure 1*. It shows that the most commonly affected sites are the pelvis, femur, tibia and fibula, accounting for 60% of all primary sites.[13]

Neuroblastoma

Neuroblastoma is the most common extracranial solid tumour in childhood.[14,15] It arises from sympathetic nervous tissue, the most common site of origin being the adrenal medulla. This tumour is characterised by excess catecholamine secretion, and many children present with associated features of flushing, anxiety and hypertension. Approximately 60% of children with neuroblastoma present with metastatic (Stage 4) disease (*Table 1*); the outcome for this group is below 20% survival at 5 years despite current aggressive multimodality therapy.[16] The outcome for children with more localised disease varies from > 90% survival at 5 years for Stage 1 and 2 disease with surgery alone, to approximately 65% 5 year survival for Stage 3 disease using a combination of surgery, chemotherapy and radiotherapy.[16] There is a small group of young patients, < 1 year old, who appear to have disseminated disease but in whom the cancer regresses with little or no therapy, and are sometimes denoted Stage 4S (*Table 1*).

Treatment policies and options in paediatric oncology

Treatment for ESFT includes radiotherapy, surgery and chemotherapy, but success is mixed.[17] However, the combination of a safe local control with surgery and/or radiation plus effective systematic combination chemotherapy has been able to improve the disease-free survival rates from approximately 10% with only local therapy to its present 50–70%. This survival rate necessitates a recognition of patients' characteristics related to prognosis, since knowledge of these factors may have important implications for treatment stratification.[18] Such factors are sex, site of primary disease and tumour markers.

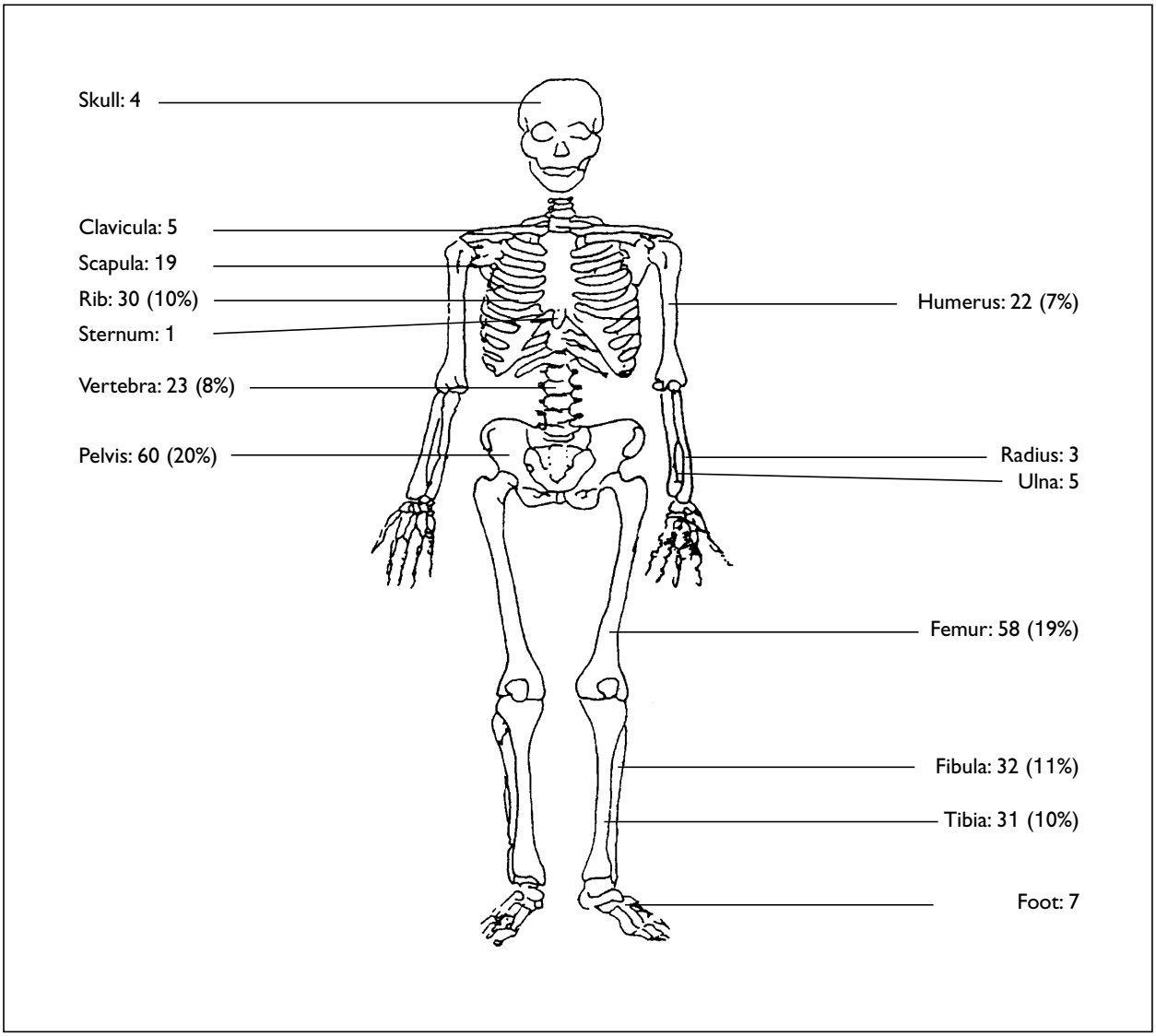


FIGURE 1 *The skeletal distribution of Ewing's sarcoma in a cohort of 300 patients*[13]

TABLE 1 *Distribution of neuroblastoma cases in UK between 1987 and 1991*[15]

Age at diagnosis (years)	< 1	1–4	5–9	10–14		
%	25.9	59.5	12.8	1.9		
Sex	Male	Female				
%	52.9	47.1				
Stage	I	II	III	IV	IVS	Unknown
%	4.5	9.9	14.2	61.5	5.1	4.7

Progress to improve the outlook for patients with neuroblastoma has been slow, with only modest gains being made. Nevertheless, the more recent advent of prognostic markers has allowed for the development of management plans based on risk assessment. This seems to provide a better means of selecting patient protocols rather than stage alone.

Low-risk patients are managed with surgery alone because excellent cure rates are achieved even when some tumour is left behind. Intermediate-risk patients are still at low risk of succumbing to disease but probably require some chemotherapy (together with surgery). The amount of chemotherapy is determined in part by the biological features.

High-risk patients include those for whom little progress has been made. Many centres have used dose-intensification regimens including the use of autologous bone marrow transplant (ABMT).

Tumour markers in oncology

It is hard to produce an all-encompassing definition of a tumour marker because it can refer to anything that distinguishes types of patient and aids clinical practice. In this project we evaluated the use of genetic or biological markers. These are measurable parameters by which a transformed cell can be differentiated from its corresponding progenitor cell,[19] and they can be detected in abnormal amounts in the blood, urine, body tissue or, indeed, the tumour itself. They include abnormal levels of proteins and catecholamines, and unusual gene aberrations or changes – basically anything measurable in the body that becomes abnormal when a tumour is present. Histological characteristics of tumours (e.g. the presence of differentiated ganglia in neuroblastoma) can also be thought of as markers, but these were not evaluated in this review.

Tumour markers are currently used in four areas in cancer generally; screening, diagnosis, prognosis and monitoring of patients.[20,21] In **screening** asymptomatic populations for cancer the use of tumour markers has been relatively limited,[22] though their use in cancer/genetic epidemiology has also been advocated.[23] In **diagnosis** the practical use of such markers is determined by their ability to identify specific cancers correctly, and various proposals for the evaluation in such settings have been advocated.[24,25] However, to date most work has involved the identification of patients who may or may not benefit from a specific treatment regimen, that is, **prognosis**/targeting of treatment. This often involves the development of algorithms possibly using a number of different markers.[26] The final area in which tumour markers are used is in the **monitoring** of patients following primary therapy. This may be either to identify recurrence of the primary tumour via specific tumour characteristics or for the detection of malignant disease more generally using systemic markers, so that evidence of disease may be obtained earlier than would otherwise be possible clinically, thus producing a lead time, which could ensure improved patient prognosis.[27]

Use of tumour markers in paediatric oncology

Whilst tumour markers are currently being used in a variety of areas within paediatric oncology this report will concentrate upon two specific tumour types – ESFT and neuroblastomas – which collectively account for approximately 20% of all paediatric cancers in the UK, and are associated with a range of 5 year survival probabilities and response rates, reflecting the overall clinical diversity of paediatric cancers. The relatively poor prognosis for these patients ensures that early diagnosis and detection of relapse is vital for improvements in patient outcome. Current tumour markers used in these tumour types include biochemical, immunoassay and polymerase chain reaction (PCR)-based methods.

The tumours of the Ewing's sarcoma family are characterised by *EWS* gene rearrangements, involving 22q12. The most frequent abnormality is t(11.22)(q24.12), found in over 85% of tumours. This translocation results in expression of a chimeric RNA product, coding for a protein that contains the amino terminal of EWS fused to the DNA-binding domain of FLI1. Cloning of the specific chromosome translocations has led to the development of RT–PCR (reverse transcription – PCR)-based assays for the detection of these fusion transcripts.[28]

Neuroblastoma is relatively unusual in terms of paediatric cancers in that there have been a number of proposals for the use of tumour markers to screen asymptomatic patients for the disease.[29–31] Use of markers in the specific diagnosis of disease have been considered,[32] but the most researched use of tumour markers has been in the development of prognostic risk groups in order to target therapy.[33–38] Use of tumour markers in the monitoring of patients following primary therapy has also been considered.[39,40]

Rationale for a systematic review of tumour markers in paediatric oncology

A persistent difficulty in conducting primary research in paediatric oncology, not just in relation to the use of tumour markers, is the relatively small number of children who develop disease, and the therefore low statistical power which individual primary studies have of detecting

either treatment benefits or survival benefits arising out of prognostic staging (using tumour markers). It is therefore appropriate that the use of systematic review methodology, and in particular meta-analysis techniques, is assessed in this setting in order to provide a means of synthesising evidence relating to the use of tumour markers from a variety of primary studies and thereby having greater statistical power.

However, in any meta-analysis heterogeneity will almost certainly be present, and the exact nature of such heterogeneity will to some extent dictate the clinical usefulness of the results obtained. Heterogeneity can be broadly thought of as being: (1) statistical, (2) clinical, or (3) structural or methodological. Statistical heterogeneity arises out of the fact that individual studies may report quantitatively different results purely because of random variation, and random effects meta-analysis methods are frequently used to allow for this. Clinical heterogeneity refers to the populations studied, and differences between studies that may be present, for example severity of disease or age. Whilst methods such as meta-regression are sometimes used to adjust for such clinical heterogeneity, this may not always be feasible. Finally, structural or methodological heterogeneity refers to differences in the actual study methodology or reporting of results/analysis used by the different primary studies, for example prospective/retrospective studies, adjusted/unadjusted results, or different cut-offs. As with clinical heterogeneity, it may be possible to use meta-regression or hierarchical modelling techniques to overcome some or all of the methodological heterogeneity, but the assumptions made are frequently more stringent. When any combination of the three types of heterogeneity outlined is present in a meta-analysis a judgement has to be made as to the clinical/policy validity that can be attached to the results of such an analysis. Even when it is felt that clinical or policy decisions cannot be based upon the results of a systematic review/meta-analysis because of the level/types of heterogeneity present, a key role of such analyses/studies to is to identify those factors which prevent such decisions from being made, and recommend strategies for overcoming them, thereby framing the future research agenda. These recommendations may be either in terms of undertaking new primary studies or the requirement for greater collaboration and pooling of individual patient-level data, or possibly both.

Chapter 2

Tumour markers in the Ewing's sarcoma family

Introduction

Tumour origin

ESFT (which include Ewing's sarcoma of the bone, extraosseous Ewing's sarcoma, pPNETs and Askin's tumour) are poorly differentiated small round cell tumours.

These are aggressive malignant tumours of children and young adults, which arise in bone and soft tissue.[41] Ewing's sarcoma is predominantly of skeletal origin, and pPNET (or neuroepithelioma) usually arises in soft tissue. In recent years it has become apparent that they share not only similar morphological and biological features but also specific chromosomal rearrangements between the *EWS* gene on chromosome 22 and various members of the ETS gene family,[41,42] and hence have been collectively called the Ewing's sarcoma family of tumours (ESFT).

Incidence and age

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 UK is 13 per million of 0–24 year olds per year, but it is rarely described in children under 5 years old and adults over 30 years of age.[43]

Clinical presentation

Ewing's sarcoma may affect any bone but the most common sites are the lower extremity (45%), followed by the pelvis (20%), the upper extremity (13%), the axial skeleton and ribs (13%) and the face (2%).[44] The femur is the most frequently affected bone, the tumour usually arising in the mid-shaft. Tumours with similar morphology may arise in soft tissue, and are designated extraosseous Ewing's sarcoma.

pPNET is the second most common soft tissue malignancy in childhood, accounting for 20% of sarcomas.[45] It shows a predilection for the chest wall (Askin's tumour), followed by paraspinal tissues, the abdominal wall, head and neck, and the extremities.[45] Soft tissue extension is common in osseous ESFT, and infiltration of adjacent bone is frequent in soft tissue ESFT, so that it can often be difficult clinically to determine the primary site of tumour origin.

Prognosis

ESFT are typically aggressive, with an overall 5-year disease-free survival of only 45–60%.[46] Approximately 30% of patients with ESFT have metastases at presentation; for these patients, overall survival is 10–20%, compared with 60% in those with localised disease.[47] Relapse is very common in ESFT, even occurring within 3 years of diagnosis in up to 50% of patients with localised disease.[48]

Pathology

ESFT are histologically similar to other small round cell tumours, but they have a distinct clinical behaviour and therefore require different therapeutic management. Consequently, accurate diagnosis of ESFT is essential, often requiring light and electron microscopy, and immunocytochemistry.

ESFT are thought to be of neural origin, largely based on the presence of limited neuronal differentiation, in the form of Homer–Wright rosettes, ganglion cells and neurofibrillary structures observed by conventional light microscopy. Neuronal differentiation is more readily detectable following immunohistochemical analysis for neuronal antigens and electron microscopy for the presence of neurosecretory granules or primitive neurites.

Tumour biology and cytogenetics

Cytogenetics

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. 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.[49]

The presence of *EWS–ETS* gene rearrangements is increasingly used to define ESFT. The

breakpoint on chromosome 22q at the location of the *EWS* gene is consistent; this can partner with a number of different *ETS* gene family members from various chromosomes, as shown in *Table 2*.

Growth factors and their receptors in ESFT

A number of different growth factors and their receptors are thought to play a role in the development and progression of ESFT. In particular, insulin-like growth factor 1 is thought to be an important growth factor for ESFT, acting as a survival through an autocrine growth loop.[50]

Additional growth factors which have been investigated and are thought to be important include stem cell factor and its receptor c-kit, tumour necrosis factor, human gastrin-releasing peptide and neurotensin.

Prognostic factors

At the time of diagnosis a number of clinical features have been shown to correlate with a poor prognosis in patients with ESFT, and 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.[47] 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-cut, and single factors may be important. It is in this context that tumour markers need to be viewed.

Treatment

Improvements in the prognosis for Ewing's sarcoma only started to occur following the introduction of effective systemic chemotherapy during the late 1960s and 1970s. It is now clear that successful treatment of Ewing's sarcoma demands an integrated team approach to the use of radiotherapy, surgery and chemotherapy in order to offer the best prospects for both primary tumour and systemic disease control.

Improved radiology and the advent of molecular markers have redefined the process of diagnosis for ESFT. Successive clinical trials[51] suggest that dose intensification of chemotherapy regimens may improve survival, and the use of high-dose therapy is now being formally evaluated. Surgical advances are reflected in the active use of surgery in local control associated with a more selective use of radiotherapy. While the benefits of modern multimodal 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.

Study aim

The aim of this part of the study was to conduct a systematic review of studies of tumour markers described in ESFT, and to establish an **evidence-based** perspective on their predictive clinical power. The power of markers to monitor disease status has also been evaluated, as this may also impact on patient management and outcome. A systematic review is the preferred means of identifying and combining existing evidence.[52,53] 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 ESFT. 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.

Methods

The systematic review followed the guidelines published by the NHS Centre for Reviews and

TABLE 2 EWS–ETS *gene rearrangements in ESFT*

Translocation	Gene fusion	Tumour type (frequency of *EWS* gene rearrangement)
t(11;22)(q24;q12)	*EWS–FLI1*	ESFT (85%)
t(21;22)(q22;q12)	*EWS–ERG*	ESFT (10%)
t(7;22)(p22;q12)	*EWS–ETV1*	ESFT (rare)
t(17;22)(q12;q12)	*EWS–E1AF*	ESFT (rare)
t(2;22)(q33;q12)	*EWS–FEV*	ESFT (rare)

Dissemination,[54] and had an overall philosophy to maintain breadth, synthesise the evidence qualitatively and then, only where appropriate, use quantitative methods.

Search strategy

The three on-line bibliographic databases 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 3*). A paper was included if a word from the set {Ewing's Sarcoma}, a word from the set {Tumour Marker} and a word from the set {Clinical Area} were included anywhere in the paper. 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.

TABLE 3 *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	PAS	Prognostic
	c-Myc	Diagnostic
Primitive peripheral neuroectodermal tumour	Cytokeratin	Pediatric
	HNK-1	Paediatric
PNET	Beta2-integrin-linked	Screening
protein kinase	Infant(s)	
	pPNET	
	MIC-2	
	Mitotic index	
	RT-PCR	
	Translocation	
	Plasma viscosity	
	ESR	
	EWS	
	EWS-ERG	
	EWS-FLI1	
	EWS-ETS	
	Neuronal differentiation	

* *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*

Inclusion criteria

To be included 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 paper had to be based on a primary research study of 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 because, although most patients were aged less than 18 years, most studies reported an age range up to about 30 years.

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.

Exclusion criteria

Papers that reported only laboratory work, methodology for identifying new markers or results from animal studies were excluded. Furthermore, if multiple 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. Histological characteristics of tumours were not included in the markers reviewed.

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

Copies of the accepted papers together with those for 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 and in which clinical area, that is, screening, diagnosis, prognosis or monitoring.

Amongst the covariate information extracted from each paper was the sampling method used to measure the marker, whether survival was overall (OS) or disease-free (DFS), the marker cut-off level if applicable and, if so, the total number of patients and events within each cut-off group.

Meta-analysis was performed, where possible, in order to combine all the relevant results found from the literature search and explain between-subject heterogeneity,[52] but for each clinical area only those tumour markers on which three or more papers provided data were considered. Both fixed and random effects meta-analyses were used, with the former preferred if there was no significant evidence of heterogeneity. For the meta-analysis of data from the prognosis papers, the extraction of the log of the hazard ratio ($\log_e$(HR)) and its variance was desired. These statistics were chosen because of the meaningful interpretation of the HR, the variety of methods available to indirectly estimate them and the approximate normal distribution of $\log_e$(HR) for large samples. Three methods, based on the approach of Parmar and colleagues,[55] were used to try and obtain an unadjusted or, if this was not possible, an adjusted estimate where required:

(1) using the direct estimates of these quantities given
(2) calculating indirect estimates using summary information available within the paper itself
(3) using the IPD to calculate unadjusted estimates from a Cox proportional hazards model.[56,57]

Even though markers were only considered for meta-analysis if they were reported in three or more papers, problems in extracting data from the papers often meant that for some of these markers only two estimates were available to be pooled in a meta-analysis.

A meta-regression was also performed, where appropriate, to estimate the effect of the cut-off point chosen on the HR.[52]

If there was 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.

Economic and psychosocial effects of tumour markers

A set of keywords was used to screen the abstracts of the selected papers for any economic or psychosocial results relating to the use of a tumour marker in ESFT for any of the clinical areas (see appendix 2).

Results

Literature search results

A total of 1089 papers were identified from the searches: 781 were first identified in MEDLINE, then an additional 273 in EMBASE and a further 35 from CANCERLIT (see appendix 3). 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 out of 107), and agreed that 80 of 82 (98%) 'not relevant' papers were indeed not relevant (*Figure 2*). The classification produced 82 papers of relevance but a 'reference explosion' (i.e. checking the references of the 82 papers for any more relevant papers which were not yet included) of important prognostic marker papers highlighted six more articles of which two were relevant (*Figure 2*). This gave a final total of 84 relevant papers (see appendix 1).

Tumour markers identified and the reporting of their clinical value

A total of 70 different tumour markers were studied in the 84 relevant papers in relation to the diagnosis, prognosis or monitoring of ESFT, but no marker was used for screening purposes (*Table 4*). 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 diagnosis papers because the results mostly only gave the number of ESFT patients with high/positive marker levels to those with low/negative 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 in three or more papers.

Prognostic tumour markers, data extraction and meta-analysis

More consistent and detailed information was available from the prognostic papers; of the markers studied further in this area, LDH, 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 (*Figure 3* and appendix 1). In the papers from which it was possible to obtain $\log_e$(HR) and its variance, the sampling method of marker levels proved to be consistent within each tumour marker. LDH values were measured in serum from patients with ESFT; all other markers were detected in the tumour. All the prognostic meta-analysis results below are subject to problems of poor reporting and likely publication bias. Therefore, **the following meta-analysis results should be treated with caution, and serve only as a guide for identifying the most important prognostic markers for clinical practice**.

Individual estimates of the HR 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 to those without. Of the 14 papers studied, only two provided sufficient information for a meta-analysis.[58,59] 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 = 2.397, 95% confidence interval (CI) = 0.495 to 11.616); however, this was not statistically significant, which may in part be a sign of the small sample size, reflected in the wide CIs (*Figure 3*).

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.[60,61] 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 = 0.171, 95% CI = 0.079 to 0.373); for patients with metastatic disease at diagnosis there was no statistically significant evidence of an association (HR = 0.418, 95% CI = 0.093 to 1.874) (*Figure 3*). When this DFS data was combined with that from a paper[62] reporting OS, the presence of EWS–FLI1 type 1 was still significantly associated with an improved outcome in patients with localised but not metastatic disease (localised, HR = 0.215, 95% CI = 0.116 to 0.396; metastatic, HR = 0.507, 95% CI = 0.235 to 1.092).

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 small cytogenetic IPD and was not analysed because of the concerns about the quality and reporting bias of such IPD.

Individual and overall pooled estimates of the HR are shown for all the markers in *Figure 3* and appendix 5. Serum LDH was associated with both

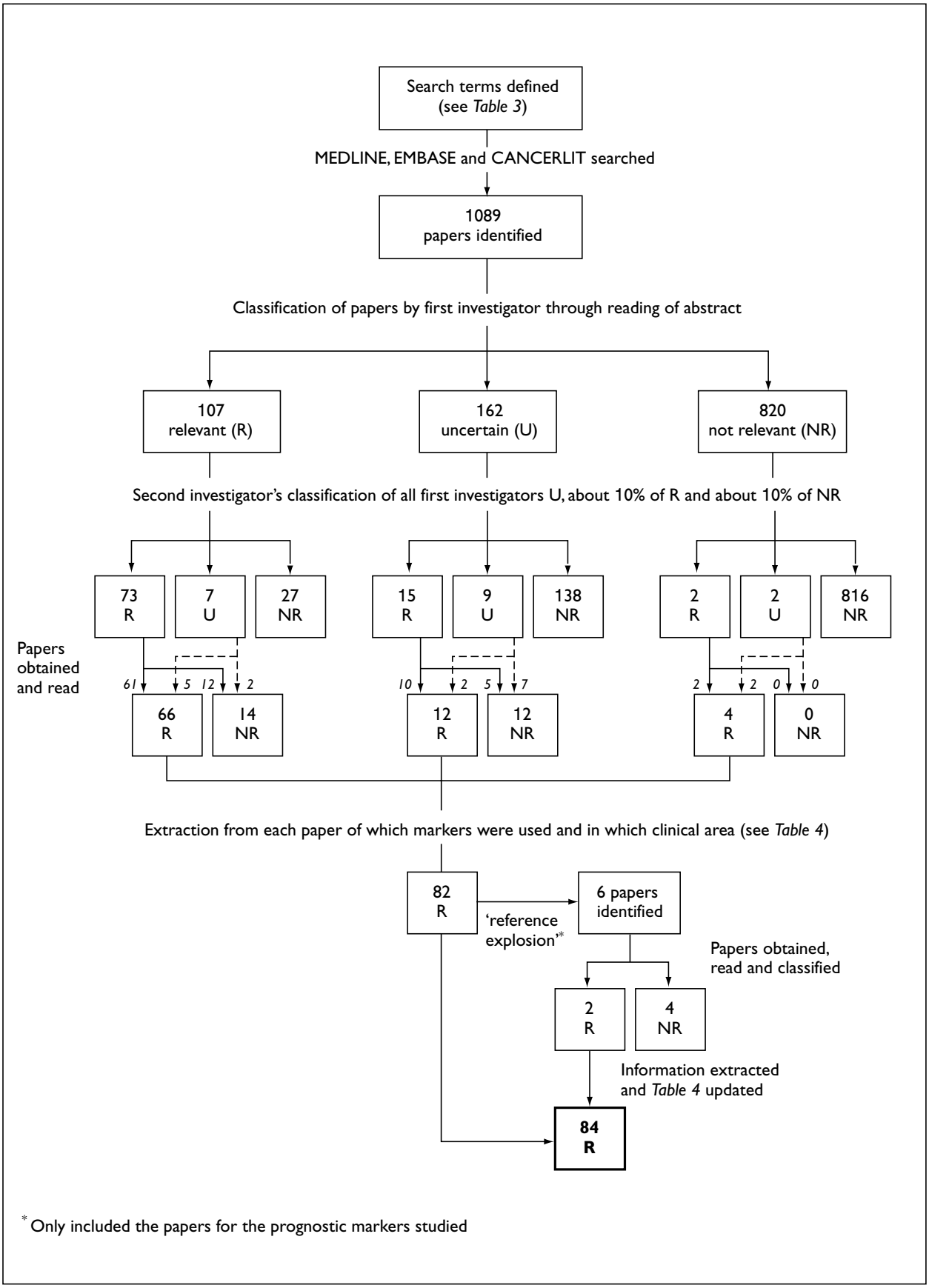
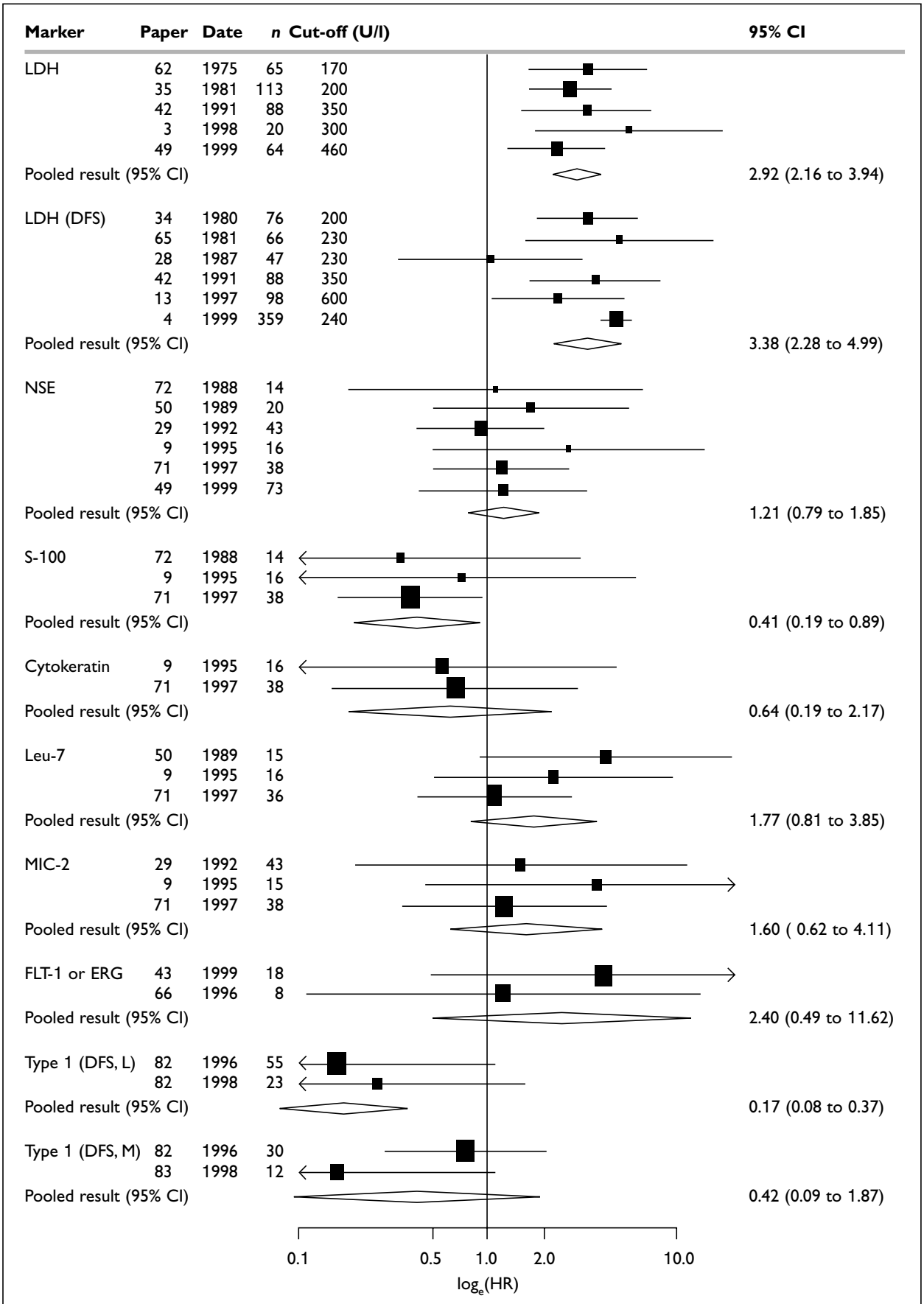


FIGURE 2 *Flow chart showing the results at each stage of the process used to identify the final set of relevant papers in the ESFT review. A paper was classified as 'relevant' if it provided a quantitative result or gave tabulated IPD, from humans about a tumour marker in ESFT in relation to the clinical area of screening, diagnosis, prognosis or monitoring*

TABLE 4 *List of tumour markers in ESFT identified by the systematic review together with the number of papers overall and within each clinical area*[*]

Tumour marker	Total papers	Diagnosis	Prognosis	Monitoring
EWS–FLI1 or t(11;22) (or chromosome 11 or 22 relating specifically to FLI1 or t1122)	35	35	13	2
Neurone-specific enolase (NSE)	22	22	12	0
MIC-2, CD99, HBA71 or 12E7	18	18	5	0
EWS–ERG or t(21;22) (or chromosome 21 or 22 relating specifically to ERG or t2122)	16	16	8	2
Lactate dehydrogenase (LDH)	15	14	15	2
Desmin	10	10	3	0
Leukocyte 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	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
β actin	2	2	0	0
Chromogranin, chromogranin A, chromogranin 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 investigated in only a single paper, looking at one or more of the clinical areas (see appendix 4)*


Marker Paper Date n Cut-off (U/l) 95% CI
LDH 62 1975 65 170
35 1981 113 200
42 1991 88 350
3 1998 20 300
49 1999 64 460
Pooled result (95% CI) 2.92 (2.16 to 3.94)
LDH (DFS) 34 1980 76 200
65 1981 66 230
28 1987 47 230
42 1991 88 350
13 1997 98 600
4 1999 359 240
Pooled result (95% CI) 3.38 (2.28 to 4.99)
NSE 72 1988 14
50 1989 20
29 1992 43
9 1995 16
71 1997 38
49 1999 73
Pooled result (95% CI) 1.21 (0.79 to 1.85)
S-100 72 1988 14
9 1995 16
71 1997 38
Pooled result (95% CI) 0.41 (0.19 to 0.89)
Cytokeratin 9 1995 16
71 1997 38
Pooled result (95% CI) 0.64 (0.19 to 2.17)
Leu-7 50 1989 15
9 1995 16
71 1997 36
Pooled result (95% CI) 1.77 (0.81 to 3.85)
MIC-2 29 1992 43
9 1995 15
71 1997 38
Pooled result (95% CI) 1.60 (0.62 to 4.11)
FLT-1 or ERG 43 1999 18
66 1996 8
Pooled result (95% CI) 2.40 (0.49 to 11.62)
Type 1 (DFS, L) 82 1996 55
82 1998 23
Pooled result (95% CI) 0.17 (0.08 to 0.37)
Type 1 (DFS, M) 82 1996 30
83 1998 12
Pooled result (95% CI) 0.42 (0.09 to 1.87)
0.1
0.5
1.0
2.0
10.0
log$_e$(HR)

LEFT: FIGURE 3 *Forest plot for Ewing's sarcoma showing individual and pooled HRs* with 95% CIs for each tumour marker, with details from the primary papers (number relates to the papers as listed in appendix 1) 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) are shown; and the area of each block is proportional to the precision of the HR. FLI–1 or ERG = EWS–FLI1 or EWS–ERG gene rearrangements; type 1 = EWS–FLI1 type 1. (* HR > 1 indicates a greater instantaneous risk of death (for OS) or disease recurrence/ death (for DFS) for patients with high/positive marker levels or those with presence of the marker, compared with those patients with low/negative levels or without the presence of the marker, respectively)*

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 (HR = 2.92, 95% CI = 2.16 to 3.94, $p < 0.0001$). Furthermore, patients with high LDH levels had an approximately 3.4 times greater risk of disease recurrence or death (HR = 3.38, 95% CI = 2.28 to 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,[63] there was a significant increased risk of death approximately 3.2 times greater for those patients with high serum LDH levels compared with those with low levels (HR = 3.21, 95% CI = 2.43 to 4.26, $p < 0.0001$).

There was also statistically 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 to 0.89, $p = 0.024$). However, there was no statistical evidence that expression of NSE (HR = 1.21, 95% CI = 0.79 to 1.85), cytokeratin (HR = 0.64, 95% CI = 0.19 to 2.17), Leu-7/HNK-1/CD57 (HR = 1.77, 95% CI = 0.81 to 3.85) or MIC-2/HBA71/CD99/12E7 (HR = 1.60, 95% CI = 0.62 to 4.11]) were associated with OS. IPD for both NSE and S-100 was given in three papers.[64–66] However, when these data were pooled, a Cox regression 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 to 4.11) (see appendix 5).

Cut-off points

In the above analyses the definition of high or low serum LDH levels varied considerably from paper to paper (*Figure 3*). Plotting $\log_e$(HR) against cut-off point weakly suggested that a higher HR was obtained when the cut-off point was between 200 and 350 U/l (see appendix 6). However, a meta-regression including cut-off point as a covariate was not statistically significant (estimate for cut-off = 0.0010, 95% CI = –0.0031 to 0.0010, $p = 0.32$). Study size and year of publication also did not appear to be associated with the estimate of the HR for LDH, or any of the other markers investigated (*Figure 3*).

Economic and psychosocial results

The 84 selected papers were examined for their relevance to an economic evaluation of tumour markers in ESFT either by their reporting cost information, usable resource information, economic outcome measures such as quality-adjusted life-years or cost-effectiveness (cost–benefit) analysis. Disappointingly, no studies reported such economic evaluation.

Equally disappointing, the 84 selected papers did not report any results regarding the psychosocial consequences for children and their families of using tumour markers clinically in ESFT.

Discussion

Appraisal of the systematic review

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, perhaps reflecting the lack of clinically useful markers in ESFT. 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, but we acknowledge the review may not be fully comprehensive. Only about 10% of the first investigators 'not relevant' papers were double checked, so it is possible that some papers of relevance were excluded unintentionally. Furthermore, other databases (e.g. SIGLE) and other sources of information (e.g. consultation with researchers) should ideally have been used, but this was not feasible given the time and resources available, particularly in light of the large literature already identified. 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 prognostic markers analysed. We did manage to exclude multiple papers which were written on the same or overlapping datasets, including just one based on the largest number of patients, the most detailed results and the longest follow-up time. However, this was a very difficult process due to the fact that many patient groups overlapped, but not completely.

Our initial search strategy included the names of those markers known *a priori* to be potentially important; however, we acknowledge that this list will not be comprehensive in light of the large number of markers identified during the review, although we did include more general terms such as 'marker' that will have limited this problem. We do not proclaim either that the review identifies the entire set of markers that have ever been studied. On the contrary, the fact that we identified so many suggests there will be some more that have been missed unintentionally. In terms of the meta-analyses, the decision to only assess markers studied in three or more papers for each clinical area may have led to some important markers being excluded from analysis, even though they were studied in two very large studies. Future reviews should seek to base meta-analysis decisions on numbers of individuals in studies rather than number of studies. Another concern is that the extraction of summary statistics, required for the estimation of $\log_e$(HR) and its variance, were not double checked, and thus this could mean some unintentional mistakes have been made. It should be noted that none of the aforementioned flaws in our search strategy and analysis decisions are likely to detract from the main methodological issues (such as poor reporting or the need for individual patient data) we identified.

We also recognise that there may be the common problem of publication or reporting bias; in particular, results that do not generate formal statistically significant or clinically valuable findings may not be in the public literature. This often happens because of a reluctance of journals and/or researchers to present or report negative findings. However, we were encouraged to find that the HRs we observed for NSE, S-100 protein, cytokeratin, MIC-2 and Leu-7 were not statistically significant in 16 out of 17 primary studies included, suggesting that publication bias may not be too much of a problem, at least for some of the markers. We were still concerned, though, that the presentation of cytogenetic data could be particularly biased, with papers just reporting data/results about the better-known cytogenetic abnormalities and not the entire set studied. This problem and that of poor presentation and small study sizes were the reasons why we did not assess the prognostic impact of the chromosome markers just presented within cytogenetic IPD, with the exception of those relating to the more commonly reported *EWS–ETS* gene rearrangements.

Publication bias in the choice of cut-offs

Another facet of publication or dissemination bias is in the choice of marker cut-off point to define groups of patients, whereby the choice of cut-off level or status is specifically chosen to optimise the difference between the groups and produce a result with the maximum statistical or clinical significance possible. This is most likely practised to ensure the most striking result and improve the chances of the study being published. This approach leads to wide variability in reporting, making it extremely difficult to assess the overall evidence from across studies and make clinical decisions of how best to use a marker to distinguish groups of patients for different types of treatment and care. For example, eight different cut-off levels of serum LDH were observed in only ten different prognosis papers used in the meta-analysis for this marker. We strongly advocate a move away from this approach, and appeal to that of **evidence-based** medicine. Research groups should work together to identify the most appropriate cut-off point for each marker and then be consistent in using it to assess the clinical value of the marker. This is particularly important for rare diseases, such as ESFT and other childhood cancers, because sample sizes are most often very small and so the clinical potential of a marker needs to be assessed across a larger number of studies. If the majority of these studies were to use different cut-off points, as was the case in the ESFT literature, then it becomes very hard to form a general consensus or decision about the tumour marker's value. Alternatively, if the agreement on a common cut-off point is not practical, the problem could be overcome by the presentation of full IPD, either within the paper or made available on the Internet, including the **exact** tumour marker value for each patient.[67,68] Calculations could then be made independently across studies using the cut-off point of interest.

Treatment received by patients

Another aspect of our review to be considered is that we did not account for the type of treatment

that patients received during follow-up. It would have been extremely difficult to incorporate treatment into the meta-analyses because treatments change over time and vary considerably between studies. The complexity of different treatments adds to the problem of clinically interpreting the results we found, a process already made difficult by the general poor standard of reporting. We recommend that type of treatment is reported for each patient within IPD so that the clinical value of markers for specific treatments can be evaluated more easily, and across studies to obtain bigger patient numbers.

Data extraction, quality of reporting and recommendations

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.[69] For example, for the purposes of the meta-analyses, 132 attempts were made to obtain estimates of $\log_e$(HR) and its variance from the data/results provided but only 83 of these proved successful. Furthermore, only six of these 83 HRs were provided directly in a paper, ten had to be calculated indirectly, and the remaining 67 were calculated using the raw individual patient data available. The variance of $\log_e$(HR) was never directly reported. The HR and its CI (or $\log_e$(HR) and its variance or CI) provide an important estimate of the difference in risk of death (for OS) or disease recurrence/death (for DFS) between two groups of patients, but this was mostly not acknowledged in the selected papers, which often quoted only an inexact *p* value.

The indirect methods suggested by Parmar and colleagues[55] proved particularly crucial, and ensured that estimates were obtained despite the poor statistical reporting, enabling a greater number of studies to be included in the results than would otherwise have been possible. However, due to the very nature of these methods, the estimates they provide are only approximate and simply make the best possible use of the results presented. Availability of these and other indirect methods does not overcome inadequate statistical reporting because they only enable more of the available evidence to be included rather than providing the direct estimates required. Questions still exist of how best to combine indirect estimates with direct estimates, and it would be far better to have as many direct estimates as possible.

Furthermore, Tierney and colleagues[70] have compared indirect estimates of the HR with direct estimates from IPD and have shown that the indirect estimates often poorly approximate their IPD equivalent.

Taking these points into consideration we chose to use only those indirect methods presented by Parmar and colleagues.[55] For example, given some assumptions, we could possibly have used estimates of proportion surviving to 2, 3, 5 or 10 years to obtain estimates of $\log_e$(HR) and variance. In fact, Vale and colleagues[71] have shown how event rates at fixed-time points can be combined with assumptions about censoring to produce indirect estimates. However, given the finding of Tierney and colleagues,[70] we felt that using this or other indirect methods would add further heterogeneity to our results and make the clinical interpretation of the meta-analysis results even more tenuous.

Adjustment factors

Our decision to obtain unadjusted estimates of $\log_e$(HR) and its variance in preference to adjusted estimates was made out of prior concerns regarding the wide variability in adjustment factors used, and this problem was indeed observed during the assessment of the selected papers. It is clear that once important prognostic markers have been identified they need to be evaluated against other clinically useful prognostic tools, such as histological characteristics or, indeed, other tumour marker levels. However, if authors are inconsistent in the adjustment factors they use it becomes very difficult and impractical to pool results across studies and make a proper evaluation of markers **over and above** other factors. We recommend research groups collaborate and identify the most important clinical factors that need to be considered whenever assessing the benefits of a marker for clinical practice. These factors will often need updating and must be made accessible (e.g. on the Internet) so that they can be consistently used as adjustment factors whenever markers are assessed. Presentation of these identified factors within IPD, alongside the exact marker levels and complete survival information (see below), is also recommended because it would allow adjusted estimates to be made independently across studies using the adjustment factors of interest.

Recommendations for improved reporting and benefits of IPD

It is imperative that the quality of statistical reporting improves if clear conclusions and policy recommendations are to be formed about tumour

markers. Altman and Lyman[72] have proposed important guidelines for both conducting and evaluating prognostic marker studies. Alongside these, we have developed simple guidelines on how to report results to facilitate both interpretation of individual studies and the undertaking of systematic reviews, meta-analysis and, ultimately, evidence-based practice (*Box 1*). We recommend that in primary studies which evaluate the use of a tumour marker for prognosis that the HR is reported together with its 95% CI (or $\log_e$(HR) and CI/variance) and also an **exact** *p* value when comparing two or more groups of patients (Collett[73] provides details of how to do this). Furthermore, it is important to define explicitly the prognostic groups being compared by reporting the specific marker cut-off level or status that defines them; and also to report the group sizes and the number of events in each patient group. If a *p* value is presented it is crucial to report its **exact** value and/or provide the **exact** value of the appropriate test statistic (e.g. χ^2 statistic) from which it is calculated. If it is not reasonable to report the exact *p* value because it is very small, say less than 0.001, then '$p < 0.001$' should be presented together with the **exact** test statistic. These recommendations should be applied to **all markers** studied, not just those that are significant.

Presentation of full IPD **for all markers** considered is also desirable, where possible including the exact initial marker level, time of disease recurrence (if appropriate), follow-up period and final status, so that $\log_e$(HR), its variance and other information may be calculated if required. The availability of IPD has allowed important evidence-based reviews to be made in other cancer settings,[74,75] and the capability to provide IPD for rare diseases such as ESFT is high because of the small study sizes. If it is not appropriate to provide IPD within a paper itself then there is the opportunity to publish on the Internet.[67] Of course, even making individual patient data available on the web is not

BOX 1 Guidelines for reporting prognostic tumour marker studies

Objective: to improve reporting of prognostic marker results and facilitate access to individual patient data

Results of **all** the marker analyses should be presented – both significant and non-significant results – and we recommend the following:

Summary data

Essential – present:

(1) The **HR** and its **CI**, or at least the $\log_e$(HR) and its variance

(2) Both **unadjusted** and **adjusted** results for each marker. For adjusted results, clearly state what variables have been adjusted for. Ideally, a consistency in the set of adjustment factors used across studies should be sought through **collaborative groups**

(3) The prespecified **cut-off level** used to define the groups (if used), and also the **number of patients** and **number of events** within each group

Highly desired – present:

(4) **Exact *p* values**. Reporting of results as 'significant' or 'not significant' is insufficient. Very small *p* values can be given as $p < x$ (e.g. $p < 0.0001$), but in this case the exact χ^2 statistic is also needed

(5) **Survival curves** showing the difference in survival over time between the groups, with clear **step** and **censoring points**; also, the initial numbers in each group, and the number of events and remaining numbers at various time points during follow-up, are needed

(6) **Percentage/survival at *n* years** with a CI using Kaplan–Meier or other methods that allow for censoring, together with the number of patients at risk at that time in each group

Individual patient data

Present **individual patient data** in the paper or on the Internet, or make available with details clearly indicated within the paper. Data on markers that were not analysed should be included. Subject to any restrictions imposed by data protection laws and guidelines, include

- exact initial marker level and how the marker was measured
- time of disease recurrence (if appropriate)
- follow-up period
- final disease status
- levels of other existing prognostic markers of recognised and accepted importance for current clinical practice
- patient subgroup information, e.g. age, stage of disease and type of treatment received

without its problems, with the non-permanency of individual web pages, and so perhaps a **central repository to collate and manage individual patient data** is needed within each disease area. The United Kingdom Children's Cancer Study Group has already initiated this type of approach within paediatric oncology.[76]

The presentation of full IPD would overcome all the following problems of poor reporting:

- no appropriate analysis presented
- no presentation of the HR and its CI (or $\log_e$(HR) and its variance and/or CI)
- group numbers and events not given
- inexact *p* values presented
- variability in marker cut-off level chosen
- variability in type of estimates made, that is, unadjusted and adjusted
- different outcome assessed, that is, OS and DFS
- variability in adjustment factors
- results only given for a few of the markers considered (publication/reporting bias).

In defence of some of the poor reporting observed, some authors may argue that the analysis/presentation of prognostic data was only a secondary part of their study. However, it is clearly important to analyse and report prognostic data to the guidelines above whenever or however it is studied. Presentation of IPD would, of course, remove this area of concern by enabling estimates to be made where prognostic data was available but not used or reported as needed.

The potential for substantial differences in meta-analysis of survival data when using results provided within the literature instead of IPD has recently been shown in the head and neck cancer literature.[77] In addition, Stewart and Parmar[78] recommend that, whenever possible, meta-analysis using IPD is preferred because it produces the least biased answers and the most appropriate way of addressing questions that have not been or could not be resolved by individual clinical trials. In the area of tumour markers, presentation of IPD would enable more appropriate meta-analysis and thus an improved way to synthesise the literature and develop an **evidence-based** perspective of the most appropriate markers to use. Furthermore, IPD would also allow an evaluation of combinations of markers, which may enable more specific and accurate prognostic assessments. For those researchers considering future systematic reviews of tumour markers we recommend they seek to obtain IPD wherever possible, as this is likely to be the most productive.

Clinical interpretation of markers and results

This systematic review has evaluated tumour markers in ESFT for which there is currently a literature database. 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 it is also used to monitor other malignancies including ovarian dysgerminoma, testicular germ cell tumour, Hodgkin's disease and non-Hodgkin's lymphoma.[79] 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 need to be compared. However, uncertainty on the optimal clinically significant cut-off value for serum LDH was reflected in the range of values quoted across the literature (170–600 U/l). 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 HR. Providing exact marker levels in the IPD would facilitate using a common cut-off point.[68]

The presence of *EWS–ETS* gene rearrangements is increasingly used to define ESFT, although a small proportion of tumours in this pathologically defined group do not express these gene rearrangements. Whether these tumours contain novel *EWS* gene rearrangements yet to be defined or represent a subset of tumours that do not contain such rearrangements is a critical question. The presence of EWS–FLI1/t(11;22)(q24;q12) or EWS–ERG/ t(21;22)(q22;q12) may be associated with a worse outcome for patients than for those with tumours that do not have this rearrangement, emphasising the potential importance of EWS gene rearrangements in the progression of ESFT. At the molecular level, EWS–FLI1/t(11;22)(q24;q12) rearrangements show great diversity, many 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[60,61] and OS.[62] Further studies are required to substantiate this and evaluate the clinical significance of different fusion transcript types.

Of concern is how difficult it is to draw clinically relevant conclusions from a large number of the studies reviewed in this report. We had hoped to assess the overall benefits of individual markers using decision models that incorporated the clinical, economic and psychosocial findings; however, this was not possible given the poor reporting and lack of informative data in the literature. The present drive to improve the long-term prognosis allied with advances in molecular understanding emphasise the need for multicentre studies to evaluate critically both the more established markers and new molecular markers in the context of modern therapy. While large multicentre studies are complex to organise and run, smaller studies may overcome some of their limitations by better and clearer reporting of tumour marker results. This may allow pooling of data and a more meaningful evaluation. The use of tumour markers to monitor patients with ESFT is an area that needs considering, given the small literature identified in this clinical area.

Of course, with the current rapid growth of genetic epidemiology, many new genetic markers and genetic sequences may be identified that supersede the markers we have identified in this review. Studies of genetic markers need to consider the guidelines we form in this review for reporting results, in particular the need to make IPD available, if they are to avoid the problems we have identified and facilitate evidence-based reviews.

Economic and psychosocial issues

The clinical implications of the results must also be considered together with economic and psychosocial aspects of tumour markers. Once a tumour marker has been identified as clinically effective, the decision to use the marker in practice (e.g. for screening or monitoring) also involves the cost of its implementation and the psychological impact it has on patients; hence, it was disappointing to identify such large gaps in these areas in the ESFT literature. However, this perhaps reflects the uncertainty about which markers have enough clinical effectiveness and importance to warrant subsequent economic and psychosocial studies.

It was disappointing that none of the 84 selected papers reported an economic evaluation. Additionally, NHS EED, DARE, the HTA database and IBSS (International Bibliography of the Social Sciences) were also searched. Again, no relevant papers were found which either included an economic assessment or contained information that would be of use in developing an economic model. In the absence of such information, a clear prescription for future trials and studies is to include an economic evaluation element, either in terms of a cost-effectiveness study or the identification of resource use that would permit a decision model to be developed.

Equally disappointing was that our search found no published evidence on the psychosocial consequences for children and their families of using tumour markers clinically in ESFT. Psychosocial evaluations of tumour markers are clearly important and have been performed for other disease settings.[80] The lack of work on the psychosocial outcomes of using tumour markers, particularly for prognosis and monitoring, is an obvious gap in the literature in the light of evidence pointing to the psychological vulnerability of patients who survive bone tumours,[81] and the potential for regular follow-up monitoring of tumour markers to generate anxiety. Given the extent to which tumour markers were assessed for prognosis, it was surprising that no psychosocial evaluation was performed in this clinical area, but this again probably reflects the lack of certainty as to which are clinically important. Knowledge about a tumour marker level may have severe consequences for the psychological well-being of patients and their families following diagnosis or treatment, and so this importantly needs to be assessed. Once a marker has been deemed clinically effective, future research on the marker should include an assessment of the psychosocial outcomes of using it in practice, particularly for prognostic and monitoring purposes. We also recommend more general work to investigate how the use of markers should be best communicated to children with ESFT and their families.

Recommendations for future research

This systematic review emphasises the uncertainty on the use of many of the studied tumour markers in ESFT, reflecting the small size of many studies and poor statistical reporting, and also the need for large, multicentre quality-controlled studies. This would enable the potential of individual markers in prognosis, monitoring and diagnosis to be evaluated, and also allow combinations of markers to be assessed. It would also enable markers in subgroups of patients (e.g. different

ages or treatments) to be studied more easily. Comparison of marker levels in serum from patients with ESFT to those in a healthy population is critical, whilst cost and psychosocial issues should also be measured and evaluated. Histological markers should also be assessed and compared with the more genetic/biological markers we have established in this review.

We have already discussed how the reporting of data can be improved, although we again draw attention to the potential of IPD to overcome the majority of problems we have found if it is reported for **all the markers** studied, even those that were not significant. Presentation of full cytogenetic IPD for all abnormalities studied is especially recommended. Histological characteristics and other important clinical factors should also be given in the IPD, such as stage and age, so that their clinical power can be evaluated and compared with other markers. The type of treatment received by each patient should also be reported.

We emphasise the importance of **evidence-based** medicine and encourage research groups to collaborate in order to establish the most important aspects of the markers currently available, such as the most appropriate cut-off points, whilst working towards the identification of new markers. This is particularly important for rare diseases, such as ESFT and other childhood cancers, because sample sizes are often very small and so the clinical potential of a marker needs to be assessed across a larger number of studies. Agreement would also be needed as to which markers to measure; we have provided a base that highlights the ones reported in greatest detail so far.

Chapter 3

Tumour markers in neuroblastoma

Introduction

Neuroblastoma is an enigmatic tumour demonstrating diverse clinical and biological characteristics and behaviour.[82] On the one hand tumours may demonstrate spontaneous regression, as well as spontaneous and induced differentiation to benign tumours, while on the other hand they may exhibit extremely malignant behaviour with very low cure rates.

Over the past 30 years the prognosis for infants (i.e. < 1 year old) and children (i.e. > 1 year old) with local or regional disease has improved modestly. However, for children with metatstatic disease at diagnosis the prognosis remains poor despite the advent of intensive multimodal therapy. As a consequence there has been a lot of interest in understanding the biology of neuroblastoma and finding better prognostic factors.

Tumour origin

Neuroblastoma is an embryonal cancer of the postganglionic sympathetic nervous system and most commonly arises in the adrenal gland but may also arise from sympathetic ganglia and other sites.

Incidence and age

Neuroblastoma is the commonest extracranial solid tumour of childhood, comprising between 8 and 10% of all childhood cancers (although comprising 15% of childhood cancer deaths, indicating the poor prognosis of many of the tumours). The incidence in the UK and the USA is approximately one in 7000 live births, and there is a slight sex predominance in most series with a male-to-female ratio of 1.2:1.

Neuroblastoma is predominantly a disease of the first decade, with about 80% of patients aged up to 4 years and a median age of 22 months.

Clinical presentation, including staging

Because neuroblastoma has a high propensity to rapidly disseminate and may originate in a variety of sites, the mode of presentation is diverse. In 65% of patients the primary tumour arises in the abdomen, with the adrenal gland being the single most common site. Infants tend to have more neck and chest primaries. However, most patients present with the consequence of metastatic disease, which may involve the bone marrow, bone, liver and skin.

A number of different staging systems have been used. The most widely accepted is the International Neuroblastoma Staging System (INSS):[83]

- Stage 1: localised tumours.
- Stage 2: unilateral tumours, with or without complete excision.
- Stage 3: tumour infiltrating across the midline ± regional node involvement.
- Stage 4: dissemination to a distant site.
- Stage 4S: localised primary tumour (as in Stages 1 and 2) with dissemination to skin, liver and/or bone marrow in infants less than 1 year of age. Typically this group of tumours has the ability to mature spontaneously.

Prognosis

Patients with Stage 1, 2 or 4S have a 3 year event-free survival of 75–90%. Infants with Stage 3 or 4 tumours have a cure rate of 80–90% or 60–75%, respectively. In contrast, children with Stage 3 or 4 tumours have 3 year survival rates of 50 or 15%, respectively.

The age of 1 year is used as an important cut-off point, and delineates a significant change in the behaviour of the tumours. *Table 5* shows that in infants (i.e. those aged under 1 year) most tumours are localised, whereas in children (i.e. those aged over 1 year) most tumours are metastatic at diagnosis. Overall, the majority of tumours are metastatic, and it is this group for whom the prognosis is poor, with a survival rate of the order of 10–20%. In addition to the fact that infants have more localised disease, the relative prognosis, even accounting for Stage, is better in infants, suggesting that biological factors are contributing both to the behaviour and to the prognosis.

Pathology

There are three classical histopathological patterns of neuroblastoma tumours (neuroblastoma, ganglioneuroblastoma and ganglioneuroma). These reflect a spectrum of

TABLE 5 *Distribution of extent of neuroblastoma disease by age of patient*[82]

Extent of disease at diagnosis	Age at diagnosis (years)	
	% patients aged < 1 year	% patients aged > 1 year
Localised	39	19
Regional	18	13
Disseminated (Stage 4)	25	68
Stage 4S	18	

maturation, differentiation and clinical behaviour. Neuroblastoma is one of several 'small round blue cell' tumours, and is identified by a number of histological and immunohistochemical features. The most widely used histopathological classification system was developed by Shimada and colleagues,[84] and recently revised by Joshi and colleagues.[85]

Tumour biology and cytogenetics

A number of genetic and biological features have been investigated in recent years, in an effort to improve understanding of the biological behaviour of neuroblastoma and to find markers that would be more predictive of prognosis or response to treatment. Current narrative reviews suggest these include *MYC-N* copy number, ploidy, deletion or loss of heterozygosity of chromosome 1p and gain of chromosome 17q, all of which have been linked with prognosis. Neurotrophins and the expression of related receptor genes have also shed light on the tumour biology. A large number of additional factors have been investigated, but many of these are likely to reflect secondary changes.

Catecholamine metabolism

Reflecting its origin within the sympathetic nervous system, the tumour usually secretes dopamine and its chief metabolite, homovanillic acid (HVA), in excess. Dopamine, HVA and vanillylmandelic acid (VMA), measured in both serum and urine, have all been used as tumour markers for neuroblastoma. Since 90–95% of tumours produce sufficient catecholamines to increase urinary metabolites, this has been of great diagnostic value. Levels are typically raised at the time of diagnosis, and can be used to follow disease activity.[86] Attempts have been made to correlate levels at diagnosis with prognosis but studies have been variable. The ability to measure urine catecholamines in untimed urine samples,[87] rather than 24 hour urine collections, have made this an easy test to use in monitoring of disease activity and subsequently to detect relapse.

Non-specific markers

Various serum markers, including ferritin, NSE and LDH, have been studied. Current evidence suggests that none of these markers are specific for neuroblastoma, and the levels measured will depend on overall tumour burden. Early studies suggested a strong relationship of these factors to stage, reflecting tumour burden.

MYC-N *copy number*

MYC-N is a proto-oncogene normally expressed in the developing nervous system and selected other tissues. It had previously been noted that some neuroblastoma tumours contained extra-chromosomal clusters of DNA, known as double minutes. These were subsequently shown to represent amplification (extra copies) of *MYC-N* (also referred to as N-*myc*, in the literature). Amplification of *MYC-N* was noted to occur in about 25% of primary, untreated neuroblastomas and associated predominantly with an advanced stage of disease at diagnosis. Nearly all patients with *MYC-N* amplification experience rapid tumour progression and a poor prognosis after conventional therapy.

Chromosome 1p

The deletion or allelic loss of the short arm (p) of chromosome 1 has been correlated with a poor prognosis. However, most cases also have *MYC-N* amplification, and so loss of 1p may not be a truly independent prognostic factor. Nevertheless, the 1p region is of interest as it contains likely neuroblastoma genes.

Gain of chromosome 17q

There is increasing evidence that gain of 17q genetic material is perhaps the commonest genetic abnormality in primary neuroblastoma. This region of the chromosome is likely to contain a gene (or genes) that contributes to neuroblastoma tumorigenesis when present in increased copy number. Gain of 17q has been shown to be associated with an unfavourable outcome, and in some cases may act independently to MYC-N.[88–91]

DNA content

The majority of advanced primary tumours have either a near-diploid (normal DNA content or a DNA index of 1) or near-tetraploid DNA content. In contrast, favourable neuroblastomas, especially those in infants (i.e. those aged < 1 year), usually have a hyperdiploid (increased DNA content with a DNA index of > 1). Infants with hyperdiploid tumours are more likely to have lower stages of disease at diagnosis and to respond better to treatment. The favourable prognostic association is likely the result of whole chromosomal gains, in contrast to children (i.e. those aged > 1 year) in whom chromosomal structural changes are more likely to occur.

Neurotrophins

Because some neuroblastoma tumours undergo spontaneous differentiation, mimicking neuronal differentiation, some investigators have considered whether the malignant phenotype may be partially caused by a suboptimal response to signals regulating differentiation. Investigators have focused on the neurotrophin family, and in particular on nerve growth factor and its receptors (TrkA and low-affinity nerve growth factor receptor). In the laboratory, neuroblastoma cells that express TrkA are able to differentiate when nerve growth factor is present. At a clinical level, neuroblastomas with normal TrkA expression are associated with favourable biological features (normal *MYC-N* copy number) and cure rates.

Other biological factors

Amongst the other biological factors that have been investigated are genes related to drug resistance and genes relating to invasion and metastasis.

Prognostic factors

In an attempt to explain the heterogeneity of neuroblastoma, Brodeur[92] proposed a model based on composite clinical and genetic features (*Table 6*). It should be noted that this model does not include gain of chromosome 17q, and future models will need to take this into account. Essentially, these models are trying to reconcile a range of clinical and biological features, all of which appear to be linked with prognosis. No single factor appears to predominate, and this would also indicate both the complex nature and our incomplete understanding of this tumour.

Treatment

As indicated earlier, progress to improve the outlook for patients with neuroblastoma has been slow, with only modest gains being made. Nevertheless, the recent advent of prognostic markers has allowed for the development of management plans based on risk assessment. This seems to provide a better means of selecting patient protocols than relying on Stage alone.

Low-risk patients are managed with surgery alone because excellent cure rates are achieved even when some tumour is left behind.

Intermediate-risk patients are still at low risk of succumbing to disease but probably require some chemotherapy (together with surgery). The amount of chemotherapy is determined in part by the biological features.

High-risk patients include those for whom little progress has been made. Many centres have used dose intensification regimens including the use of ABMT, although the use of ABMT is not based on clear evidence of efficacy.

Screening

Screening for neuroblastoma has been extensively studied for the past 30 years.[93] The case for screening revolves around the evidence that

TABLE 6 *Composite clinical and genetic features in neuroblastoma*[92]

Feature	Type 1	Type 2	Type 3
MYC-N gene	Normal	Normal	Amplified
Ploidy	Hyperdiploid	Near-diploid	Near-diploid
	Triploid	Near-tetraploid	Near-tetraploid
Chromosome 1p loss of heterozygosity	Absent	Present	Present
trk expression	High	Variable (low)	Low or absent
Age (years)	< 1	≥ 1	1–5
INSS stage	1, 2, 4S	3, 4	3, 4
3-year survival (%)	~95%	25–50%	~5%

neuroblastoma is a disease of early childhood, with the majority of patients presenting with advanced stage disease and having a poor prognosis, and some suggestion that the disease could progress from localised good prognosis disease to advanced stage disease, linked with the easy availability of urine tumour metabolites for screening. As a consequence, large screening programmes were established in Japan, North America and Europe.

Sawada and colleagues were the first to report, in 1971, the application of urine testing to the early detection of neuroblastoma at a population level.[94] Screening was introduced at the age of 6 months in Sapporo, Kyoto and districts of Tokyo, in Japan, using increasingly sophisticated biochemical methods of detection of the urine catecholamines VMA and HVA. Screening resulted in an increased rate of detection of neuroblastoma. However, the majority of these cases turned out to have localised disease, and this was a good prognosis with high survival rates. The tumours nearly all had favourable biological features without *MYC-N* amplification or 1p deletion.[95] Moreover, there was no associated reduction in incidence or survival of infants presenting subsequently with advanced, poor-prognosis disease. The implication of these findings was that the screening detected tumours that would have undergone spontaneous regression and would not require active intervention.

In addition, screening produced a significant number of false-positive cases, with children being subjected to unnecessary and stressful hospitalisation and further investigation. The false-negative rate has been harder to identify, but is felt to be under-reported with the possibility that it is this group who contribute to the children presenting later with poor-prognosis disease. Large-scale screening programmes were also established in the north of England, France, Austria, Germany and Canada (Quebec).

Study aim

The aim of this part of the project was to conduct a systematic review of studies of tumour markers described in neuroblastoma, and to establish an **evidence-based** perspective on their predictive clinical power. The power of markers to monitor disease status has also been evaluated, as this may also impact on patient management and outcome. A systematic review is the preferred means of identifying and combining existing evidence.[52,53] 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 childhood cancers. 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.

Methods

The systematic review followed the guidelines published by the NHS Centre for Reviews and Dissemination,[54] and had an overall philosophy to maintain breadth, synthesise the evidence qualitatively and then, only where appropriate, use quantitative methods.

Search strategy

The three on-line bibliographic databases 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 7*). A paper was included if a word from {Neuroblastoma}, a word from {Tumour Marker} and a word from {Clinical Area} were included anywhere in the paper.

The keywords in {Neuroblastoma} 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 first in MEDLINE, then in EMBASE and, finally, in CANCERLIT, with any duplicates being eliminated.

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

Inclusion

To be included a paper had to provide a quantitative result or give tabulated IPD evaluating the use of a tumour marker in neuroblastoma. The paper had to be based on a primary research

TABLE 7 *Sets of keywords used in the neuroblastoma literature search of MEDLINE, EMBASE and CANCERLIT*

{Neuroblastoma}	{Tumour Marker}		{Clinical Area}
Neuroblastoma	Tumour marker(s)	Neuropeptide(s)	Patient(s)
Ganglioneuroblastoma	Tumor marker(s)	Somatostatin receptors	Child
Ganglioneuroma	Marker(s)	Telomerase	Children
	N-Myc	CD44	Prognosis
	NMYC	Mitotic index	Diagnosis
	MYC-N	RT-PCR	Monitoring
	MYCN	Dopamine	Follow-up
	Tyrosine hydroxylase	NB84	Prognostic
	TH	Noradrenaline	Diagnostic
	Dopa-decarboxylase	Adrenaline	Pediatric
	DDC	Vanillylmandelic acid	Paediatric
	Phenylethanolamine-N-methyl transferase	VMA	Screening
	PNMT	Epinephrine	Infant(s)
	PGP9.5	Homovanillic acid	
	Dopamine-beta-hydroxylase	HVA	
	DBH	Normetanephrine	
	Phenylalanine	NM	
	Drug resistance	Metanephrine	
	MRP	MN	
	Tyrosine	3-methoxy tyramine	
	3,4-dihydroxyl phenyl alanine	3-MT	
	1p deletion	Vanillacetic acid	
	DNA diploidy	VPA	
	17q	Vanillglycol	
	14q	VG	
	DOPA	Vanillglycol acid	
	Neuron-specific enolase	VGA	
	NSE	Catechol acetic acid	
	Ferritin	CAA	
	Lactate dehydrogenase	VAA	
	LDH	Norepinephrine	
	Ganglioside(s)	Vanilalamine	
	Monosialoganglioside	VA	
	Disialoganglioside	TrkA	
	c-Neu	TrkB	
	c-Myc	TrkC	

study of humans relevant to the clinical area of screening, diagnosis, prognosis or monitoring. There was no restriction on the age of patients in the study, although approximately 90% of papers reported an age range of less than 18 years. Papers were only included if they were written in English.

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 apparently healthy population
- **diagnosis** – tumour marker levels considered of diagnostic value
- **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.

Exclusion

Papers that reported only laboratory work, methodology for identifying new markers or results from animal studies were excluded. Review articles and foreign language papers were also excluded. Histological characteristics of tumours were not included in the markers reviewed, for example the presence of differentiated ganglia in neuroblastoma (Shimada index). We did not use any cytogenetic IPD.

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

Copies of the accepted papers together with those for which the relevance remained unclear after assessment by the three investigators were obtained and then read thoroughly to make a final decision as to their inclusion. From the accepted papers, information was extracted on the tumour marker used and in which clinical area (i.e. screening, diagnosis, prognosis or monitoring).

Amongst the covariate information extracted from each paper was the sampling method used to measure the marker, whether survival was OS or DFS, the marker cut-off level if applicable and, if so, the total number of patients and deaths within each cut-off group. The age range and stages of neuroblastoma disease represented by the patients were also recorded for each study, as these were known *a priori* to be important clinical features.[92]

Meta-analysis was performed, where possible, in order to combine all the relevant results found from the literature search and explain between-subject heterogeneity.[52] For each of the areas of screening, diagnosis and monitoring, only those tumour markers on which three or more papers provided data were considered. For the area of prognosis, due to the large number of prognostic markers and prognostic studies identified, meta-analysis was limited to those reported in ten or more papers. Both fixed and random effects meta-analyses were used, with the former preferred if there was no significant evidence of heterogeneity. Meta-analysis for clinically relevant subgroups of patients was also considered, but was only performed where enough data were available.

Obtaining $\log_e$(HR) and its variance

For the meta-analysis of data from the prognosis papers, the extraction of $\log_e$(HR) and its variance was desired. These statistics were chosen because of the meaningful interpretation of the HR, the variety of indirect estimation methods available[55] and the approximate normal distribution of $\log_e$(HR) for large samples.

The papers studying prognosis commonly reported more than one result by relating one or more marker to both OS and/or DFS and then providing unadjusted and/or adjusted results on each occasion. For the meta-analysis of the prognosis papers, extraction of $\log_e$(HR) and its variance was sought from all the different occasions a tumour marker level at the baseline was related to either OS or DFS through a result or IPD. A sequential process using five different attempts in turn, based on the approach of Parmar and colleagues,[55] was used to try and obtain an estimate from each occasion (*Figure 4*). An unadjusted estimate was preferred for each occasion but if this could not be obtained an adjusted estimate was then sought. The sequential process used is described below.

Note: The following may be of greater interest to statisticians and those interested in the methods of data extraction; others may wish to omit the following description of the sequential process below and go straight to the heading 'Publication bias' (p. 29).

The five attempts

For all sample sizes:

(1) Use the direct estimates of $\log_e$(HR) and variance given (see appendix 14, method 1)

or

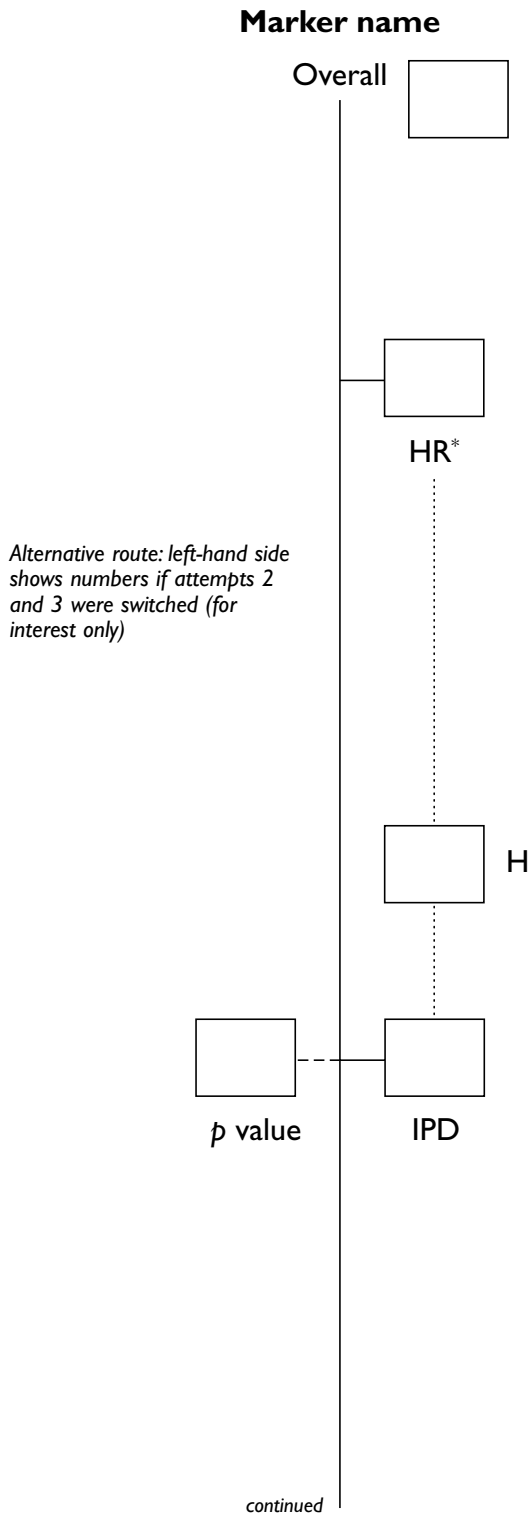
calculate indirect estimates using summary information provided **explicitly** within the paper itself (i.e. no estimation or interpolation (e.g. from figures or tables) of the summary statistics needed to obtain an indirect estimate was allowed here – they had to be explicitly given) (see appendix 14, methods 2–8 and 11). If an unadjusted estimate was not possible here **and** no IPD or unadjusted p value (or χ^2 statistic) from a log-rank/Wilcoxon test or Cox regression analysis was presented, then an adjusted result was sought using attempt 1.

For all sample sizes greater than 25 patients:

(2) Use the IPD to calculate unadjusted estimates from a Cox proportional hazards regression model[56,57] (see appendix 14, method 9). If the choice of cut-off to define the two groups was arbitrary, a value was chosen to be comparable to the most common value observed in attempt 1.

(3) Calculate indirect estimates by combining the p value (or χ^2 statistic) presented from a

Overall aim: To obtain a single direct or indirect estimate of the $\log_e$(HR) and its variance for each occasion possible throughout the relevant papers. Five possible attempts were made to obtain each estimate; unadjusted estimates were sought at each stage unless otherwise stated



The name of the prognostic marker of interest

The total No. of occasions that baseline levels of this marker have been related by results or IPD to either OS or DFS across the papers identified; an attempt was made from each of these to obtain an estimate of $\log_e$(HR) and its variance

ATTEMPT 1

The total No. of estimates of $\log_e$(HR) and its variance obtained directly, or indirectly by using the methods of Parmar* et al.[55] (see appendix 14, methods 1–8 and 11). Unadjusted estimates were sought, but if this proved unsuccessful and no IPD or *p* value/χ^2 statistic was available (for attempts 2 and 3), then an adjusted estimate was sought using the attempt 1 approach. (*Subtotals are also shown for unadjusted/adjusted results within each outcome together with No. of patients*)

NB. The indirect estimates at this stage were only calculated using summary information provided explicitly within the paper

The No. of occasions IPD were presented where a direct/indirect estimate was also available from attempt 1 (for interest only)

ATTEMPT 2

*The total No. of occasions remaining where IPD were available that could potentially be used to calculate a direct unadjusted estimate of the required $\log_e$(HR) and its variance by a Cox regression analysis **given** no estimate was calculated through attempt 1* (see appendix 14, method 9). (*Subtotals are also presented within each outcome together with No. of patients*)

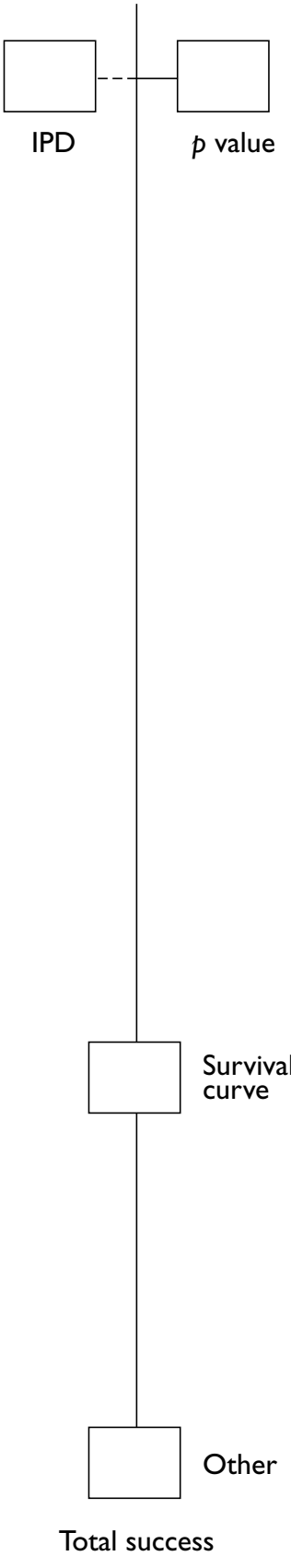
Quoted
Attempts made to use IPD for No. of patients (*n*) > 25

No. of successes
Whether attempts 3–5 could be used for the failures

Reasons attempts may fail here
Partial event times; marker groups too small; levels reported in a (clinically) inappropriate format; etc.

FIGURE 4 *Description of the sequential process used to obtain estimates of $\log_e$(HR) and its variance, with an explanation of the components (in* italics*) of the flow-charts in* Figure 6 *and appendix 10*

continued



ATTEMPT 3

Total No. of occasions remaining where a p *value (or* χ^2 *statistic) was available from log-rank or Wilcoxon test or Cox regression analysis but not all the other summary statistics required were directly available for attempt 1. (Subtotals also presented with No. of patients)*

Aim: to estimate or interpolate the required summary statistics from figures or tables, in order to combine with *p* value and estimate $\log_e$(HR) and its variance (see appendix 14, methods 7–8)

Quoted
No. of attempts made to obtain information for $n > 25$

No. of successes and therefore No. of estimates of $\log_e$(HR) and its variance obtained

Reasons attempts may fail here
Overall No. of events and/or group sizes not given; '*p* value > 0.05' or 'not significant' reported; etc.

ATTEMPT 4

As attempt 1 and 3 but now seeking an adjusted estimate for those occasions where an adjusted result was available alongside an unadjusted result, and the unadjusted estimate had proved impossible through attempts 1–3

Quoted
No. of adjusted results alongside unadjusted ones

Attempts required after unadjusted failed ($n > 25$)

No. of successes for adjusted estimates

ATTEMPT 5

Total No. of occasions remaining where a survival curve was available comparing two groups defined by the tumour marker, from which it may be possible to estimate $\log_e$*(HR) and its variance* (see appendix 14, method 10). *(Subtotals also shown for each outcome with No. of patients)*

Quoted
No. of attempts made for $n > 25$

No. of successes

Reasons attempts may fail here
No censoring on curve; presentation poor; etc.

No. of occasions remaining where an estimate of $\log_e$*(HR) and its variance proved impossible from attempts 1 to 5*

Sum of the successes from attempts 1 to 5

* *No. of occasions both* $\log_e$*(HR) and its variance directly given (if any) presented here*

***FIGURE 4* contd** *Description of the sequential process used to obtain estimates of* $\log_e$*(HR) and its variance, with an explanation of the components (in* italics*) of the flow-charts in* Figure 6 *and appendix 10*

log-rank/Wilcoxon test or Cox regression analysis with summary information from the paper, at least one component of which was not explicitly given in the paper and required estimation or interpolation from figures or tables (see appendix 14, methods 7 and 8).

(4) Where an adjusted result was presented alongside an unadjusted result, and an unadjusted estimate has proved impossible from attempts 1–3, calculate an adjusted estimate using attempt 1 or 3.

(5) Calculate an indirect unadjusted estimate from a survival curve comparing two groups of patients defined by a single tumour marker's status (see appendix 14, method 10). However, no survival curves without censoring points on it were considered because of the further uncertainties introduced, such as the time when patients dropped out.

The following points must be noted:

- During attempts 1 and 3, the χ^2 statistic was preferred to the p value if given a choice; equally, a result from a Cox regression analysis was preferred to an equivalent result from a log-rank/Wilcoxon test.
- IPD for prognosis were defined as such only if they gave baseline marker levels, the time to death or recurrence of disease or end of follow-up, and also final disease status at that time.
- If IPD were presented but estimates using attempt 2 were still not possible, the rest of the paper was screened to assess whether attempt 3, 4 or 5 could be used (i.e. was there a p value, adjusted result or survival curve available).
- Attempt 5 was not applicable if attempt 3 had failed, because if a survival curve with censoring points had been presented this would have been used to calculate the missing statistics required in attempt 3 (e.g. the number of deaths).
- If a study gave two or more results comparing the same outcome and patients but used different cut-off points in each case, then each of these was counted as a different occasion. Any subsequent meta-analysis would only include one of these results.
- If an occasion only presented results that compared three or more groups of patients (defined by two or more cut-off points) these were not used to estimate $\log_e$(HR) and its variance because this would have required further estimation methods and introduce even more heterogeneity into the results.
- An unadjusted estimate was preferred because prior knowledge of the literature indicated that adjusted results were highly inconsistent in what they adjusted for.

A graphical representation of the extraction process is presented in *Figure 4*. It shows each stage of the process, what method of estimation each of attempts 1–5 used and the reasons why estimates were often not possible. It also explains the flow-charts presented to show the results of extraction for each marker (see *Figure 6* and appendix 10).

A major problem encountered during the extraction process was the reporting of inexact p values. For example, it was common for the p value relating to the HR to be quoted as '$p < 0.05$' or '$p < 0.001$' or '$p > 0.05$', with no equivalent χ^2 statistic provided. The use of the p value was often crucial in the methods to indirectly estimate $\log_e$(HR) and its variance when direct estimates were not given (attempts 1 and 3), and it was important to utilise as much of the available data from the literature as possible. Hence, it was decided to assume $p = x$ where '$p < x$' had been reported, which would thus lead to a conservative estimate of $\log_e$(HR). However, where '$p > 0.05$' or 'p not significant' was reported, these results were not used because any estimate was open to more inaccuracy and could produce a potentially large overestimate of $\log_e$(HR).

Publication bias

An assessment of the publication bias in the prognosis literature was made where appropriate. Funnel plots were constructed by plotting $\log_e$(HR) against its standard error (SE) for each of the estimates used in the meta-analysis. In addition, two statistical tests, as described by Begg and colleagues[96] and Egger and colleagues,[97] were used to aid the subjective interpretation of the funnel plots. Finally the trim and fill method was applied to the data;[98] this is an **exploratory** method which 'adjusts' for funnel plot asymmetry and is used as a sensitivity analysis to assess the likely impact publication bias will have on the pooled results.

Economic and psychosocial effects of tumour markers

A set of keywords was used to screen the abstracts of the selected papers for any economic or psychosocial results relating to the use of a tumour marker in neuroblastoma for any of the clinical areas (see appendix 2).

Results

Literature search results

A total of 3415 papers were identified from the searches; 1536 were first identified in MEDLINE, then an additional 473 in EMBASE and then a further 1406 in CANCERLIT (see appendix 8). These were then classified by three investigators (*Figure 5*). The second and third investigators agreed that 85.7% of the first investigator's 'relevant' papers were indeed relevant or uncertain (42 out of 49). They also agreed that 193 of 222 (86.9%) 'not relevant' papers were indeed not relevant, and classified the 29 others as eight 'relevant' papers and 21 'uncertain' papers. After obtaining and reading the entire articles, 15 of these 21 'uncertain' papers were ultimately classified as 'not relevant'. Thus, 208 of the first investigator's 222 'not relevant' papers had been correctly

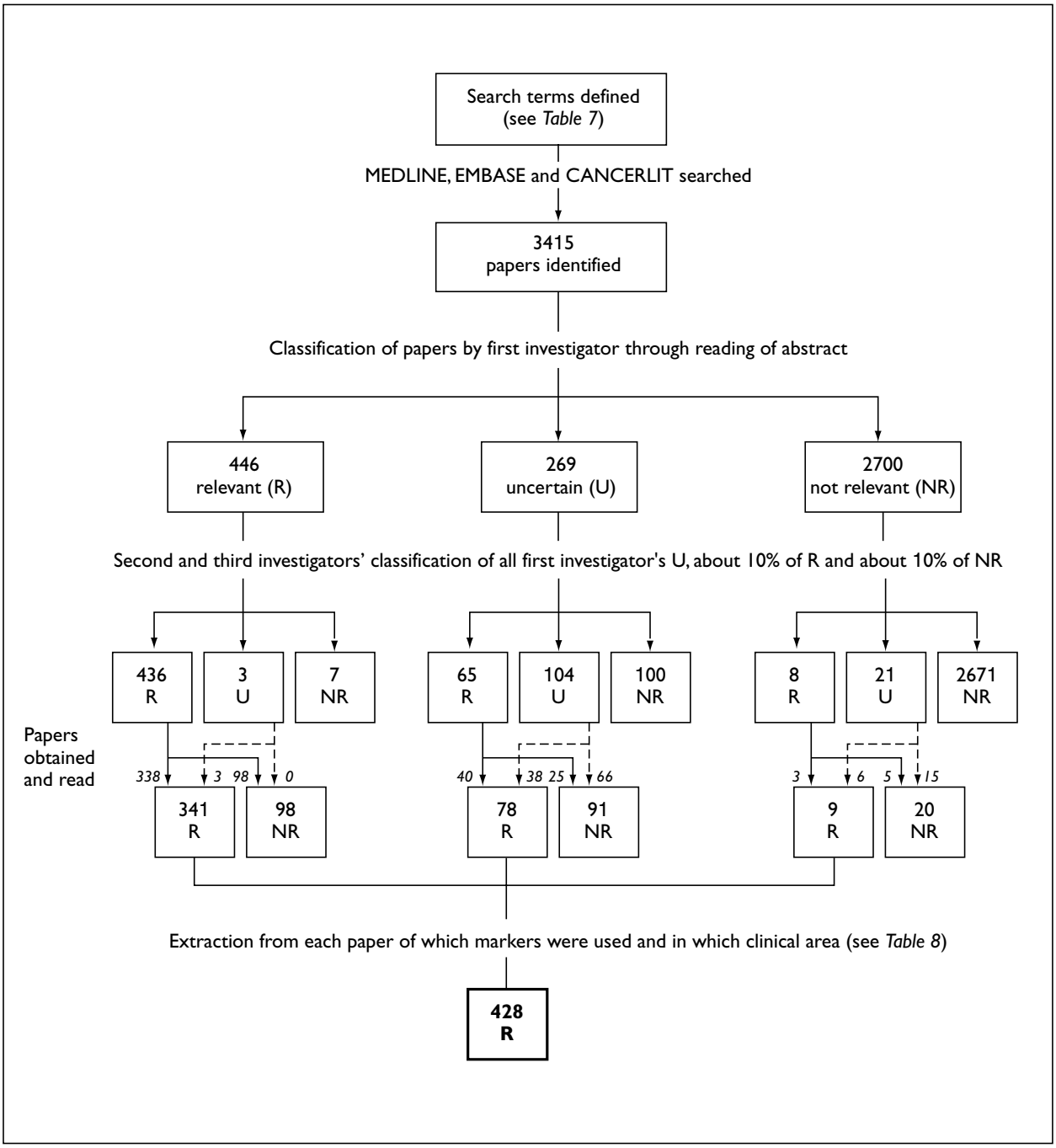


FIGURE 5 *Flow chart showing the results at each stage of the process used to identify the final set of relevant papers in the neuroblastoma review. A paper was classified as 'relevant' if it provided a quantitative result or gave tabulated IPD from humans about a tumour marker in neuroblastoma in relation to the clinical area of screening, diagnosis, prognosis or monitoring*

classified (93.7%). The finished classification produced 428 relevant papers (*Figure 5* and appendix 7). Interestingly, CANCERLIT identified 32 of these papers over and above what had already been found in MEDLINE and then in EMBASE, including articles from well-established journals such as the *Lancet* and the *Journal of Clinical Oncology* (see appendix 8).

Tumour markers identified overall and within each clinical area

A total of 195 different tumour markers were studied in the 428 relevant papers in relation to the screening, diagnosis, prognosis or monitoring of neuroblastoma (*Table 8*). There were 49 different papers on screening, 288 on diagnosis, 260 on prognosis and 51 on monitoring; 201 of the 428 papers covered

TABLE 8 *List of tumour markers in neuroblastoma that were identified by the systematic review together with the number of papers overall and within each clinical area*[*]

Tumour marker	Overall	Screening	Diagnosis	Prognosis	Monitoring
MYC-N	201	7	148	151	9
VMA	125	44	78	45[†]	18
HVA	105	38	64	35[‡]	16
DNA index/ploidy/diploidy/triploidy/aneuploid/ hyperdiploidy	56	5	37	44	1
Chromosome 1p or 1p36	47	4	34	40	1
Ferritin or isoferritin	49	3	36	33	5
NSE	45	2	33	28	9
LDH	32	1	22	26	4
Dopamine	24	2	22	10	4
TrkA (nerve growth factor receptor)	25	0	16	16	0
Adrenaline/epinephrine	15	0	15	5	4
Multidrug-resistance/associated protein/p-glycoprotein	16	0	7	16	0
Nonadrenaline/noradrenaline/norepinephrine	13	0	13	5	2
CD44	10	0	7	8	0
Neuropeptide Y	12	0	10	9	0
Tyrosine hydroxylase	12	0	11	3	3
Chromosome 17q	11	0	9	8	0
Ha-Ras P21/H-ras/c-Ha-Ras	11	0	8	6	1
Telomerase/telomeric repeats	11	0	6	7	0
Chromosome 14q	8	0	6	7	0
Ganglioside GD2	8	0	7	5	2
S100 protein	7	1	5	5	0
Chromosome 11q	6	0	5	6	1
Low-affinity nerve growth receptor	6	0	3	6	0
Metanephrine	6	0	6	1	1
TrkC	6	0	3	5	0
3-Methoxy-4-hydroxyphenyl glycol	5	1	3	1	0
4-Hydroxy-3-methoxymandelic acid	5	0	5	1	2
Dihydroxyphenylalanine	5	1	5	1	3
Dopamine β-hydroxylase	5	0	3	2	2
Proliferating cell nuclear antigen/proliferation index/ Ki67/KiS5 protein	5	0	5	4	0
3-Methoxytyramine	4	0	4	1	0
Ganglioside Bcl2	4	0	2	3	0
Ganglioside GD1a	4	0	3	3	1
Ganglioside GD1b	4	0	3	3	1
Ganglioside GM1	4	0	3	3	1

continued

***TABLE 8* contd** *List of tumour markers in neuroblastoma that were identified by the systematic review together with the number of papers overall and within each clinical area*[*]

Tumour marker	Overall	Screening	Diagnosis	Prognosis	Monitoring
Ganglioside GM3	4	0	3	3	1
Leukocytes	4	0	0	4	0
Normetanephrine	4	0	4	1	1
PGP9.5 protein	4	0	4	1	2
Somatostatin	4	0	1	3	0
Synaptophysin	4	0	4	1	0
TrkB	4	0	4	2	0
3-Methoxytyrosine	3	0	0	1	0
Chromosome 1q	3	0	2	3	0
Chromosome 3p	3	0	3	3	0
Ganglioside GD3	3	0	2	3	0
Ganglioside GM2	3	0	2	3	0
Ganglioside Gt1b	3	0	2	2	1
Haemoglobin	3	0	1	3	0
Platelets	3	0	1	3	0
Vannilactic acid	3	0	3	1	0
3,4-Dihydroxyphenylacetic acid	2	0	2	0	0
α subunit of GTd protein	2	0	1	2	1
Carcinoembryonic antigen	2	0	2	1	0
Chromogranin	2	0	2	1	0
Chromosome 2p	2	0	2	0	0
Chromosome 9p	2	0	0	2	0
c-Myc	2	0	1	2	0
c-Src	2	0	2	1	0
DDX1 gene copy number	2	0	2	2	0
Desmin	2	0	2	0	0
Dihyrdroxyphenylacetic acid	2	0	2	0	0
Erythrocyte sedimentation rate	2	0	0	2	0
Ganglioside Gq	2	0	1	2	0
Hydroxymandellic acid	2	1	2	0	0
Hydroxymethoxyphenylethylenglycol	2	0	2	0	0
Interleukin-1β enzyme	2	0	2	1	0
Lymphocyte counts	2	0	0	2	0
Mage1	2	0	0	2	0
Mage3	2	0	0	2	0
Metadrenaline	2	0	2	1	0
Metaiodobenzylguapidine	2	0	2	0	0
MHC class I gene expression	2	0	0	1	1
Nb84	2	0	2	0	0
NM23-h1	2	1	2	2	0
Normepinephrine	2	0	2	1	1
N-Ras	2	0	1	2	0
P16 gene/mutation	2	0	0	2	0
P75 gene	2	0	2	0	0
PP60csrcn	2	0	1	2	0
Serum creatine kinase BB	2	0	1	1	0
Sialic acid	2	0	2	1	1
Tyramine	2	0	2	0	0
Vasoactive intestinal peptide	2	0	2	1	0

[*] *A further 110 markers were studied in a single paper, looking at their use in one or more the clinical areas (see appendix 9)*
[†] *Thirty-six of these studied VMA; 20 studied the VMA:HVA ratio*
[‡] *Twenty-six of these studied HVA; 20 studied the VMA:HVA ratio*

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.

Screening

The review identified 49 papers which gave quantitative data relating to the use of tumour markers in screening, and potentially considered the evaluation of a population-based screening programme for neuroblastoma. These papers covered programmes established in geographical regions of Austria, Canada, France, Germany, Japan and the UK. The studies considered a variety of outcomes, including (1) feasibility/uptake rate, (2) the number of false-positive and false-negative cases, (3) incidence, (4) stage distribution and (5) mortality. In terms of outcomes (3), (4) and (5), some studies have undertaken, or are designed to enable in the future, a comparison between screened and control (non-screened) populations. The heterogeneity in outcome, combined with general poor quality in reporting results, meant that no quantitative synthesis was performed.

Diagnosis

It was not possible to perform a meta-analysis of the data from the diagnosis papers because the results mostly only compared the number of neuroblastoma patients with high/positive marker levels to those with low/negative levels respectively. Marker levels from patients with neuroblastoma were rarely compared with those from a sample of healthy controls in any of the diagnosis papers, for example none of the 22 papers reporting levels of LDH at diagnosis compared patients with neuroblastoma to healthy controls.

Prognostic tumour markers and data extraction

It was possible to collate and synthesise relevant data and summary statistics for prognostic tumour markers. The 12 most commonly studied prognostic markers were each selected for an in-depth study to establish their individual value as a prognostic tool; each marker was studied in ten or more prognosis papers. The prognostic value of CD44 expression was also evaluated, because all of its eight prognostic studies were contained within those papers of the other 12 markers to be evaluated and thus relatively little additional effort was thought necessary. Hence, 13 markers overall were evaluated as prognostic tools. These covered 211 (81.2%) of the selected prognosis papers, and within them there were 575 occasions where levels of any of these tumour markers were related to OS or DFS by summary statistics or IPD.

From each of these occasions $\log_e$(HR) and its variance were sought. This proved an extremely difficult process because the statistical analyses and reporting of prognostic data was most often inadequate, inappropriate and incomplete. The following describes the results and problems at each stage of the data extraction procedure. **Those not interested in the data extraction method and problems may wish to omit the description below and proceed to the heading 'Overall' (p. 36).**

Attempt 1

Both $\log_e$(HR) and its variance were directly provided for only three of the 575 available occurrences (*Figure 6* and *Table 9*), and all these three direct estimates were from a single paper[35] out of the 211 studied. This rather concerning finding increased the importance of available indirect methods of estimation that used other summary statistics provided, such as CIs, *p* values, group numbers and numbers of events.[55] Using attempt 1 a further 121 indirect estimates were obtained using the summary statistics explicitly given within a paper (*Figure 6* and *Table 9*). This meant that in total only 124 direct/indirect estimates of $\log_e$(HR) and its variance were obtainable using summary statistics directly reported from the 575 occurrences (21.6%).

Attempt 2

Further attempts were then made to obtain the 451 remaining estimates required, beginning with attempt 2. Fortunately, a large amount of IPD were available throughout the literature because the low incidence of neuroblastoma leads to small study sample sizes and thus greater feasibility for inclusion of IPD within articles. IPD were presented for 173 of the remaining occasions required, and extracted with the premise to calculate a direct estimate of $\log_e$(HR) and its variance using a Cox regression analysis. However, this modelling technique was only considered appropriate if the sample size was greater than 25, which was the case for 70 of the 173 studies. From these, 41 successful direct estimates of $\log_e$(HR) and its variance were made; the other 29 failed because, on inspection, the IPD were often incomplete, reducing the sample size to less than 25 (e.g. some patients had missing marker levels or event times), or the marker levels were not presented in a clinically appropriate format (e.g. VMA in milligrams per day). Thus, 165 successful estimates of $\log_e$(HR) and its variance were ultimately made from attempts 1 and 2 (*Figure 6* and *Table 9*). The 29 failures from attempt 2 were also checked

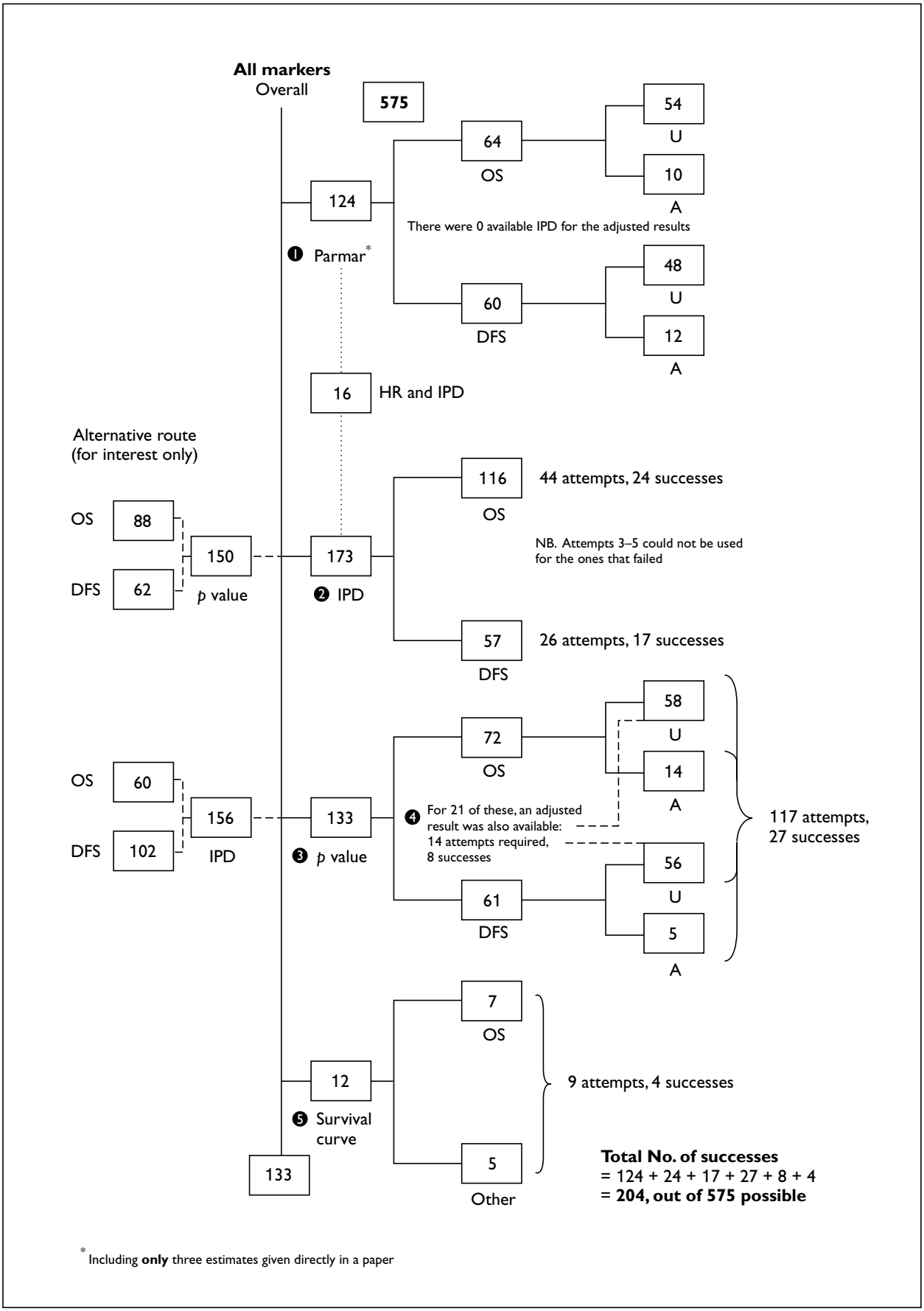


FIGURE 6 *Overall results of the extraction process for the 13 prognostic markers; attempts 1–5 are represented by black circles, and* Figure 4 *explains each component of this flow chart*

TABLE 9 *Description of the summary statistics required for each indirect estimation method (see appendix 14) together with the number of times each method was successfully used in attempts 1–5*

Method No.	Method	Attempt 1	Attempt 2	Attempt 3	Attempt 4	Attempt 5	Total
1	$\log_e$(HR) and its variance given	3	–	0	0	–	3
2	HR and its variance given	0	–	0	0	–	0
3	HR and CI given	30	–	0	3	–	33
4	$\log_e$(HR) and CI given	0	–	0	1	–	1
5	HR and *p* value given	10	–	2	2	–	14
6	$\log_e$(HR) and *p* value given	2	–	0	2	–	4
7	*p* value, group numbers and total events given	67	–	21	0	–	88
8	χ^2 statistic, group numbers, and total events given	10	–	4	0	–	14
9	IPD given	–	41	–	–	–	41
10	Survival curve given	–	–	–	–	4	4
11	HR (no variance or CI or *p* value or χ^2), group numbers and total events given	2	–	0	0	–	2
Total		124	41	27	8	4	204

to see if attempts 3, 4 or 5 could be used, but unfortunately these approaches were not possible either.

Attempt 3

One very important indirect method of Parmar and colleagues[55] enables an estimate of $\log_e$(HR) and its variance when a *p* value/χ^2 statistic is given (from a log-rank/Wilcoxon test or Cox regression) alongside group numbers and total group events (see appendix 14, methods 7 and 8). However, a common trait in the neuroblastoma literature is to provide a *p* value/χ^2 statistic directly without the group numbers and/or total events. One hundred and thirty-three of the remaining occurrences demonstrated this, and so these articles were studied further in order to try and indirectly estimate the missing statistic(s) needed, which would then enable estimates of $\log_e$(HR) and its variance (attempt 3). For example, the most common statistic missing was the number of events in the two groups, which could often be estimated by survival curves or summary percentages. For attempt 3 it was again only considered sensible to make estimates where the sample size was > 25, and this criteria left 117 occurrences to investigate. Unfortunately, successful estimation of the missing statistics was possible in only 27 of these occurrences (*Figure 6* and *Table 9*). The running total of estimates from attempts 1 to 3 was 192.

Attempt 4

Attempt 4 sought to use the occasions where an adjusted result was presented together with an unadjusted result but the unadjusted estimate of $\log_e$(HR) and its variance had not been obtained from attempts 1 to 3. In this case, and if the sample size was greater than 25, the adjusted estimate was now sought using the methods of Parmar and colleagues,[55] as in attempts 1 and 3. Eight indirect adjusted estimates were made from 14 occurrences (*Figure 6* and *Table 9*).

Attempt 5

By this stage there were 145 remaining occasions where attempt 2, 3 or 4 had not been considered because no IPD, *p* value/χ^2 statistic or adjusted result was available. One final method used to estimate $\log_e$(HR) and its variance for these occasions was to extract information from any available survival curve (attempt 5). In 12 of these 145 remaining occurrences a survival curve was presented in the article, showing the difference in survival for the two marker groups, and nine of these were for occasions with > 25 patients. Only four successful indirect unadjusted estimates of $\log_e$(HR) and its variance were possible from these nine occurrences; the remaining five failed because either no censoring was present or the steps and censoring points on the curve were too numerous to manage (*Figure 6* and *Table 9*).

Overall

Thus, a total of 204 estimates of $\log_e$(HR) and its variance were calculated from attempts 1 to 5 for the 13 markers studied. This meant there were just 35.5% successes from the 575 available occurrences, and 133 occasions (23.1%) remained where one of the markers had been related to prognosis by summary data and yet no appropriate statistical analysis had been done to enable attempts 1 to 5 to even be considered (*Figure 6*).

The methods used to obtain each of the 204 estimates are shown in *Table 9*. It is interesting to look at which of the indirect methods were most frequently used for the 159 estimates obtained during attempts 1, 3 and 4. The methods using a direct estimate of HR or $\log_e$(HR) with a CI were used just 34 times (21.4%), whilst those using a direct estimate of HR or $\log_e$(HR) with a *p* value were only used 18 times (11.3%). The most frequent methods needed were those that used a *p* value/χ^2 statistic in combination with group numbers and total events (102 times (65.2%)). This demonstrates that the calculation and/or presentation of a HR or $\log_e$(HR) was not appreciated across the prognostic literature, not to mention the small importance placed on also providing variance, a CI or even a *p* value.

Meta-analysis for prognostic markers

For all 13 individual markers, the results of the extraction process and of each successful estimate obtained are shown in appendices 10 and 11, respectively. For each estimate, other important clinical and statistical information relating to it was also recorded (see appendix 11), six components of which created severe problems in terms of pooling together estimates and developing clinically meaningful results. These were stage of disease, age of patients, cut-off used to define the two marker groups, outcome (OS or DFS), type of result (unadjusted or adjusted) and adjustment factors. There was great diversity in the combination of these features for each estimate available. For example, for marker MYC-N there were 94 estimates of $\log_e$(HR) and its variance available, but this involved nine different cut-off points, nine different stage groups, three different age groups, 15 adjusted estimates and two different outcomes (*Table 10*). Furthermore, for the 15 estimates that were adjusted for other clinical features (within a Cox regression model) there were only two that adjusted for all the same ones, and that was because the estimates came from the same article.[99] This inconsistency and variability of reporting was reflected equally in the estimates obtained for the other 12 markers (see appendix 11), and justified our decision to obtain unadjusted results in preference to adjusted results where possible.

TABLE 10 *Demonstration of the large heterogeneity in the estimates of $\log_e$(HR) and its variance. The number (n) of the 94 prognostic results for MYC-N is shown within each outcome, result type, stage of disease, cut-off point and age of patients*

Parameter		n
Outcome	DFS	46
	OS	48
Result type	Unadjusted	77
	Adjusted	17
Stage	All	68
	1	3
	2	2
	4	4
	1, 2, 3	3
	1, 2, 3, 4	5
	2, 3, 4, 4S	2
	3, 4	3
	Unknown	5
Cut-off point	1 copy	23
	2 copies	1
	3 copies	17
	4 copies	5
	5 copies	2
	10 copies	18
	Mean gene expression	2
	Positive versus negative protein (or staining versus no staining)	9
	Unknown	17
Age	All	78
	< 1year	2
	> 1 year	5
	Unknown	9

The extent and nature of this large heterogeneity meant it was difficult to overcome, or even account for, using current sophisticated statistical methodology. Even the analysis of subgroups of estimates was not realistic because it was virtually impossible to obtain subgroups that reflected patients with similar features. Nevertheless, it was very important to utilise the data extracted for each marker and thus make the most of what the literature over the last 35 years has reported. It was decided that a meta-analysis should be performed for each of the 13 markers separately within each outcome (OS and DFS) for estimates from cut-off points that, although not identical, were broadly similar in the two groups they defined. Furthermore, if a paper provided two or more estimates within an outcome

for the same marker and same patients by using **different cut-offs** then only one of these estimates was used in the meta-analysis, that based on the greatest number of patients and/or the cut-off that was the most comparable with other studies. Full details of the estimates included and excluded from the meta-analyses are given in *Figure 7*.

The meta-analysis results for each marker are presented in *Table 11* and *Figure 7*. Their interpretation is now discussed for three types of marker class. However, all the results are subject to problems of poor reporting, heterogeneity and likely publication bias. Therefore, **the following results must be treated with caution and should only serve as a guide for identifying the most important markers to be used in practice**.

DNA/chromosome abnormalities

MYC-N provided the most estimates from the literature, with the majority of studies reporting that amplification of the *MYC-N* copy number was associated with a worse outcome. A smaller number of papers looked at *MYC-N* RNA and protein levels, with high expression again indicative of a poorer prognosis. On the assumption that high expression of RNA levels was related to amplification of the *MYC-N* copy number, the results for this marker were pooled, and there was strong statistically significant evidence that amplification of the *MYC-N* gene was associated with a worse OS and DFS. The risk of death was 5.48 times greater for patients expressing *MYC-N* amplification compared with those that did not (HR = 5.48, 95% CI = 4.30 to 6.97]), and similarly for risk of disease recurrence/death (HR = 4.28, 95% CI = 3.34 to 5.49).

Patients expressing a deletion of chromosome 1p also had a significantly worse OS (HR = 3.12, 95% CI = 1.95 to 4.98) and DFS (HR = 3.93, 95% CI = 2.31 to 6.68) than patients who did not have this abnormality. This was the only marker where the cut-off was consistent throughout because either the patients did or did not exhibit this deletion.

There was consistent evidence across the literature that patients who **did not** express diploid cells (i.e. DNA index = 1) in tumour had a significantly improved OS (HR = 0.31, 95% CI = 0.20 to 0.48) and DFS (HR = 0.33, 95% CI = 0.20 to 0.56).

Biological markers

There was statistically significant evidence that high expression of LDH was associated with a worse OS and DFS. The risk of death was 3.36 times more for those patients who had high expression of LDH compared with those with normal levels (HR = 3.36, 95% CI = 1.72 to 6.57) and similarly for risk of disease recurrence/death (HR = 3.20, 95% CI = 2.06 to 4.98). High levels of serum ferritin were also associated with a worse outcome (DFS, HR = 4.26, 95% CI = 2.42 to 7.53; OS, HR = 2.74, 95% CI = 1.92 to 3.91), as was low tumour expression of TrkA (DFS, HR = 0.26, 95% CI = 0.16 to 0.42; OS, HR = 0.09, 95% CI = 0.05 to 0.16). Furthermore, patients with abnormally high levels of NSE in tumour were associated with a significantly worse OS (HR = 5.22, 95% CI = 3.12 to 8.73) and DFS (HR = 5.56, 95% CI = 2.11 to 14.7) in comparison with patients with low levels.

Patients who had expression of the multidrug resistance protein in tumour were associated with a significantly worse DFS (HR = 6.37, 95% CI = 3.71 to 10.93) and OS (HR = 3.52, 95% CI = 1.19 to 10.46) compared with those who did not. Furthermore, low tumour expression of the CD44 gene was significantly associated with a worse DFS (HR = 0.06, 95% CI = 0.02 to 0.21).

Urinary catecholamines

There was no significant evidence that urinary levels of VMA or HVA were associated with OS, and no meta-analysis was possible within the DFS outcome (*Table 11*). However, there was evidence that low urinary levels (normally < 1) of the VMA:HVA ratio were associated with a worse OS and DFS. Patients expressing such low levels had a risk of death 2.27 times greater than for those who had higher levels (HR = 0.44, 95% CI = 0.18 to 1.06), and similarly for risk of disease recurrence/death (HR = 0.35, 95% CI = 0.17 to 0.72) (*Table 11*). It was not possible to perform any meta-analyses for the urinary catecholamine marker dopamine because there was only one estimate available within each outcome.

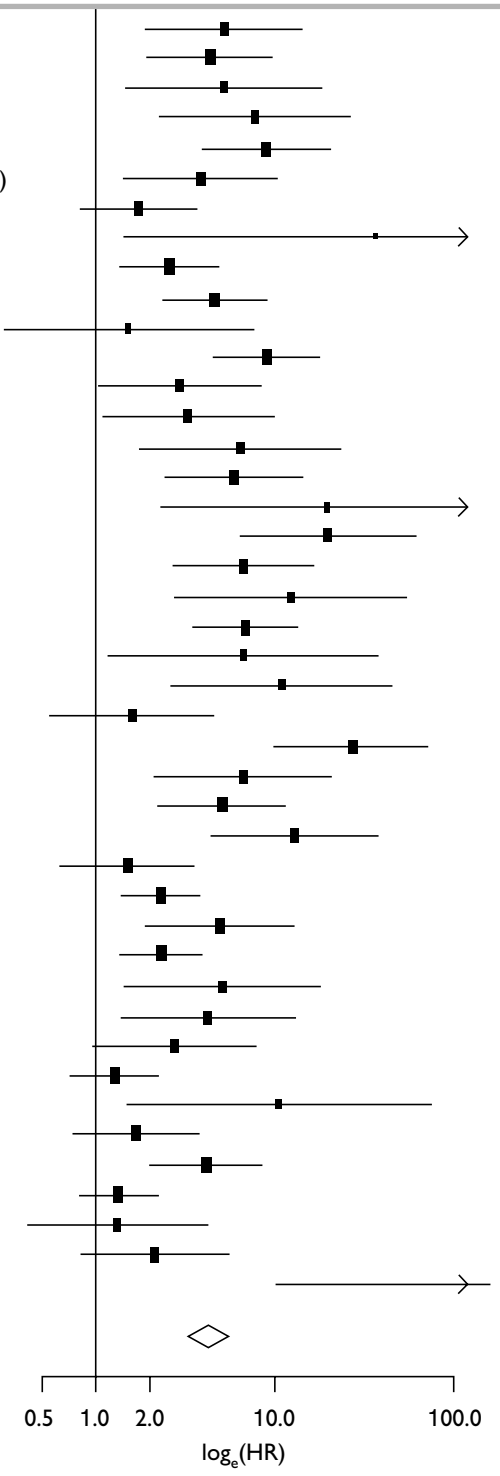
Monitoring

In terms of monitoring for neuroblastoma, the review identified 51 papers which provided quantitative data evaluating the serial use of tumour markers to aid the clinical management of patients with neuroblastoma. However, there was considerable heterogeneity between the studies in terms of:

- tumour markers considered (e.g. VMA, HVA, MYC-N, ferritin, NSE, LDH)
- outcome (OS, DFS)
- statistical analyses undertaken and reporting of results
- length of follow-up, for example treatment phase or long term follow-up

(a) MYC-N (disease-free survival)

Paper	*n*	u/a	Age	Stage	Cut-off (copies)
44	50	u	All	All	1
80	48	u	Unknown	All	Negative, positive
87	60	u	All	All	Mean expression
102	59	a	All	All	3
109	59	u	All	All	1
122	34	a	Unknown	All	Expression (no/yes)
142	42	u	Unknown	All	10
145	28	u	< 1 year	All	3
145	61	u	> 1 year	All	3
152	89	u	All	All	1
173	30	u	All	All	Unknown
188	225	u	All	1, 2, 3	10
200	41	u	All	All	10
239	27	u	All	All	1
246	38	u	All	All	1
254	232	u	All	All	10
260	28	u	All	1, 2, 3, 4	1
276	81	u	All	All	10
277	48	u	All	1, 2, 3, 4	10
278	47	u	All	All	10
280	89	u	All	All	1
288	28	u	All	1, 2, 3, 4	1
295	34	u	All	All	3
306	60	a	All	All	3
315	121	u	All	All	2
317	225	u	All	All	10
332	48	u	All	All	10
335	Unknown	a	All	3	1
337	37	u	All	All	1
378	147	u	All	All	5
386	167	u	Unknown	1, 2, 3	4
386	126	u	Unknown	4	4
387	60	u	All	Unknown	1
388	149	a	All	1, 2, 3	4
388	87	a	All	4	4
465	85	u	> 1 year	4	Unknown
474	12	u	> 1 year	3	10
495	122	a	All	All	3
505	102	u	All	4	10
506	237	a	All	All	Unknown
528	67	a	All	All	3
540	32	u	All	All	Unknown
548	319	u	All	1	3



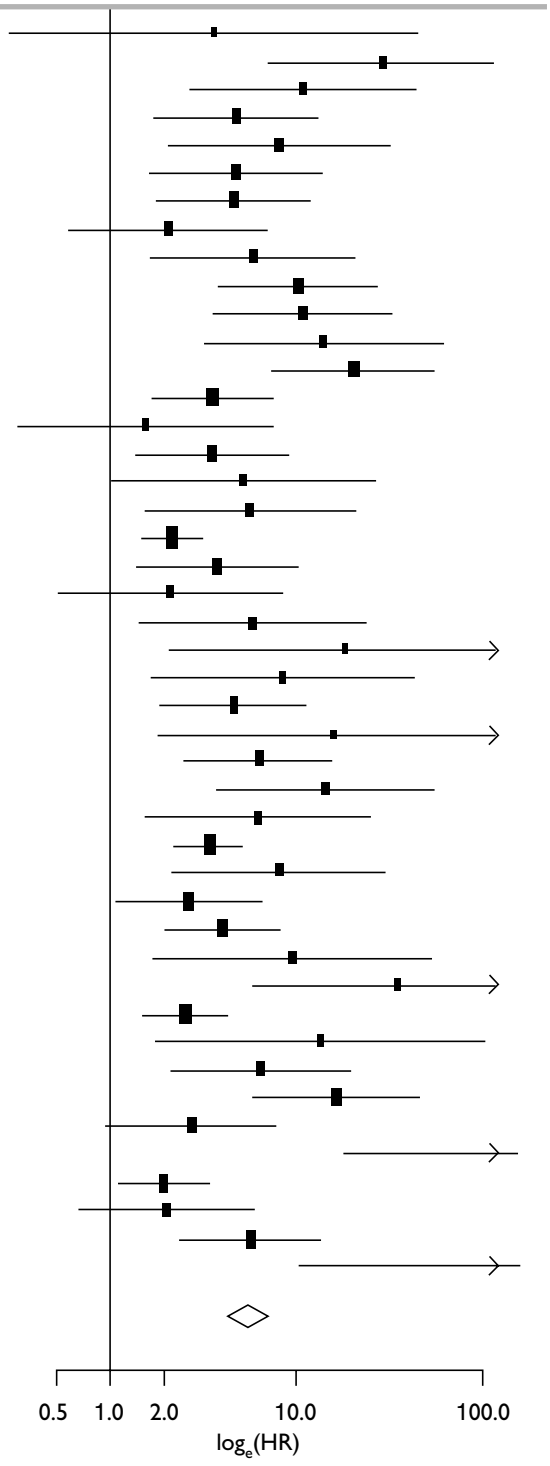
Pooled HR (95% CI): 4.28 (3.34 to 5.49)

NB. For the two results for DFS from paper 122 the one for gene rather than N-Myc mRNA was used; for the two results from paper 200 the result for a cut-off of ten copies rather than positive/negative was used; and for the two results from paper 337 the one for one copy rather than positive/negative was used. See *Table 12*

FIGURE 7 *Individual and pooled HR results for the 13 prognostic markers evaluated in neuroblastoma. The figures show individual and pooled HR results for the prognostic markers, together with the* ***paper*** *number,* ***outcome*** *(OS or DFS),* ***age*** *of patients and their* ***stage*** *of disease, and whether the estimate was unadjusted (u) or adjusted (a). Also, information as to those studies omitted from the meta-analysis is given. HR > 1 indicates a greater instantaneous risk of death (for OS) or disease recurrence/death (for DFS) for patients with high/positive marker levels or those with a marker present, compared with those patients with low/negative levels or without a marker present, respectively. The variable* ***cut-off*** *gives the cut-off level or the (lower, upper) groups. DNA/ chromosome aberrations: (a) MYC-N; (b) DNA index; (c) chromosome 1p. Biological markers: (d) LDH; (e) CD44; (f) NSE; (g) TrkA; (h) ferritin; (i) MDR. Urinary catecholamines: (j) VMA; (k) HVA; (l) VMA: HVA. No meta-analysis was possible for dopamine*

MYC-N (overall survival)

Paper	*n*	u/a	Age	Stage	Cut-off (copies)
27	66	a	All	All	10
39	33	u	All	All	1
40	94	u	All	All	1
44	48	u	All	All	1
50	48	u	All	All	Negative, positive
59	30	a	< 1 year	2, 3, 4, 4S	3
80	48	u	Unknown	All	Negative, positive
87	60	a	All	All	3
93	21	u	All	Unknown	1
106	48	u	All	All	1
107	35	u	All	All	1
108	77	a	Unknown	All	Unknown
109	59	u	All	All	Unknown
111	153	u	All	All	3
173	31	u	All	All	Unknown
181	29	u	All	All	Staining (no/yes)
193	40	u	All	All	4
194	18	u	All	All	Negative, positive
199	492	u	All	All	Unknown
200	41	u	All	All	1
214	26	u	All	3,4	1
216	31	u	All	All	1
246	38	u	All	All	1
247	26	u	All	2, 3, 4, 4S	10
256	32	u	All	3, 4	10
260	28	u	All	1, 2, 3, 4	1
277	48	u	All	1, 2, 3, 4	10
285	30	u	All	All	3
288	29	u	All	3, 4	10
297	295	u	All	All	3
306	60	u	All	All	3
316	43	u	All	All	3
337	57	u	All	All	Negative, positive
356	58	u	All	All	10
373	68	u	All	All	Unknown
376	237	a	All	All	Unknown
387	60	u	All	Unknown	1
393	45	u	All	All	Unknown
396	110	u	Unknown	All	10
469	37	u	> 1 year	Unknown	5
501	18	u	All	All	3
506	237	a	All	All	Unknown
540	32	u	All	All	Unknown
544	81	u	All	All	Unknown
548	319	u	All	1	3



Pooled HR (95% CI): 5.48 (4.30 to 6.97)

NB. For the two results for OS from paper 87 the one for three copies rather than mean of gene was used; for the two results from paper 200 we chose the one for one copy rather than positive/negative; for the two results from paper 469 we chose the one for five copies rather than protein. See *Table 12*

FIGURE 7* contd** *Individual and pooled HR results for the 13 prognostic markers evaluated in neuroblastoma. The figures show individual and pooled HR results for the prognostic markers, together with the* ***paper *number,* ***outcome*** *(OS or DFS),* ***age*** *of patients and their* ***stage*** *of disease, and whether the estimate was unadjusted (u) or adjusted (a). Also, information as to those studies omitted from the meta-analysis is given. HR > 1 indicates a greater instantaneous risk of death (for OS) or disease recurrence/ death (for DFS) for patients with high/positive marker levels or those with a marker present, compared with those patients with low/negative levels or without a marker present, respectively. The variable* ***cut-off*** *gives the cut-off level or the (lower, upper) groups. DNA/ chromosome aberrations: (a) MYC-N; (b) DNA index; (c) chromosome 1p. Biological markers: (d) LDH; (e) CD44; (f) NSE; (g) TrkA; (h) ferritin; (i) MDR. Urinary catecholamines: (j) VMA; (k) HVA; (l) VMA: HVA. No meta-analysis was possible for dopamine*

(b) DNA index

Outcome	Paper	*n*	u/a	Age	Stage	Cut-off
DFS	280	89	u	All	All	Diploid/aneuploid
	102	59	u	All	All	Diploid/near triploid
	295	34	u	All	All	Diploid or tetraploid/aneuploid
	239	27	u	All	All	Diploid/aneuploid
	109	51	u	All	All	1 > 1
	548	31	u	All	Stage 1	1 > 1
	410	52	u	< 1 year	All	Diploid/triploid
Pooled HR (95% CI): 0.33 (0.20 to 0.56)						
OS	117	41	u	All	All	Diploid/aneuploid
	46	68	a	All	All	Aneuploid (no/yes)
	199	654	u	All	All	1 > 1
	393	45	u	All	All	Diploid or tetraploid/atriploid
	93	21	u	All	All	Diploid or tetraploid/hyperploid or triploid
	40	92	u	< 2 years	All	1 > 1
	469	57	u	> 1 year	Unknown	1.2
	234	54	u	All	All	Diploid/aneuploid
	109	51	u	All	All	1 > 1
	548	31	u	All	Stage 1	1 > 1
	193	40	u	All	All	Diploid/aneuploid
Pooled HR (95% CI): 0.31 (0.20 to 0.48)						

NB. The DFS estimate of triploid versus tetraploid from paper 410 was omitted because it was not classifying the same groups as the other estimates, which compared diploid (DNA index = 1) versus others. See *Table 13*

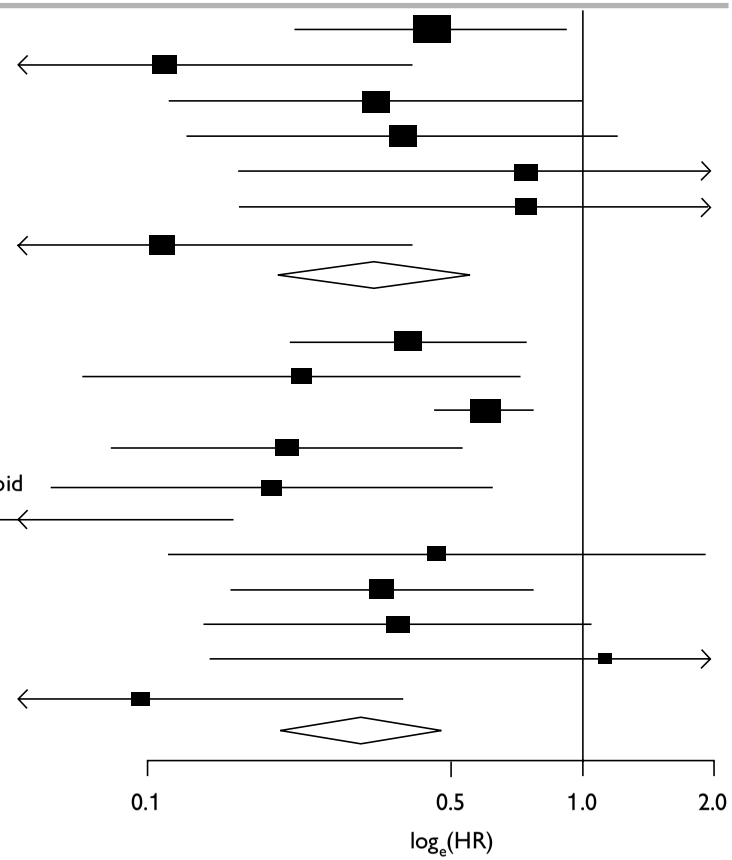


FIGURE 7* contd** *Individual and pooled HR results for the 13 prognostic markers evaluated in neuroblastoma. The figures show individual and pooled HR results for the prognostic markers, together with the* ***paper *number,* ***outcome*** *(OS or DFS),* ***age*** *of patients and their* ***stage*** *of disease, and whether the estimate was unadjusted (u) or adjusted (a). Also, information as to those studies omitted from the meta-analysis is given. HR > 1 indicates a greater instantaneous risk of death (for OS) or disease recurrence/ death (for DFS) for patients with high/positive marker levels or those with a marker present, compared with those patients with low/negative levels or without a marker present, respectively. The variable* ***cut-off*** *gives the cut-off level or the (lower, upper) groups. DNA/ chromosome aberrations: (a) MYC-N; (b) DNA index; (c) chromosome 1p. Biological markers: (d) LDH; (e) CD44; (f) NSE; (g) TrkA; (h) ferritin; (i) MDR. Urinary catecholamines: (j) VMA; (k) HVA; (l) VMA: HVA. No meta-analysis was possible for dopamine*

(c) Chromosome 1p

Outcome	Paper	*n*	u/a	Age	Stage
DFS	188	91	u	All	1, 2, 3
	280	89	u	All	All
	386	74	a	Unknown	1, 2, 3
	295	32	u	All	All
	403	52	u	All	Unknown
	506	238	u	All	All
	80	35	u	Unknown	All
	173	30	u	All	All
	277	50	u	All	1, 2, 3, 4
Pooled HR (95% CI): 3.93 (2.31 to 6.68)					
OS	338	53	u	All	All
	111	156	u	All	All
	109	59	u	All	All
	544	58	u	All	2, 3, 4, 4S
	93	21	u	All	All
	80	35	u	Unknown	All
	106	48	u	All	All
	173	30	u	All	All
	277	50	u	All	1, 2, 3, 4
	506	237	a	All	All
	188	91	u	All	1, 2, 3
Pooled HR (95% CI): 3.12 (1.95 to 4.98)					

NB. For all these estimates the groups were 1p deletion not present versus 1p deletion present. No estimates were excluded from the meta-analysis

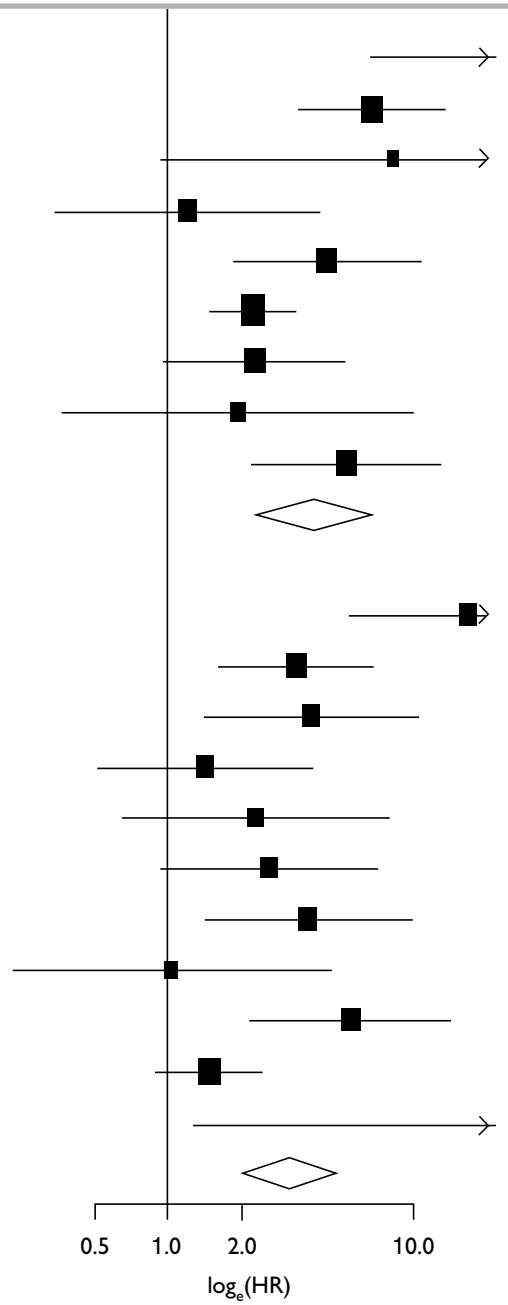
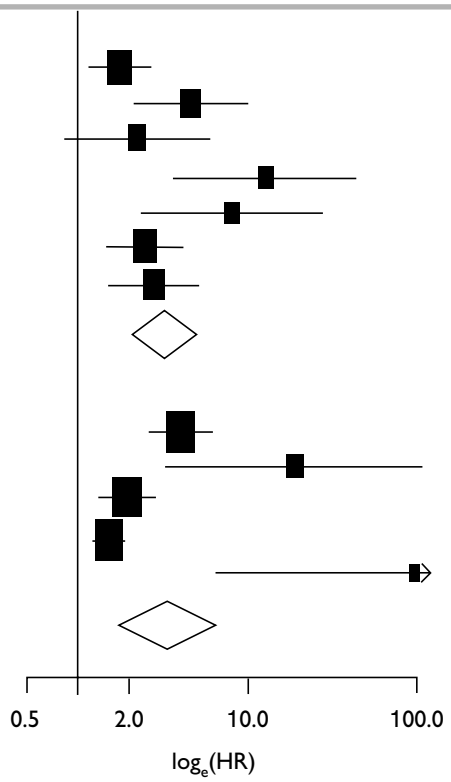


FIGURE 7* contd** *Individual and pooled HR results for the 13 prognostic markers evaluated in neuroblastoma. The figures show individual and pooled HR results for the prognostic markers, together with the* ***paper *number,* ***outcome*** *(OS or DFS),* ***age*** *of patients and their* ***stage*** *of disease, and whether the estimate was unadjusted (u) or adjusted (a). Also, information as to those studies omitted from the meta-analysis is given. HR > 1 indicates a greater instantaneous risk of death (for OS) or disease recurrence/ death (for DFS) for patients with high/positive marker levels or those with a marker present, compared with those patients with low/negative levels or without a marker present, respectively. The variable* ***cut-off*** *gives the cut-off level or the (lower, upper) groups. DNA/ chromosome aberrations: (a) MYC-N; (b) DNA index; (c) chromosome 1p. Biological markers: (d) LDH; (e) CD44; (f) NSE; (g) TrkA; (h) ferritin; (i) MDR. Urinary catecholamines: (j) VMA; (k) HVA; (l) VMA: HVA. No meta-analysis was possible for dopamine*

(d) LDH

Outcome	Paper	*n*	u/a	Age	Stage	Cut-off (U/l)
DFS	388	341	a	All	1, 2, 3	Unknown
	280	87	u	All	All	1500
	495	122	a	All	All	300/400
	188	104	u	All	1, 2, 3	2 x normal
	317	104	u	All	1, 2, 3	2 x normal
	8	182	a	All	4	Unknown
	9	96	a	All	1, 2, 3	Unknown
Pooled HR (95% CI): 3.20 (2.06 to 4.98)						
OS	297	268	u	All	All	1000
	14	120	u	All	1, 2, 3, 4S	1500
	14	162	u	All	4	1500
	199	1149	u	All	All	1500
	40	92	u	All	All	1500
Pooled HR (95% CI): 3.36 (1.72 to 6.57)						

NB. No estimates were excluded from the meta-analysis



(e) CD44

Paper	*n*	u/a	Age	Stage	Cut-off
279	52	u	Unknown	All	Negative/positive
315	121	u	All	All	Negative/positive
384	52	u	All	All	Negative/positive
Pooled HR (95% CI): 0.06 (0.02 to 0.21)					

NB. No estimates were excluded from the meta-analysis

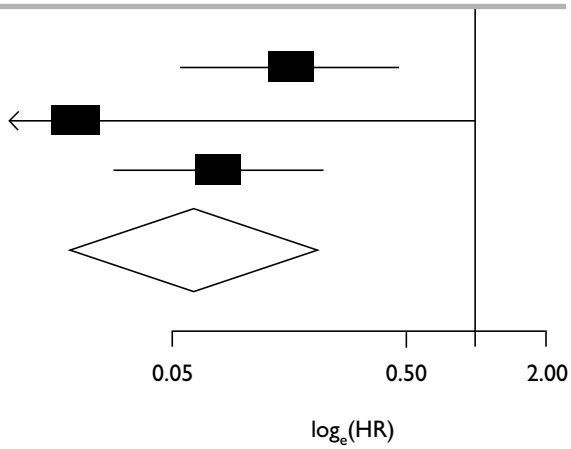
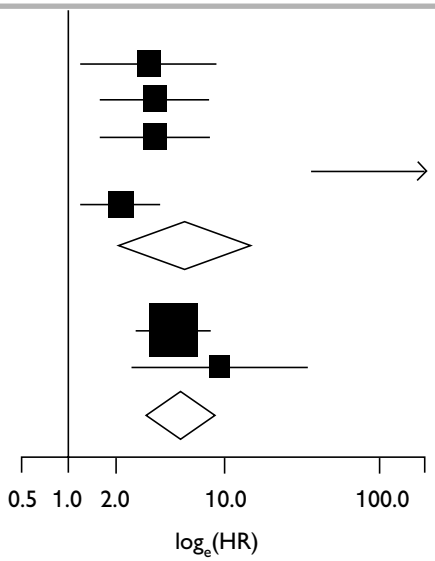


FIGURE 7* contd** *Individual and pooled HR results for the 13 prognostic markers evaluated in neuroblastoma. The figures show individual and pooled HR results for the prognostic markers, together with the* ***paper *number,* ***outcome*** *(OS or DFS),* ***age*** *of patients and their* ***stage*** *of disease, and whether the estimate was unadjusted (u) or adjusted (a). Also, information as to those studies omitted from the meta-analysis is given. HR > 1 indicates a greater instantaneous risk of death (for OS) or disease recurrence/ death (for DFS) for patients with high/positive marker levels or those with a marker present, compared with those patients with low/negative levels or without a marker present, respectively. The variable* ***cut-off*** *gives the cut-off level or the (lower, upper) groups. DNA/ chromosome aberrations: (a) MYC-N; (b) DNA index; (c) chromosome 1p. Biological markers: (d) LDH; (e) CD44; (f) NSE; (g) TrkA; (h) ferritin; (i) MDR. Urinary catecholamines: (j) VMA; (k) HVA; (l) VMA: HVA. No meta-analysis was possible for dopamine*

(f) NSE

Outcome	Paper	*n*	u/a	Age	Stage	Cut-off (ng/ml)
DFS	278	47	u	All	All	0.4
	188	155	u	All	1, 2, 3	2 x normal
	317	155	u	All	1, 2, 3	2 x normal
	482	63	u	All	1, 2, 3	80
	482	78	u	All	4	80
Pooled HR (95% CI): 5.56 (2.11 to 14.66)						
OS	297	169	u	All	All	100
	407	35	a	All	4	100
Pooled HR (95% CI): 5.22 (3.12 to 8.73)						

NB. The OS estimates from papers 185 and 259 were excluded from the meta-analysis because they compared different groups of NSE patients than the other estimates (see *Table 17*)



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- number of patients
- age/stage distribution
- number of serial measurements.

The combination of these problems with the relatively small number of papers identified meant that any meta-analysis using these studies was not worthwhile, both practically and clinically.

Publication bias

An assessment was made of the possible publication bias in the neuroblastoma prognosis literature by studying the estimates of $\log_e$(HR) and its variance obtained for MYC-N. *Figure 8* presents a funnel plot for the OS estimates of $\log_e$(HR) for MYC-N. Visual inspection indicated a considerable degree of asymmetry, which suggested some studies with small HRs were missing at the bottom right hand corner of the plot. Such an assessment was confirmed by the two statistical tests for asymmetry which both produced p values < 0.001.

Seventeen studies were estimated as missing in addition to the 45 included in the analysis. The missing studies are displayed in the funnel plot of *Figure 9*. As an **exploratory** analysis, we included these studies, using a random effect model, and the pooled HR reduced from 5.48 (95% CI = 4.30 to 6.97) to 3.44 (95% CI = 2.64 to 4.49). Hence, although a prognostic benefit of MYC-N remained after adjusting for potential publication bias, it appears that the effect size from the original meta-analysis might be biased upwards, that is, an overestimate of the true underlying $\log_e$(HR).

The assessment of the DFS estimates for MYC-N produced similar results with the funnel plot, again indicating considerable asymmetry, which was confirmed by both Eggers' ($p < 0.001$)) and Begg's ($p = 0.005$) tests (see appendix 12). Adjustment of the funnel using trim and fill suggested that nine studies were missing (in addition to the original 43). The funnel plot displaying these imputed studies is presented in appendix 12.

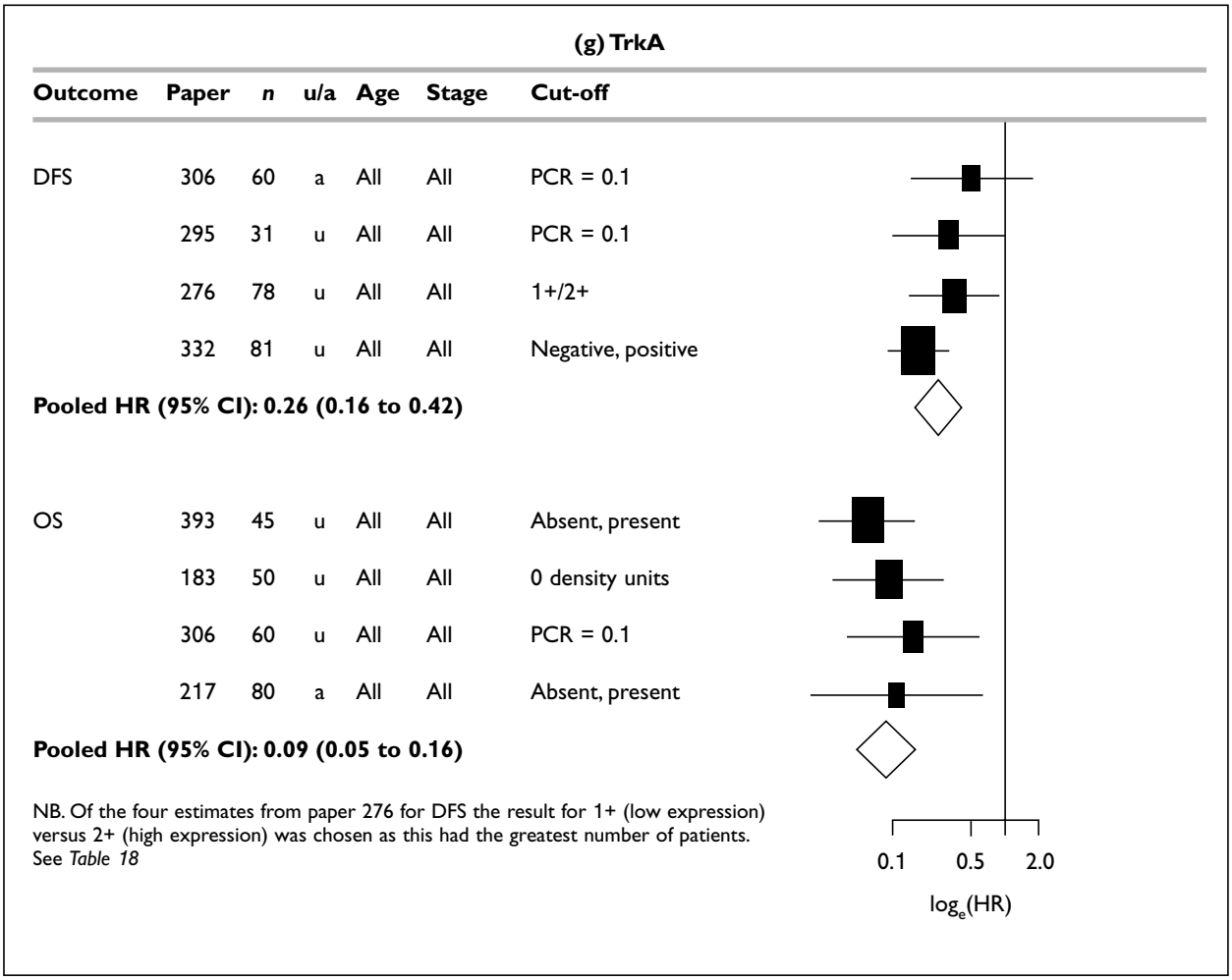


FIGURE 7* contd** *Individual and pooled HR results for the 13 prognostic markers evaluated in neuroblastoma. The figures show individual and pooled HR results for the prognostic markers, together with the* ***paper *number,* ***outcome*** *(OS or DFS),* ***age*** *of patients and their* ***stage*** *of disease, and whether the estimate was unadjusted (u) or adjusted (a). Also, information as to those studies omitted from the meta-analysis is given. HR > 1 indicates a greater instantaneous risk of death (for OS) or disease recurrence/death (for DFS) for patients with high/positive marker levels or those with a marker present, compared with those patients with low/negative levels or without a marker present, respectively. The variable* ***cut-off*** *gives the cut-off level or the (lower, upper) groups. DNA/ chromosome aberrations: (a) MYC-N; (b) DNA index; (c) chromosome 1p. Biological markers: (d) LDH; (e) CD44; (f) NSE; (g) TrkA; (h) ferritin; (i) MDR. Urinary catecholamines: (j) VMA; (k) HVA; (l) VMA: HVA. No meta-analysis was possible for dopamine*

Again in an **exploratory** analysis, adjusting for funnel plot asymmetry in this way reduced the pooled HR from 4.28 (95% CI = 3.34 to 5.49) to 3.43 (95% CI = 2.65 to 4.45). Hence, a prognostic effect still exists after adjusting for publication bias, but the effect size is smaller than that originally found.

Economic and psychosocial results

No papers made an economic evaluation of the use of tumour markers in neuroblastoma, but two papers reported cost data in relation to screening.[100,101] They are both somewhat dated and contain few details about cost calculations, which makes it difficult to assess the accuracy of the claims made or the relevance of the findings to current practice. A more detailed description of these findings is given in appendix 13.

Even more disappointing, the 428 'relevant' papers did not report any results regarding the psychosocial consequences for children and their families of using tumour markers clinically in neuroblastoma.

Discussion

Appraisal of the systematic review

This is the first systematic review of tumour markers that has been undertaken in neuroblastoma, and

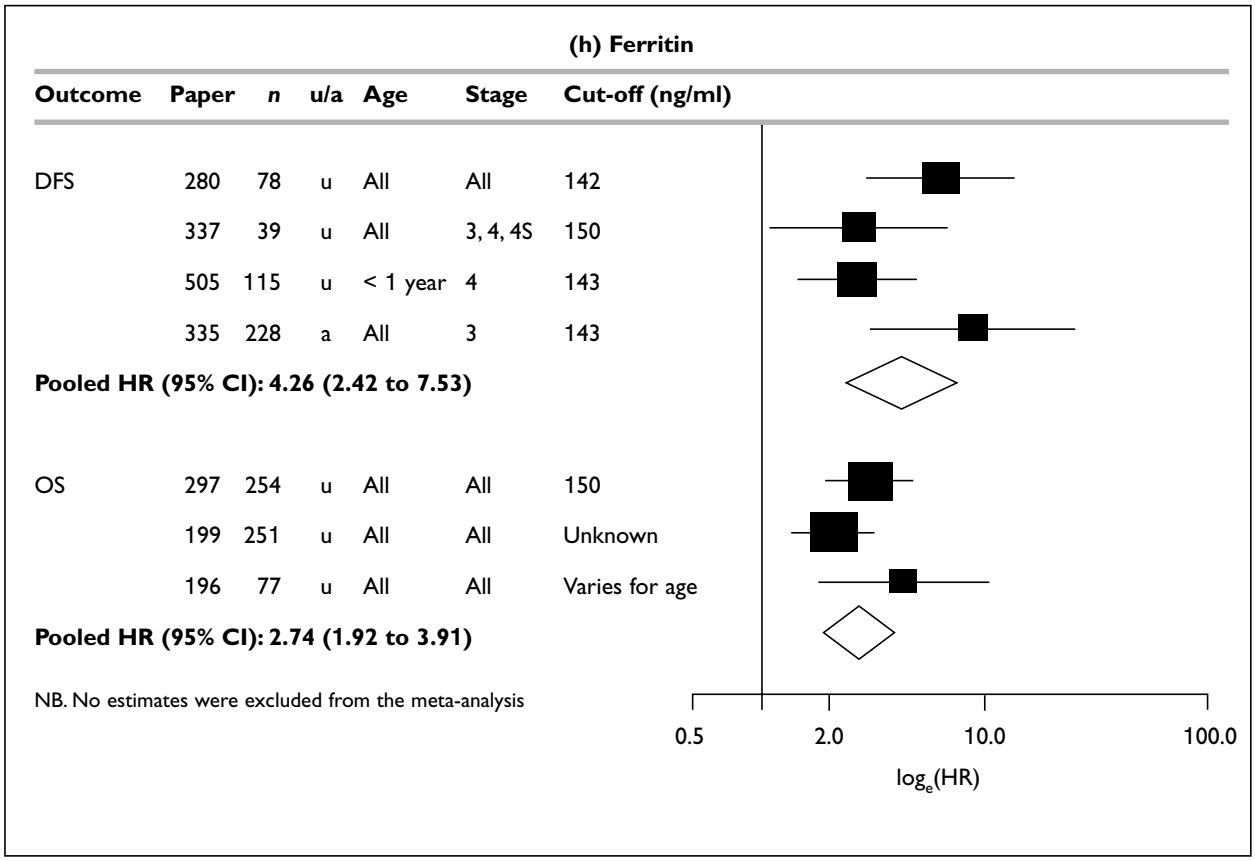


FIGURE 7* contd** *Individual and pooled HR results for the 13 prognostic markers evaluated in neuroblastoma. The figures show individual and pooled HR results for the prognostic markers, together with the* ***paper *number,* ***outcome*** *(OS or DFS),* ***age*** *of patients and their* ***stage*** *of disease, and whether the estimate was unadjusted (u) or adjusted (a). Also, information as to those studies omitted from the meta-analysis is given. HR > 1 indicates a greater instantaneous risk of death (for OS) or disease recurrence/death (for DFS) for patients with high/positive marker levels or those with a marker present, compared with those patients with low/negative levels or without a marker present, respectively. The variable* ***cut-off*** *gives the cut-off level or the (lower, upper) groups. DNA/chromosome aberrations: (a) MYC-N; (b) DNA index; (c) chromosome 1p. Biological markers: (d) LDH; (e) CD44; (f) NSE; (g) TrkA; (h) ferritin; (i) MDR. Urinary catecholamines: (j) VMA; (k) HVA; (l) VMA: HVA. No meta-analysis was possible for dopamine*

forms a knowledge base, pooling information from different studies to obtain overall measures of potential clinical value. We identified 428 papers, which showed diversity in primary interest, methodology, analysis of data and quality of reporting. There were 195 different tumour markers studied, of which 169 were looked at in five or fewer papers. This perhaps reflects the need for better clinical markers in neuroblastoma, leading to an emphasis placed by journals and other publications, and thus clinicians, on investigation of new markers rather than consolidating the knowledge on and applicability of existing markers. This novel review should facilitate the development of future research strategies and improve the standard of scientific reporting by establishing the markers studied so far and specifying the gaps in the literature that need filling in order to properly evaluate tumour markers and make decisions about their clinical value.

During the systematic review we classified 3415 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, although we acknowledge the review may not be fully comprehensive. Only about 10% of the first investigator's 'not relevant' papers were double checked, so it is likely that some papers of relevance were excluded unintentionally. Furthermore, MEDLINE, EMBASE and CANCERLIT were the only databases chosen to identify the literature, although ideally other databases (e.g. SIGLE) and other sources of information (e.g. consultation with researchers) should have been used; this was not considered feasible given the time and resources available in light of the large literature already identified. CANCERLIT provided important articles over

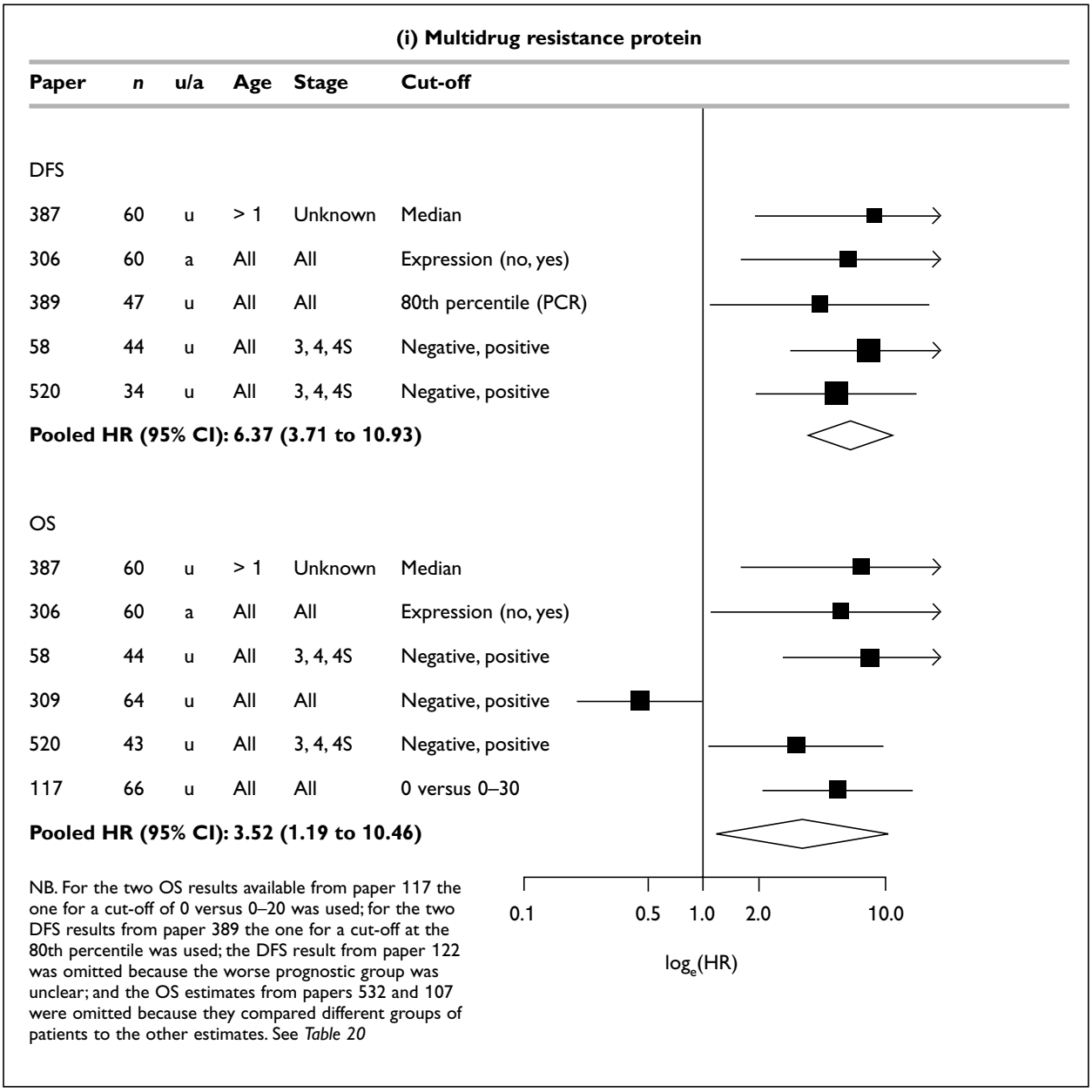
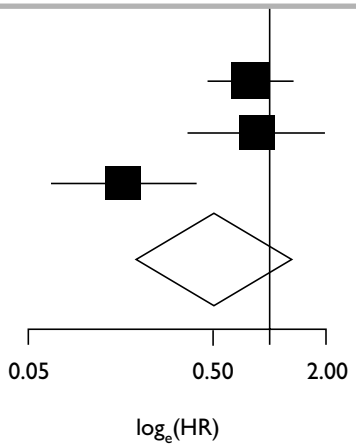


FIGURE 7* contd** *Individual and pooled HR results for the 13 prognostic markers evaluated in neuroblastoma. The figures show individual and pooled HR results for the prognostic markers, together with the* ***paper *number,* ***outcome*** *(OS or DFS),* ***age*** *of patients and their* ***stage*** *of disease, and whether the estimate was unadjusted (u) or adjusted (a). Also, information as to those studies omitted from the meta-analysis is given. HR > 1 indicates a greater instantaneous risk of death (for OS) or disease recurrence/ death (for DFS) for patients with high/positive marker levels or those with a marker present, compared with those patients with low/negative levels or without a marker present, respectively. The variable* ***cut-off*** *gives the cut-off level or the (lower, upper) groups. DNA/ chromosome aberrations: (a) MYC-N; (b) DNA index; (c) chromosome 1p. Biological markers: (d) LDH; (e) CD44; (f) NSE; (g) TrkA; (h) ferritin; (i) MDR. Urinary catecholamines: (j) VMA; (k) HVA; (l) VMA: HVA. No meta-analysis was possible for dopamine*

(j) VMA (overall survival)

Paper	*n*	u/a	Age	Stage	Cut-off
297	269	u	All	All	2.5 SD
544	74	u	All	2, 3, 4, 4S	11 µ/mmol of creatinine
256	32	u	All	3, 4	4.6 mg/day of VMA (urinary value)

Pooled HR (95% CI): 0.50 (0.19 to 1.29)



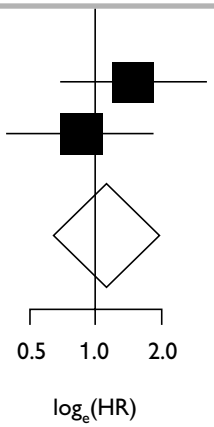
2.5 SD, 2.5 standard deviations from the mean normal value

NB. No estimates were excluded from the meta-analysis; no DFS meta-analysis was possible

(k) HVA (overall survival)

Paper	*n*	u/a	Age	Stage	Cut-off
297	232	u	All	All	2.5 SD
544	73	u	All	2, 3, 4, 4S	26 µmol/mmol of creatinine

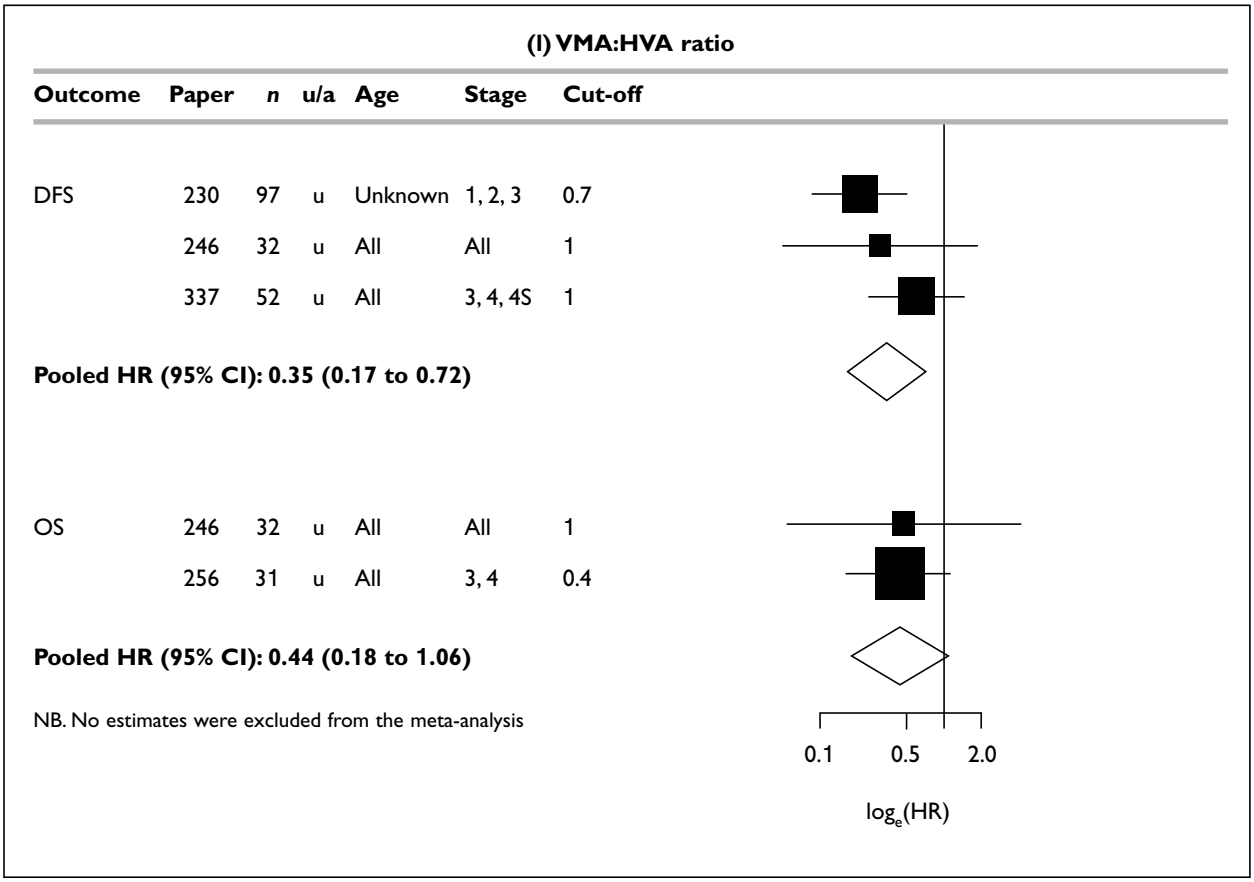
Pooled HR (95% CI): 1.14 (0.65 to 1.98)



2.5 SD, 2.5 standard deviations from mean normal value

NB. No estimates were excluded from the meta-analysis; no DFS meta-analysis was possible

FIGURE 7* contd** *Individual and pooled HR results for the 13 prognostic markers evaluated in neuroblastoma. The figures show individual and pooled HR results for the prognostic markers, together with the* ***paper *number,* ***outcome*** *(OS or DFS),* ***age*** *of patients and their* ***stage*** *of disease, and whether the estimate was unadjusted (u) or adjusted (a). Also, information as to those studies omitted from the meta-analysis is given. HR > 1 indicates a greater instantaneous risk of death (for OS) or disease recurrence/ death (for DFS) for patients with high/positive marker levels or those with a marker present, compared with those patients with low/negative levels or without a marker present, respectively. The variable* ***cut-off*** *gives the cut-off level or the (lower, upper) groups. DNA/ chromosome aberrations: (a) MYC-N; (b) DNA index; (c) chromosome 1p. Biological markers: (d) LDH; (e) CD44; (f) NSE; (g) TrkA; (h) ferritin; (i) MDR. Urinary catecholamines: (j) VMA; (k) HVA; (l) VMA: HVA. No meta-analysis was possible for dopamine*



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and above those found in the more established MEDLINE and EMBASE databases and should certainly be considered for future systematic reviews relating to cancer. Our initial search strategy included the names of those markers known *a priori* to be potentially important, but this list was certainly not exhaustive in light of the large number of markers identified during the review, although we did include more general terms such as 'marker' that will have limited this problem. We do not proclaim either that the review identified the entire set of markers that have ever been studied. On the contrary, the fact that we identified so many suggests there will be some more that have been missed unintentionally.

We had hoped to evaluate the references of all the selected papers to identify any other papers missing from our database but this was not feasible with the time available. Similarly it was not possible to check for duplicates of patients across papers. Our experience with the ESFT review (see chapter 2), which involved only 84 papers, demonstrated the difficulties of assessing duplicate patients, especially when patient populations appear to overlap but not completely. We also did not include foreign language papers because of the difficulties in translation, and this may have introduced bias if only statistically or clinically significant foreign language studies/papers were (re)written for publication in an English language journal. An example of this type of problem has been reported by Egger and colleagues,[102] who showed that investigators based in German-speaking countries tend to publish clinical trials in English language journals if the results are statistically significant but choose German language journals if the results are negative.

TABLE 11 *Meta-analysis results for the 13 prognostic markers evaluated in neuroblastoma*

Marker type	Tumour marker	No. of prognosis papers	No. of occurences of reports	Outcome	No. of estimates obtained	Pooled HR*	95% CI	*p* value
DNA or chromosome abnormalities	MYC-N	151	194	DFS	46	4.28	3.34 to 5.49	< 0.0001
				OS	48	5.48	4.30 to 6.97	< 0.0001
	DNA index	44	62	DFS	8	0.33	0.20 to 0.56	< 0.0001
				OS	11	0.31	0.20 to 0.48	< 0.0001
	Chromosome 1p	40	49	DFS	9	3.93	2.31 to 6.68	< 0.0001
				OS	11	3.12	1.95 to 4.98	< 0.0001
Urinary catecholamines	VMA	36	40	DFS	1	NA	NA	NA
				OS	3	0.50	0.19 to 1.29	0.15
	HVA	26	29	DFS	0	NA	NA	NA
				OS	2	1.14	0.65 to 1.98	0.65
	VMA:HVA	20	28	DFS	3	0.35	0.17 to 0.72	0.0043
				OS	2	0.44	0.18 to 1.06	0.068
	Dopamine	10	11	DFS	1	NA	NA	NA
				OS	1	NA	NA	NA
Biological markers	CD44	8	8	DFS	3	0.06	0.02 to 0.21	< 0.0001
				OS	0	NA	NA	NA
	TrkA	16	21	DFS	7	0.26	0.16 to 0.42	< 0.0001
				OS	4	0.09	0.05 to 0.16	< 0.0001
	NSE	28	39	DFS	5	5.56	2.11 to 14.7	0.0005
				OS	4	5.22	3.12 to 8.73	< 0.0001
	LDH	26	30	DFS	7	3.20	2.06 to 4.98	< 0.0001
				OS	5	3.36	1.72 to 6.57	0.0004
	Ferritin	33	41	DFS	4	4.26	2.42 to 7.53	< 0.0001
				OS	3	2.74	1.92 to 3.91	< 0.0001
	Multidrug resistance protein	16	30	DFS	7	6.37	3.71 to 10.9	< 0.0001
				OS	9	3.52	1.19 to 10.5	0.023

NA, not applicable: meta-analysis not possible because no estimates or only one estimate was available

* *HR > 1 indicates a greater risk of death (for OS) or disease recurrence/death (for DFS) for patients with high/positive marker levels or those with a marker present, compared with those patients with low/negative levels or without a marker present, respectively*

However, whether such a bias exists for other non-English-speaking countries, or indeed observational studies, is unclear.

We also recognise that there may be the common problem of publication bias or reporting bias, in particular results that do not generate formal statistically significant or clinically valuable findings may not be in the public literature. This often happens because of either a reluctance of journals or a reluctance of researchers to present/report negative findings. We demonstrated this problem in the meta-analysis of the MYC-N prognosis results, where there was evidence of asymmetry about the estimate of the HR for both the OS and DFS groups leading to overestimates of the true HR. Adjusting for this using the exploratory trim and fill method reduced our estimate of the HR to a value that was more likely to be closer to the true underlying value. However, this adjusted result must still be interpreted in light of the other problems of poor reporting, and, for such reasons, we did not consider it worthwhile to adjust the meta-analysis results for the other 12 markers, even though publication bias most likely still exists for their results. The concern of publication and reporting bias was one reason why cytogenetic IPD were not used, the others reasons being poor presentation and small study sizes. Hence, markers that were just presented within cytogenetic IPD have been omitted from this review.

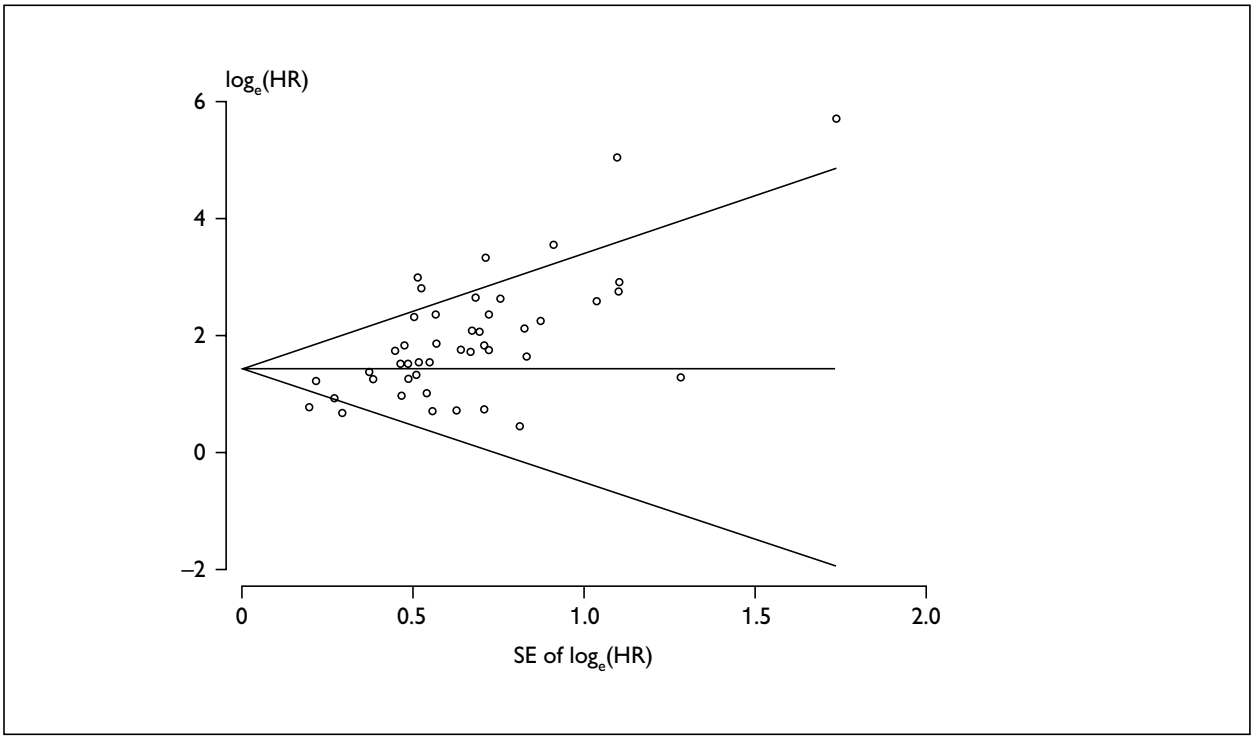

FIGURE 8 *Begg funnel plot (pseudo 95% CI) of log$_e$(HR) for MYC-N and OS*

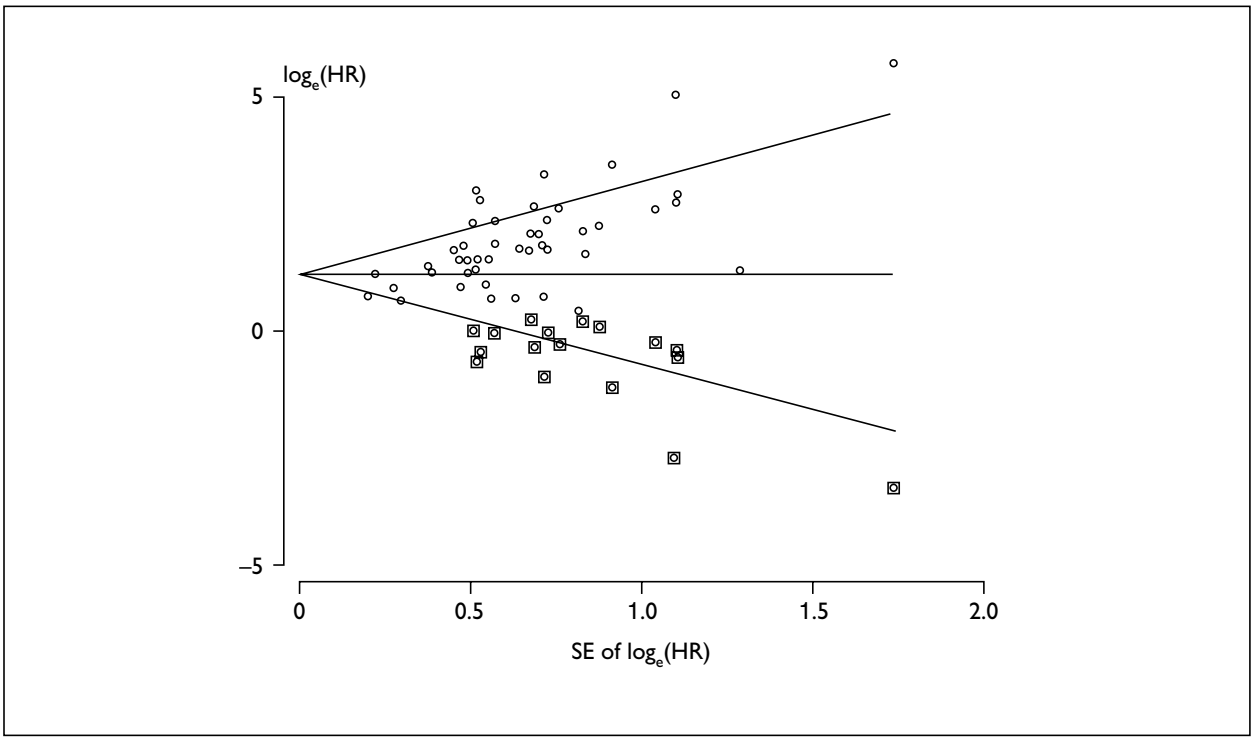

FIGURE 9 *Funnel plot (pseudo 95% CI) of log$_e$(HR) for MYC-N and OS indicating studies estimated as 'missing' by the trim and fill method*

The problems of poor reporting may have amplified the publication bias we observed because insignificant or negative results are often the most poorly reported, making estimates of desired statistics more difficult. We indeed may have added to the publication bias by assuming $p = x$ where $p < x$ was given, but not assuming $p = x$ where $p > x$ or 'not-significant' was presented, and hence excluding the more negative results. This decision was based on the former being a conservative estimate of the HR, and the latter vulnerable to large inaccuracies (e.g. if $p = 0.05$ was assumed where $p > 0.05$ was presented). Fortunately, there were only 13 reports across all the 13 prognostic markers studied for which the p value was required to make estimates of $\log_e$(HR) and $p > x$ or 'not-significant' had been given. Hence, in the light of all the other problems of poor reporting and that a greater number of 'conservative' estimates were made, this is unlikely to cause too much additional concern. In terms of the meta-analyses, the decision to only assess markers studied in three or more papers for each clinical area may have led to some markers being excluded from analysis, even though they were studied in two very large studies. Future reviews should seek to base meta-analysis decisions on numbers of individuals in studies rather than the number of studies. Finally, the extraction of summary statistics, required for the estimation of $\log_e$(HR) and its variance, were not double checked, and thus this could mean some unintentional mistakes have been made. The double-checking process was not feasible given the large literature and large number of statistics that needed extracting.

It should be noted that none of the aforementioned flaws in our search strategy are likely to detract from the main methodological issues (such as poor reporting or the need for individual patient data) we identified, and we have not made strong clinical recommendations about which makers to use based on our results (see below).

Publication bias in the choice of cut-offs

Another facet of publication or dissemination bias is in the choice of marker cut-off point to define groups of patients, whereby the choice of cut-off level or status is specifically chosen to optimise the difference between the groups and produce a result with the maximum statistical or clinical significance possible. This is most likely practised to ensure the most striking result and improve the chances of the study being published. This approach leads to wide variability in reporting, making it extremely difficult to assess the overall evidence from across studies and thus make clinical decisions of how best to use a marker to distinguish groups of patients for different types of treatment and care. For example, at least 13 different cut-offs were chosen for MYC-N and at least ten different cut-offs chosen for LDH in the prognostic literature. We strongly advocate a move away from this approach and appeal to that of **evidence-based** medicine. Research groups should work together to identify the most appropriate cut-off point for each marker and then be consistent in using it to assess the clinical value of the marker. This is particularly important for rare diseases, such as neuroblastoma and other childhood cancers, because sample sizes are most often very small and so the clinical potential of a marker needs to be assessed across a larger number of studies. If the majority of these studies were to use different cut-off points, as is the case in the neuroblastoma literature, then it becomes very hard to form a general consensus or decision about the value of the tumour marker. Alternatively, if agreement on a common cut-off point is not practical, the problem could be overcome by presentation of full IPD, either within the paper or made available on the Internet, including the **exact** tumour marker value for each patient.[67,68] Calculations could then be made independently across studies using the cut-off point of interest.

Treatment received by patients

Another aspect of our review to be considered is that we did not account for the type of treatment that patients received during follow-up. It would have been extremely difficult to incorporate treatment into the meta-analyses because treatments change over time and vary considerably between studies. The complexity of different treatments adds to the problem of clinically interpreting the results we found, a process already made difficult by the general poor standard of reporting. We recommend that the type of treatment is reported for each patient within IPD so that the clinical value of markers for specific treatments can be evaluated more easily, and across studies to obtain bigger patient numbers.

Data extraction and quality of reporting

Weakness and variability in the reporting, analysis and presentation of results were frequently observed throughout the evaluation of the 428 selected papers. The extraction and synthesis of data for the areas of diagnosis and monitoring was very difficult and practically impossible. The large heterogeneity in reporting,

combined with small study sizes, made it hard to evaluate quantitatively the clinical value of the markers studied in these areas. It was particularly disappointing to note the small number of times a healthy control group was compared with patients with neuroblastoma. Boomsma and colleagues[103] give a good example of using a control group to compare marker levels at diagnosis. Even in the commonly studied area of prognosis, the reporting and analysis was so poor that the extraction of estimates required a wide range of indirect methods and an intensive, time-consuming process that highlighted the problems addressed in the recommendations of Altman and colleagues.[69]

The HR and its CI (or $\log_e$(HR) and its variance or CI) provide an important estimate of the difference in risk of death (for OS) or disease recurrence/death (for DFS) between two groups of patients, but this was not acknowledged in the vast majority of the selected papers. The papers did not concentrate on reporting other prognostic statistics either, such as proportions surviving to 2, 3, 5 or 10 years. We looked at the reporting of percentage survival at *n* years in the 26 prognosis papers for LDH, and found that only 12 papers gave estimates of percentage survival, and only six of these also gave a CI or SE.

The indirect methods suggested by Parmar and colleagues[55] proved particularly crucial, and ensured that estimates were obtained despite the poor statistical reporting, enabling a greater number of studies to be included in the results than would otherwise have been possible. However, due to the very nature of these methods, the estimates they provide are only approximate and simply make the best possible use of the results presented. Availability of these and other indirect methods does not overcome inadequate statistical reporting because they only enable more of the available evidence to be included rather than providing the direct estimates required. Questions still exist of how best to combine indirect estimates with direct estimates, and it would be far better to have as many direct estimates as possible. Furthermore, Tierney and colleagues[70] have compared indirect estimates of the HR with direct estimates from IPD and have shown that the indirect estimates often poorly approximate their IPD equivalents.

Taking these points into consideration, we used only those other indirect methods presented by Parmar and colleagues.[55] For example, given some assumptions, we could possibly have used estimates of proportion surviving to 2, 3, 5 or 10 years to obtain estimates of $\log_e$(HR) and its variance. In fact, Vale and colleagues[71] have shown how event rates at fixed-time points could be combined with assumptions about censoring to produce indirect estimates. However, given the findings of Tierney and colleagues,[70] we felt that using this or other indirect methods would add further heterogeneity to our results and made the clinical interpretation of the meta-analysis results even more tenuous. This decision was strengthened by the large heterogeneity observed in the 12 LDH papers that reported percentage survival at *n* years. Five estimates were for OS, six were for DFS and one was unspecified; variability in follow-up time was also observed, with estimates made at 2, 3, 4 or 5 years.

We looked at 535 occurrences in the literature where one of 13 tumour markers had been related to either OS or DFS by summary statistics or IPD, and we sought an estimate of $\log_e$(HR) and its variance from each. We only managed to obtain 204 successful estimates because five common problems were found:

(1) No appropriate statistical analysis performed or results not reported. On 133 occasions an article reported quantitatively the difference in outcome between two or more groups of patients (defined by marker levels) but **either** did not perform a Cox regression analysis or log-rank/Wilcoxon test to compare the survival of the groups, **or** one of these analyses had been performed but no result at all was presented (usually because the result was not statistically significant). Instead, the paper often just reported the number of patients who had an event in one or both of the groups. Thus, on these occasions, $\log_e$(HR) and its variance could not be estimated. It is clearly important that where one of the purposes of the study is to assess the prognostic value of tumour markers that appropriate statistical analyses should also be performed to calculate an estimate of $\log_e$(HR) and its variance (or CI), or indeed another comparative group estimate.

(2) Appropriate analysis performed but the HR not calculated or not reported. On 222 occasions (77 from attempt 1, 145 from attempts 3 and 5) one of these appropriate methods had been performed and their results given, but without a HR. Instead, either a *p* value/χ^2 statistic from the analysis ($n = 210$) or only a survival curve ($n = 12$) was presented. $\log_e$(HR) and its variance

(or CI) are easily calculated from these appropriate methods using most statistical software packages.

(3) Group numbers and group events not given. Where the reporting was as in point 2, indirect methods were used. These uncovered a deeper level of poor reporting than first realised, with the observation that the number of patients and the number of events in each group was often not reported. Only 104 (53.6%) successful indirect estimates were made in 194 of the occasions where only a p value/χ^2 statistic was reported. This was because one or both of the group numbers and/or group events were not reported and could not even be estimated from figures or tables. The number of patients and events in the groups defined by tumour marker levels are often smaller than the overall numbers because of missing or incomplete patient data, and so it is important to provide information for the groups themselves.

(4) Inexact p values provided. Another aspect of the poor reporting was that p values were not given exactly, often being reported as $p < 0.01$, $p < 0.05$ or $p > 0.05$. For approximately 126 of the 273 occasions where a p value was presented '$p < x$' was reported, and only 40 out of the 273 occasions presented an appropriate χ^2 statistic. Assuming $p = x$ where actually $p < x$ was true, as we did, adds further inaccuracy to the indirect methods if the p value is used. Additionally, there were 13 other occasions where the p value was needed but $p > x$ had been presented. This shows an emphasis placed on the p value for a significant result, rather than providing the recommended HR and CI (or $\log_e$(HR) and CI and/or variance) (see point 5, below).

(5) HR not generally reported, or often reported without a CI or variance. Only 57 (27.9%) of the 204 successful estimates of the HR or $\log_e$(HR) were direct estimates provided within the paper. For only three of these was a variance also reported, which is perhaps not surprising given the difficult interpretation of the variance (or the SE) of an estimate. A CI is more interpretable, and this was provided on 37 of the remaining 54 occasions; a p value (without a CI) was provided for the other 17. So, for those articles that did present an HR a good proportion also provided a variance or CI (70%). However, 30% still place more emphasis on a p value rather than a CI. A CI is preferred because it provides a range within which we are 95% certain that the true HR we are estimating lies. It is also very easy to calculate the variance of $\log_e$(HR) when a CI is provided. An indirect estimate of the variance of $\log_e$(HR) is possible given the ($\log_e$) HR and a p value, but it often proves more difficult, especially if the p value is not given exactly.

There is some evidence that the reporting of prognostic tumour marker data has improved over the last 10 years because all the papers that did present a HR were published in 1991 or later. This is encouraging, but these papers still only represent a very small proportion of the prognostic literature available over this period, so we need to continue to strive for improvement.

Adjustment factors

Our decision to obtain unadjusted estimates of $\log_e$(HR) and its variance in preference to adjusted estimates was justified on the evidence of the wide variability in adjustment factors observed. It is clear that once important prognostic markers have been identified they need to be evaluated against other clinically useful prognostic tools, such as histological characteristics, or indeed other tumour marker levels. However, if authors are inconsistent in the adjustment factors they use it becomes very difficult and impractical to pool results across studies and make a proper evaluation of markers **over and above** other factors. We recommend research groups collaborate and identify the most important clinical factors that need to be considered whenever assessing the benefits of a marker for clinical practice. These factors will often need updating and must be made accessible (e.g. on the Internet) so that they can be consistently used as adjustment factors whenever markers are assessed. Presentation of these identified factors within IPD, alongside the exact marker levels and complete survival information (see below), is also recommended because it would allow adjusted estimates to be made independently across studies using the adjustment factors of interest.

Recommendations for improved reporting and benefits of IPD

It is imperative that the quality of statistical reporting improves if clear conclusions and policy recommendations are to be formed about tumour markers. Altman and Lyman[72] have proposed important guidelines for both conducting and evaluating prognostic marker studies. Alongside these, we have developed simple guidelines on how to report results to facilitate both interpretation of individual studies and the undertaking of syste-

matic reviews, meta-analysis and, ultimately, evidence-based practice (see *Box 1* in chapter 2). We recommend that in primary studies which evaluate the use of a tumour marker for prognosis that the HR is reported together with its 95% CI (or $\log_e$(HR) and CI/variance) and also an **exact** *p* value when comparing two or more groups of patients (Collett[73] provides details of how to do this). Furthermore, it is important to explicitly define the prognostic groups being compared, by reporting the specific marker level or status that distinguishes them; and also report the group sizes and the number of events in each patient group. If a *p* value is presented it is crucial to report its **exact** value and/or provide the **exact** value of the appropriate test statistic (e.g. χ^2 statistic) from which it is calculated. If it is not reasonable to report the exact *p* value because it is very small, say less than 0.001, then '$p < 0.001$' should be presented together with the **exact** test statistic. These recommendations should be applied to **all markers** studied, not just those that are significant.

Presentation of full IPD **for all markers** considered is also desirable, where possible including the exact initial marker level, time of disease recurrence (if appropriate), follow-up period and final status, so that the HR, and other statistics or information of interest, may be calculated if required. Forty-one of the 204 estimates obtained were direct estimates calculated from IPD that would not have otherwise been possible. The availability of IPD has allowed important evidence-based reviews to be made in other cancer settings,[74,75] and the feasibility for provision of IPD for rare diseases such as neuroblastoma is high because of the small study sizes. If it is not appropriate to provide IPD within a paper itself, then there is the opportunity to publish on the Internet.[67] Of course, even making individual patient data available on the Internet is not without its problems, with the non-permanency of individual web pages, and so perhaps **a central repository to collate and manage individual patient data is needed within each disease area**. The United Kingdom Children's Cancer Study Group has already initiated this type of approach within paediatric oncology.[76]

The presentation of full IPD would overcome all the following problems of poor reporting:

- no appropriate analysis presented
- no presentation of HR and CI (or $\log_e$(HR) and CI and/or variance)
- group numbers and events not given
- inexact *p* values presented
- variability in marker cut-off level chosen
- variability in type of estimates made, that is, unadjusted and adjusted
- different outcome assessed, that is, OS and DFS
- variability in adjustment factors
- results only given for a few of the markers considered (publication/reporting bias).

In defence of some of the poor reporting observed, some authors may argue that the analysis/presentation of prognostic data was only a secondary part of their study. However, it is clearly important to analyse and report prognostic data to the guidelines above whenever or however it is studied. Presentation of IPD would, of course, remove this area of concern by enabling estimates to be made where prognostic data were available but not used or reported as needed.

The potential for substantial differences in meta-analysis of survival data when using results provided within the literature instead of IPD has recently been shown in the head and neck cancer literature.[77] In addition, Stewart and Parmar[78] recommend that, whenever possible, meta-analysis using IPD is preferred because it produces the least biased answers and the most appropriate way of addressing questions that have not been or could not be resolved by individual clinical trials. In the area of tumour markers, presentation of IPD would enable more appropriate meta-analysis and thus an improved way to synthesise the literature and develop an **evidence-based** perspective of the most appropriate markers to use. Furthermore, IPD would also allow an evaluation of combinations of markers, which may enable more specific and accurate prognostic assessments. For those researchers considering future systematic reviews of tumour markers we recommend they seek to obtain IPD wherever possible, as this is likely to be the most productive. Our extraction procedure originally sought to obtain direct and indirect estimates (method 1) in preference to using IPD (method 2) because it was felt this would be quicker, but given the poor reporting found we would, in retrospect, now seek to obtain and use IPD first. Of course, the standard of reporting IPD also needs improving in order to maximise the potential of this approach (see *Box 1* in chapter 2).

Clinical interpretation of markers and results

Neuroblastoma is a multifaceted disease. The proliferation of biological and cytogenetic markers is an indication that the cancer process is complex,

with multiple changes taking place with a neuroblastoma cell. Research studies have tried to identify which of these markers are initiating factors contributing to the cancer phenotype and which may be regarded as secondary. Initial studies for a particular marker have inevitably sought to link that marker with survival in order to establish its importance. However, it is now becoming clear that a number of key biological markers are likely to be interlinked and need to be judged alongside one another and not in isolation.

This study produced no unexpected clinical results, but it has highlighted the problems related to reporting of an individual marker. Many of the studies do not allow for adequate extraction of data in order for comparisons to be made. For example, we had hoped to assess the overall benefits of individual markers using decision models that incorporated the clinical, economic and psychosocial findings; however, this was not possible given the poor reporting and lack of informative data in the literature. Furthermore, there was a large variability in cut-off points and other measures as indicated in the statistical/reporting discussion above, limiting evaluations of markers across all patients and in specific subgroups (e.g. age < 1 year). It is now clear that comparisons need to be made, not only with equivalent studies of the same marker but also with other key markers at the same time. Recent publications[90] reflect this understanding, and our review provides a benchmark for statistical reporting in future studies. In particular, availability of IPD would facilitate assessment of combinations of markers.

The poor and heterogeneous reporting restricted any quantitative synthesis in the areas of screening, diagnosis and monitoring, and therefore any overall clinical evaluation. Recent papers, published since the start of our review, suggest that there is currently insufficient evidence to support a screening programme for infants up to 6 months of age, and the majority of authors[104] conclude that it should be discontinued. Clinical and histopathological features, which have not been evaluated in our review, are the most informative for the diagnosis of neuroblastoma, and these include age at diagnosis, tumour histology and primary tumour site. However, the detection of catecholamine metabolites in urine is also used for the differential diagnosis of neuroblastoma from other small round cell tumours of childhood. For the use of molecular and biological markers in diagnosis, the small number of studies comparing a healthy control group to patients with neuroblastoma was particularly disappointing, and future studies need to address this. Similarly, monitoring studies need to report the differences in serial marker measurements between those who develop a recurrence of disease and those who remain disease-free, preferably for a large number of patients over a long follow-up period. Where possible, research groups need to collaborate and pool resources to enable bigger sample sizes and achieve consistency across studies, which should be targeted to address the important issues. Only then will the benefits of using tumour markers for screening, diagnosis and monitoring be properly ascertainable.

This systematic review did produce an evaluation of the most commonly reported individual markers for prognosis, although the results are subject to the problems of poor and heterogeneous reporting; MYC-N, chromosome 1p, DNA index, VMA:HVA ratio, CD44, TrkA, NSE, LDH, ferritin and multidrug resistance protein were all identified as potentially important prognostic tools. Current studies[88,90,91,105–108] have also indicated that chromosome 17q gains have important prognostic significance. A number of these studies have been published since the start of this review and consequently this cytogenetic marker was not examined in detail in this review. However, in light of this current knowledge, we have subsequently extracted, wherever possible, HR results from each of the eight prognosis papers our review identified for this marker. Meta-analysis of these suggests that patients who had gain of chromosome 17q were associated with a significantly worse DFS (HR = 4.16, 95% CI = 2.56 to 6.77) and OS (HR = 4.30, 95% CI = 2.70 to 6.86) compared with those who did not. However, these results are again subject to the problems of poor reporting, heterogeneity and bias, and thus clinical interpretation is difficult. Future studies need to include chromosome 17q alongside the other relevant prognostic markers, in particular MYC-N. Unfortunately, we did not have the time to try and use the IPD found to assess **combinations** of the prognostic markers, but this is certainly another area that needs more research in the future.

The multiplicity and complexity of these markers emphasises the need for studies to be coordinated by large cancer research groups utilising multiple laboratories if meaningful results are to be obtained. In particular, collaboration is needed to facilitate the pooling of IPD, a strategy which would adequately ensure a quantitative synthesis (rather than a merely qualitative synthesis) to address the questions of interest,

such as which combinations of markers provide the best prognostic tools, or whether monitoring patients with neuroblastoma using markers is cost-effective. Of course, with the current rapid growth of genetic epidemiology, many new genetic markers and genetic sequences may be identified that supersede the markers we have identified in this review. Studies of genetic markers and sequences also need to consider the guidelines we form in this review for reporting results, in particular the need to make IPD available, if they are to avoid the problems we have identified and facilitate evidence-based reviews.

Economic and psychosocial issues

Once a tumour marker has been identified as clinically effective, the decision to use the marker in practice (e.g. for screening or monitoring) also involves the cost of its implementation and the psychological impact it has on patients; hence, it was disappointing to identify such large gaps in these areas in the neuroblastoma literature. However, this perhaps reflects the uncertainty about which markers have enough clinical effectiveness and importance to warrant subsequent economic and psychosocial studies.

The fact that no papers considered an economic evaluation of tumour markers in neuroblastoma highlights the literature gap in this area. Two papers[100,101] reported cost data in the screening of an apparently healthy population using VMA and/or HVA levels in urine. The implications were that this screening procedure was associated with both lower costs and better outcomes. However, they are both somewhat dated (1982 and 1987) and contain few details on cost calculations, which makes it difficult to assess the accuracy of the claims made or the relevance of the findings to current practice. It is disappointing that no more of the selected papers included either an economic assessment or contained information that would be of use in developing an economic model. In the absence of such information a clear prescription for future trials and studies is to include an economic evaluation element, either in terms of a cost-effectiveness study or the identification of resource use that would permit a decision model to be developed.

Equally unsatisfactory was that our search found no published evidence on the psychosocial consequences for children and their families of using tumour markers in the screening, diagnosis, prognosis or monitoring of neuroblastoma. The lack of work on the psychosocial outcomes of using tumour markers for monitoring purposes was disappointing but reflects the major gap in the neuroblastoma literature for this clinical area. Psychosocial evaluations of tumour markers are clearly important and have been performed for other disease settings.[80] Evidence suggests that some children who survive cancer, and also the parents of such children, are vulnerable to psychological sequelae,[109,110] and may continue to experience high levels of fear about disease recurrence in the years following treatment.[111] For example, it is known that children and families worry a great deal about what they will be told during follow-up appointments and that concerns about relapse are a prominent feature of their concerns during the weeks preceding clinic visits.[112,113] In view of the lack of evidence about the psychosocial consequences of using tumour markers in follow-up monitoring, suggestions that the results of tumour marker tests may be an important source of reassurance for children and their families should be tempered by evidence of the anxiety-provoking nature of follow-up appointments.

Given the extent to which tumour markers have been assessed for prognosis, it is surprising that no psychosocial evaluation has been performed in this clinical area. Knowledge of a tumour marker level may have severe consequences for the psychological well-being of patients and their families following diagnosis or treatment, and this needs to be assessed. Once a tumour marker has been deemed clinically effective, future research on this marker should include an assessment of the psychosocial outcomes of using it in practice, particularly for prognostic and monitoring purposes. We also recommend more general work to investigate how the purpose and results of tumour marker tests should be best communicated to children with neuroblastoma and to their families.

Recommendations for future research

This systematic review has emphasised the uncertainty in the use of many of the studied tumour markers in neuroblastoma, reflecting the small size of many studies and poor statistical reporting, and also the need for large, multicentre quality-controlled studies. This would enable the potential of individual markers in prognosis, monitoring and, possibly, diagnosis to be evaluated, and also allow combinations of markers to be assessed. It would also enable markers in subgroups of patients (e.g. different ages or treatments) to be studied more easily. Comparison of marker levels from patients with neuroblastoma to those in a healthy population is critical, whilst cost and psychosocial issues should also be

measured and evaluated. Histological markers should also be assessed and compared to the more genetic/biological markers we have considered in this review.

We have already discussed how the reporting of data can be improved, although we again draw attention to the potential of IPD to overcome the majority of problems we have found if it is presented for **all the markers** studied, even for those which are not significant. The presentation of full cytogenetic IPD for all abnormalities studied is especially recommended. Histological characteristics and other important clinical factors should also be given in the IPD so that their clinical power can be evaluated and compared with other markers. The type of treatment received by each patient should also be reported.

We emphasise the importance of **evidence-based** medicine, and encourage research groups to collaborate in order to establish the most important aspects of the markers currently available whilst working towards the identification of new markers. This is particularly important for rare diseases, such as neuroblastoma and other childhood cancers, because sample sizes are most often very small and so the clinical potential of a marker needs to be assessed across a larger number of studies. Agreement would also be needed as to which markers to measure; we have provided a base that highlights the ones reported in greatest detail so far.

Chapter 4

Summary of key results

- **First systematic review of tumour markers**. We have performed the first systematic review of studies of tumour markers in ESFT and neuroblastoma, forming two knowledge bases.
- **Large numbers of papers**. We screened 4510 papers overall and identified 84 papers in the ESFT literature and 428 papers in the neuroblastoma literature that studied the use of a tumour marker for clinical purposes.
- **Large numbers of markers**. Seventy different markers in ESFT and 195 different markers in neuroblastoma were identified. The majority of markers were studied in small numbers of papers, for example 169 of the markers in the neuroblastoma literature featured in five papers or fewer.
- **Benefits of screening still uncertain**. No papers looked at the use of markers to screen apparently healthy patients for ESFT. Neuroblastoma papers reported results from screening programmes established in geographical regions of Austria, Canada, France, Germany, Japan, and the UK. The studies considered a variety of outcomes but there is still considerable uncertainty whether or not population-based screening for neuroblastoma is clinically effective, and cost-effective overall, and, if so, what the optimal age at which to screen is, and also the optimal screening strategy, that is, one stage or multistage.
- **Very poor reporting of results in primary studies**. The large heterogeneity and poor standard of reporting meant that it was not possible to synthesise quantitatively the results in the areas of diagnosis and monitoring, for example marker levels at diagnosis in patients with ESFT were never compared with those in a control group of healthy patients.
- **Inadequate and incomplete reporting of prognostic data**. The reporting of both summary data and statistical estimates to provide an assessment of the difference in outcome between two groups (defined by levels of a marker) was extremely poor, for example a direct estimate of $\log_e$(HR) and its variance was only reported on three out of 575 occasions where the prognostic impact of a marker in neuroblastoma was assessed; only 34 more reported a ($\log_e$) HR and its CI; 210 occasions just reported a p value and, of 194 of those assessed, 90 did not provide group numbers and/or group events.
- **Inconsistent reporting of prognostic data**. The meta-analysis of prognostic results was hindered by the large variability in cut-off points, age of patients, stages of disease, and adjustment factors combined with different outcomes (OS or DFS) and different estimates (unadjusted and adjusted), for example in 94 prognostic estimates obtained in neuroblastoma for marker MYC-N there were nine different cut-offs, nine different stage groups and 14 adjusted results.
- **IPD often presented and very useful**. IPD were commonly observed because the rarity of childhood cancers leads to smaller study sizes and greater feasibility for publication of IPD. They enabled estimates to be made where otherwise impossible, for example 41 of the 204 successful prognostic estimates obtained in neuroblastoma were calculated using the IPD provided (20%). Presentation of complete IPD would help overcome the following problems of poor reporting:
  - no appropriate analysis presented
  - no presentation of the HR and/or variance (CI)
  - group numbers and group events not given
  - inexact p values presented
  - variability in marker cut-off level, type of estimate (unadjusted or adjusted), outcome assessed (OS or DFS) and adjustment factors
  - results only given for a few of the markers considered (publication/reporting bias).
- **Clinical interpretation of results very difficult**. The poor and inconsistent reporting made it extremely difficult to synthesise the results and make a clinical interpretation of their meaning.
- **No psychosocial or economic evaluation performed**. Disappointingly, we found no studies that evaluated the economic or psychosocial consequences of using tumour markers for clinical purposes in ESFT or neuroblastoma. Two papers reported cost data in neuroblastoma (both for screening), but these were dated and of poor quality. Given the 260 papers that studied the prognosis of patients with neuroblastoma, it was particularly disappointing that neither cost nor psychological issues were

evaluated. However, this perhaps reflects the uncertainty as to which markers have enough clinical effectiveness and importance to warrant subsequent economic and psychosocial studies.

- **Recommendations made for future research strategies and improved reporting**. These novel reviews should facilitate the development of future research strategies and improve the standard of scientific reporting because we have established the markers studied so far and specified the gaps in the literature that need to be filled in order to properly evaluate tumour markers and make decisions about their clinical value.

Chapter 5

Conclusions and recommendations

Critical appraisal of our study

Literature search

- **Only three databases searched**. We used the databases MEDLINE, EMBASE and CANCERLIT to identify the relevant literature. Ideally more databases (e.g. SIGLE) and other sources (e.g. contact with researchers) should have been used, but this was not feasible in light of the large literature already identified.
- **Only 10% of the first investigator's 'not-relevant' classification double checked**. Due to the large literature it was not possible to double check all of the first investigator's papers that were classified as 'not relevant' (ESFT, 820 papers; neuroblastoma, 2700 papers), and so some relevant papers may have been excluded unintentionally.
- **Named markers in the search strategy keywords not comprehensive**. The large number of markers identified was far greater than those specified in the search strategy. However, we did include terms such as 'marker' that will have limited this problem.
- **No evaluation of unpublished studies**. We did not have the time to contact researchers in the field to identify and include results from work that have not been published.
- **'Reference explosion' limited**. The references of important prognostic papers in ESFT were screened in order to identify other papers we may have missed; however, we did not screen those in other clinical areas. The vast number of relevant neuroblastoma papers meant that it was not feasible to perform a 'reference explosion' for this disease.
- **Foreign language papers excluded** (neuroblastoma only). We did not have the time or resources to include foreign language papers. This may have incorporated more bias if only statistically or clinically significant foreign studies/papers were (re)written for publication in an English language journal, a problem recently shown by Egger and colleagues.[102]

Data extraction

- **Some markers may not have been identified**. Given the large number of markers found it is most likely that we have unintentionally missed others whilst identifying and then classifying the 'relevant' papers. However, if any were missed they were most likely to have been included in only one or two papers and would have added limited value in terms of meta-analysis and the synthesis of results.
- **Markers just presented in cytogenetic data were not evaluated**. We did not use the majority of the cytogenetic IPD because they were poorly presented and often very limited, and we were concerned of reporting bias. Hence, markers just presented within cytogenetic IPD were not evaluated (ESFT) or not even recorded (neuroblastoma).
- **Histological markers not reviewed**. We did not have time to review all types of marker, and draw specific attention to the omission of histological markers.
- **Data extracted and analysed for only a few of the markers found in just one clinical area**. The inconsistency and variability in the way results were reported meant that no quantitative analysis could be done for the areas of diagnosis and monitoring. For prognosis, data were only extracted for those markers in ESFT that were studied in three or more papers, and this was increased to ten or more papers for prognostic markers in neuroblastoma. Hence, it is possible that other important prognostic markers have not been analysed.
- **Markers only considered for meta-analysis in a clinical area if they were reported in three or more papers**. This may have meant that markers studied in two very large studies may have been excluded from analysis. Future systematic reviews should bases meta-analysis decisions on the number of individuals in the studies rather than on the actual number of studies available.
- **Data extraction not double checked**. The extraction of summary statistics, required for the estimation of $\log_e$(HR) and its variance, were not double checked; this introduces the potential for unintentional mistakes. However, as no firm clinical conclusions were made on the basis of the meta-analysis, this problem should not detract from the main messages of our report (e.g. better reporting and the need for IPD).
- **Unadjusted estimates preferred to adjusted estimates**. We were justified in our decision to obtain unadjusted estimates instead of adjusted estimates whenever possible on the evidence of

the wide variability in adjustment factors observed, for example for the 15 MYC-N estimates obtained that were adjusted for other clinical features (within a Cox regression model) there were only two that adjusted for all the same ones and these came from the same paper.

- **Potential of duplicates of patients across papers** (neuroblastoma only). It was not feasible with the time available to check for duplicates of patients across the selected neuroblastoma papers. Our experience with the ESFT review, which involved only 84 papers, demonstrated the difficulties in assessing duplicate patients, especially when patient populations appear to overlap, but not completely.
- **Bias in publication and choice of cut-off used**. We found much evidence of publication bias in the neuroblastoma literature. This is probably due to an emphasis by journals and clinicians on reporting only statistically or clinically significant findings. There is also a strong possibility that cut-off points are specifically chosen to obtain the most significant or positive results, in order to improve the chances of publication.

Analysis and interpretation

- **Rudimentary meta-analysis performed**. Synthesis of the prognostic data incorporated adjustment for different outcomes (OS or DFS) by looking at estimates within these subgroups, but no allowance could be made for the following problems:
  - different cut-off points
  - different stages of disease
  - different age groups of patients
  - unadjusted versus adjusted estimates
  - wide variability of adjustment factors.
- **Type of treatment not taken into account**. For the meta-analysis of the prognostic data we did not consider the type of treatment that patients received during follow-up. This adds to the problems above. It would have been extremely difficult to incorporate the treatment received into the meta-analyses because treatments change over time and vary considerably between studies.
- **Indirect methods still only approximate estimates**. The use of indirect methods to estimate $\log_e$(HR) and its variance were very important and frequently used. However, they are still only approximate estimates and are likely to become more inaccurate for those studies with small sample (and event) sizes, in particular the variance estimate. We tried to limit this problem by mostly only using indirect methods when the sample size was greater than 25.
- **Other indirect methods possible**. The indirect methods we used were taken from Parmar and colleagues,[55] but others may be possible. We chose not to use any additional indirect methods because this would have added further heterogeneity to our results and made the clinical interpretation of the meta-analysis results even more tenuous.
- **Need to consider other survival statistics**. We chose $\log_e$(HR) and its variance as important measures of survival, but other summary statistics could have been used, for example the proportion of patients surviving to 2, 3, 5 or 10 years. However, the reporting was equally poor for all survival statistics in general, for example only 12 estimates of percentage survival were given in the 26 prognosis papers for LDH, and just six of these also reported a CI or SE.

Relationship to previous work

- The **poor reporting of survival data** is analogous to the problems addressed by Altman and colleagues.[69]
- Hutchon[67] describes the **benefits of IPD** and the need to present IPD either within the paper or on the Internet.
- Stewart and Parmar[78] also recommend that a meta-analysis of IPD is preferred because meta-analysis of the literature alone may produce misleading results. They suggest that an IPD meta-analysis provides the least biased and most reliable means of addressing questions that have not been satisfactorily resolved by individual clinical trials.
- The results obtained from an **IPD meta-analysis** were recently compared with those from a **literature**-based meta-analysis in head and neck cancer.[77] This highlighted the potential to obtain substantial differences because of the way the data have been reported in the literature.
- Altman[68] discusses the **problems** arising when **meta-analysis** is applied to **prognostic variables**, with particular reference to the fact that studies report different cut-offs.
- Altman and Lyman[72] have proposed important guidelines for both conducting and evaluating prognostic marker studies.
- **Indirect estimates** of the HR from summary statistics were recently compared with their equivalent **direct estimates from IPD** by Tierney and colleagues.[70] It was shown that where events happen quickly, and so publications report mature data, and patient exclusion is modest, then HR estimates from summary statistics were similar to those from

the IPD. However, where events occur over a prolonged period, such that publications report immature data, and many patients are excluded then the HR from summary statistics is a poor approximation to the IPD equivalent.

Implications for research

Clinicians

The following should be noted:

- There is insufficient evidence at present to judge the clinical role of tumour markers in the treatment of these two childhood malignancies. A large number of markers have been studied in the literature but the majority of studies are so poorly designed and reported that definite clinical conclusions cannot be made from this systematic review. However, we did manage to identify markers which showed possible prognostic importance.
- **Potentially important prognostic makers in ESFT**. We found that high levels of serum lactate dehydrogenase and lack of S-100 protein expression in tumour were significantly associated with a worse prognosis and an increased risk of death or disease recurrence/death. Expression of the EWS–FLI type 1 fusion transcript in tumours from patients with localised disease was associated with a more favourable outcome and a reduced risk of disease recurrence/death, compared with expression of other EWS–ETS fusion transcripts.
- **Potentially important prognostic markers in neuroblastoma**. We found that all of the following were associated with patients experiencing a worse outcome: amplification of the *MYC-N* gene; expression of diploid cells (a DNA index of 1) in tumour; high expression of NSE in the tumour at diagnosis; high serum levels of lactate dehydrogenase and/or ferritin; high multidrug resistance gene product expression in tumour; gain of chromosome 17q; deletion of chromosome 1p; low tumour expression of CD44 or TrkA; and a low urinary VMA:HVA ratio.
- **Clinical interpretation of these findings is very difficult**, because of poor and inconsistent reporting across the literature identified. The benefits of using these prognostic markers in practice need to be properly studied in large, multicentre studies as described below.
- The rapid development of genetic epidemiology may quickly provide new genetic markers and genetic sequences that supersede those markers we have identified as important.

Those conducting and reporting primary studies

- **Must report results better**. Analysis and reporting of tumour marker data must improve, and it is recommended that wherever possible clinicians should work with statisticians to evaluate tumour markers. In particular, the analysis and reporting of prognostic data need to be improved. It is necessary to present results of all the markers considered – those significant and not significant – and we recommend (in order of desirability) that authors:
  - **Present IPD in the paper or on the Internet**, as recommended by Altman[68] and Hutchon.[67] In particular the exact initial marker level, time of disease recurrence (if appropriate), follow-up period and final disease status for all the markers considered. Histological characteristics and other known important clinical measures should also be given in the IPD, such as stage and age, so that their clinical power can be evaluated and compared with markers and each other. IPD should always be made accessible whenever prognostic data are available, even if their evaluation was not a primary study aim. The presentation of IPD by these guidelines would help overcome the majority of problems associated with poor reporting, for example different cut-offs and adjustment factors.
  - **Report the HR and its CI**, or the $\log_e$(HR) and its variance or CI. These provide an important estimate of the difference in risk of death (for OS) or disease recurrence/deaths (for DFS) between two groups of patients. Also report the **number of patients** and **number of events within each group**, and report explicitly the **cut-off level or status** used to define the groups.
  - **Report exact *p* values** with their appropriate **exact test statistics** (e.g. χ^2 statistic). It is important to provide an exact value of at least one of these, even if the *p* value is greater than 0.05. It is insufficient to just report results as 'significant' or 'not significant'.
  - **Present survival curves** showing the difference in survival over time between two groups. On the curves also provide **clear censoring points** to denote the time when patients were censored. The exact *p* value with its test statistic from an analysis that compares the curves should be presented.

Also, provide the numbers in each group at the start of the study and then number of events and number of remaining patients at various timepoints during follow-up.
  - **Report percentage survival at *n* years** with a **CI** using Kaplan–Meier or other methods which allow for censoring.
- **Collaboration of research groups and a move towards evidence-based medicine needed**. There is a need for research groups to collaborate to assess the clinical application of markers in studies with greater patient numbers, and to achieve consistency in reporting, for example for cut-off levels and adjustment factors used. This will enable a move towards an evidence-based use of markers. We acknowledge that it is also important to try and find new and clinically better markers. However, this should not be done at the expense of establishing how existing markers can be most effectively used in practice.
- **Need to conduct large, multicentre quality-controlled studies and assess multiple markers**. Collaboration of research groups is needed to perform multicentre studies that assess both the levels of multiple markers in patients with ESFT or neuroblastoma and those in healthy patients. This approach would supersede the majority of studies we observed in the literature because it would enable the following:
  - **Large patient numbers**, which otherwise is difficult because of the rarity of the diseases.
  - The **characteristics of markers** to be assessed, such as the most appropriate cut-off points and the range of values experienced by patients (and healthy volunteers).
  - The potential of many **individual** markers in prognosis, monitoring, diagnosis and, to a lesser extent, screening to be evaluated in a much bigger study. The benefits of each individual marker could be also compared directly with other markers in order to evaluate **individual benefits** over and above those of others.
  - **Histological markers and other clinical measures to be studied**. We did not evaluate histological markers or many other important clinical measures in this review, and these need to be assessed both individually and alongside the more genetic/biological markers we have identified. This would be useful from a clinical/biological perspective, and is important because histological markers are often the most commonly used in practice, and so the benefit of any marker would need to be compared directly with them.
  - **Combinations of markers to be studied**. Combinations of markers may provide a more accurate way of screening or diagnosing patients, a more precise way of identifying different types of prognostic groups in order to target treatment, and a more specific way of monitoring patients and identifying those likely to have a recurrence of disease.
  - **A full evaluation of cytogenetic data**. Better collection and presentation of cytogenetic data could be performed to assess markers (such as chromosomes) that we were not able to evaluate in this review because of poor presentation and possible reporting bias.
  - **Marker levels in subgroups** of patients to be studied, again with much larger patient numbers than possible previously, for example patients aged < 1 year, those receiving specific treatments, and so on. This is important because the best markers to use may change for different subgroups of patients.
- **Central repositories for IPD required**. To help collate and manage IPD, central repositories are necessary for each disease area.
- **Future genetic studies to follow our guidelines of reporting and facilitate access to IPD**. With the growth of genetic epidemiology potentially leading to identification of genetic markers and sequences that could supersede the important markers currently in use, it is very important that those studies are reported properly and make available IPD. Again, central repositories are required to collate and manage such IPD.
- **Economic and psychosocial evaluation of markers required**. There is a large gap in the literature for the economic and psychosocial impact of using tumour markers in both ESFT and neuroblastoma. However, this probably reflects the uncertainty of the clinical effectiveness of the markers investigated. Once a marker has been established as clinically effective, it is important to then evaluate the cost of using tumour markers in relation to their clinical effectiveness, and so a cost-effective analysis is required. Also, an assessment of the psychosocial outcomes of using clinically effective markers is needed, and we recommend work to investigate how the purpose and results of tumour marker tests should be best communicated to children and their families.
- **Systematic reviews in cancer should consider the CANCERLIT database**. CANCERLIT provided important articles for our literature review over and above those found in the more established MEDLINE and EMBASE databases, including articles from well-established journals such as the *Journal of Clinical Oncology* and the *Lancet*. CANCERLIT should certainly be used for future systematic reviews relating to cancer, as well as

MEDLINE and EMBASE, and many new systematic reviews in cancer do appear to use this database.

Meta-analysts

- The role of meta-analysis was **severely limited** by data/reporting limitations of primary studies.
- We need to await outcome of method comparison studies and sensitivity analyses (see the section on 'Next steps' below).

Those conducting future systematic reviews of tumour markers

- Those considering future systematic reviews of tumour markers should **seek to obtain individual patient data**, as this is likely to be the most productive.

Next steps and recommendations for future research

- **Possible assessment of prognostic markers that were covered in fewer than ten papers**. However, it is most likely that the problems of poor reporting will also affect this work.
- **Sensitivity analyses and multifactorial analyses** (as far as possible) to explore and adjust for effects of different cut-offs, stages of disease, outcomes (OS or DFS), ages and result types (unadjusted or adjusted), and adjustment factors.
- **IPD results to be compared with those from the indirect methods** to assess the reliability, validity and bias of using indirect estimation compared with IPD, which we recommend to be the 'gold standard' in the reporting of data.
- **Timescale for updating the ESFT and neuroblastoma systematic reviews**. Although the development and application of markers is potentially fast-changing, especially given development in molecular biology, the standard of reporting discovered combined with the relatively small number of patients in available studies means that the benefits of replicating this review, even in the medium term, are extremely limited.

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Appendix 1

Selected papers with study Nos. (ESFT review)

1. Ambros IM, Ambros PF, Strehl S, Kovar H, Gadner H, Salzer-Kuntschik M. MIC2 is a specific marker for Ewing's sarcoma and peripheral primitive neuroectodermal tumors. Evidence for a common histogenesis of Ewing's sarcoma and peripheral primitive neuroectodermal tumors from MIC2 expression and specific chromosome aberration. *Cancer* 1991;**67**:1886–93.

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Appendix 2

Keywords used for the economic and psychosocial evaluation (ESFT review)

Economic	Psychosocial
Cost	Quality of life
Cost-effectiveness	Anxiety
Econ*	Psychosocial
Cea	Adjustment
Cba	
Cua	

Appendix 3

Breakdown of the MEDLINE, EMBASE and CANCERLIT results (ESFT review)

The EMBASE results were conditional on the paper not being found by MEDLINE; and the CANCERLIT results were conditional on the paper not already being found in MEDLINE or EMBASE. The first investigator (1st) classified all the papers as relevant (R), uncertain (?) or not relevant (NR) by reading the abstract. The second investigator (2nd) checked all 1st-?, about 10% of 1st-R and about 10% of 1st-NR by reading the abstract. Any paper classified as still R after the second investigator assessment was obtained and read fully to decide if it was to be included in the final set of relevant papers ('final yes').

MEDLINE			EMBASE			CANCERLIT		
		Final yes			**Final yes**			**Final yes**
1st-R 90	2nd-R 65	56	1st-R 16	2nd-R 8	5	1st-R 1	2nd-R 0	0
	2nd-? 7	5		2nd-? 0	0		2nd-? 0	0
	2nd-NR 18	–		2nd-NR 8	–		2nd-NR 1	–
		61			**5**			**0**
1st-? 109	2nd-R 10	8	1st-? 52	2nd-R 5	2	1st-? 1	2nd-R 0	0
	2nd-? 8	2		2nd-? 1	0		2nd-? 0	0
	2nd-NR 91	–		2nd-NR 46	–		2nd-NR 1	–
		10			**2**			**12**
1st-NR 582	2nd-R 2	2	1st-NR 205	2nd-R 0	0	1st-NR 33	2nd-R 0	0
	2nd-? 2	2		2nd-? 0	0		2nd-? 0	0
	2nd-NR 578	–		2nd-NR 205	–		2nd-NR 33	–
		4			**0**			**0**
781		75*	273		7	35		0
Total final yes = 75 + 7 + 2 reference explosion papers = **84**								

* *One of the two 'reference' explosion papers had also been found in MEDLINE but had been classified as 2nd-NR, therefore MEDLINE covered 76 of the final 84 papers*

Appendix 4

Complete list of identified tumour markers, with numbers overall and within each clinical area (ESFT review)

Tumour marker	Total No. of papers	Diagnosis	Prognosis	Monitoring
EWS–FLI1 or t(11;22) (or chromosome 11 or 22 relating specifically to FLI1 or t1122)	35	35	13	2
NSE	22	22	12	0
MIC-2, CD99, HBA71 or 12E7	18	18	5	0
EWS–ERG or t(21;22) (or Chromosome 21 or 22 relating specifically to ERG or t2122)	16	16	8	2
LDH	15	14	15	2
Desmin	10	10	3	0
Leukocyte 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	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

continued

continued

Tumour marker	Total No. of papers	Diagnosis	Prognosis	Monitoring
β actin	2	2	0	0
Chromogranin, chromogranin A, chromogranin 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
7-β_2 2 protein	1	1	1	0
Albumin	1	1	1	0
β_2-Microglobulin	1	1	1	0
β_1-integrin-linked protein kinase	1	1	0	0
CAM5.2	1	1	0	0
CDK4	1	1	0	0
Chromosome 11	1	1	1	0
Chromosome 22	1	1	1	0
Cholecystokinin gene	1	1	0	0
DNA ploidy	1	1	1	0
γ-Glutamyltransferase	1	1	1	1
HNK-1	1	1	1	0
MB2	1	1	0	0
Myoglobin	1	1	1	0
Neuroblastoma cell surface antigen	1	1	0	0
Neurone cell surface antigen	1	1	0	0
Neurosecretary-type granules	1	1	1	0
NM23	1	1	0	0
Nmyc	1	1	0	0
Secretogranin II	1	1	1	0
T(1;16)	1	1	1	1
T(15;19)	1	1	1	0
T(2;13)	1	1	0	0
Tpa, Pai-1, U-PA	1	1	1	1
V9	1	1	1	0
von Willebrand	1	1	0	0

Appendix 5

Individual and pooled results for the 13 prognostic markers evaluated (ESFT review)

The table lists individual and pooled estimates of $\log_e$(HR)* and its SE with 95% CI for each tumour marker, with details from the primary papers (see appendix 1) of date of publication (date), number of patients (*n*), cut-off used and whether the result was adjusted (a) or unadjusted (u). The results are for OS unless DFS is stated; all disease types unless localised (L) or metastatic (M) are shown; and 'method' refers to the type of method used to estimate $\log_e$(HR) and its variance (see appendix 14).

* $\log_e$(HR) > 0 indicates a greater instantaneous risk of death (for OS) or disease recurrence/death (for DFS) for patients with high/positive marker levels or those with presence of the marker.

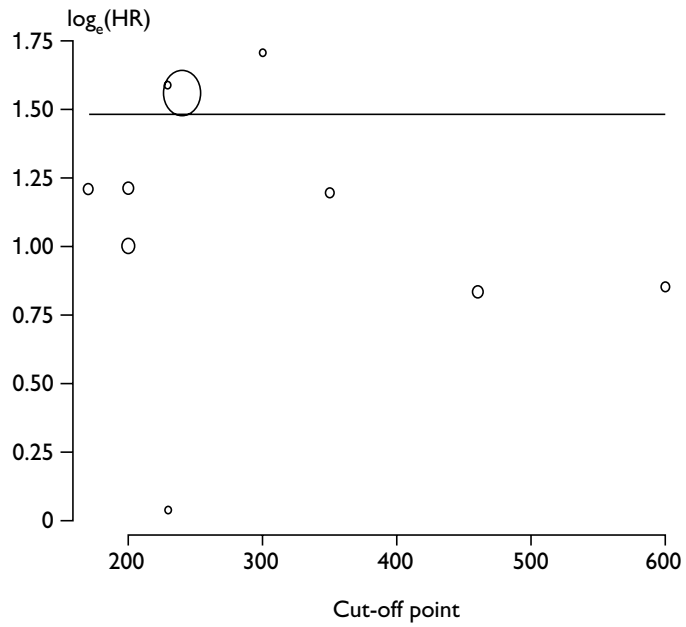
Tumour marker	Paper	log(HR)	SE (log_e(HR))	Year	*n*	Cut-off	Method	u/a
LDH	62	1.208	0.367	1975	65	170 U/l	7, 10	u
	35	0.999	0.256	1981	113	200 U/l	7	u
	42	1.194	0.4	1991	88	350 U/l	3	a*
	3	1.707	0.5815	1998	20	300 U/l	7	u
	49	0.833	0.303	1999	64	460 U/l	3	u
Meta-analysis: HR = 2.917, 95% CI = 2.161 to 3.938, $p < 0.0001$								
LDH (DFS)	34	1.21	0.31	1980	76	200 U/l	7	u
	65	1.592	0.581	1981	66	230 U/l	10	u
	28	0.031	0.57	1987	47	230 U/l	7	u
	42	1.308	0.405	1991	88	350 U/l	3	a*
	13	0.85	0.412	1997	98	600 U/l	7	u
	4	1.56	0.093	1999	359	240 U/l	3	u
Meta-analysis: HR = 3.376, 95% CI = 2.282 to 4.994, $p < 0.0001$								
OS + DFS meta-analysis: 10 papers (No. 42 OS used), HR = 3.214, 95% CI = 2.426 to 4.258, $p < 0.0001$								
OS + DFS meta-regression: 10 papers (No. 42 OS used); log_e(HR) = 1.485(0.00105) – 0.001035(0.326) x cut-off, $p = 0.34$ for cut-off								
NSE	72	0.093	0.914	1988	14	Negative, positive	9	u
	50	0.522	0.605	1989	20	Negative, positive	7	u
	29	–0.09	0.396	1992	43	Negative, positive	9	u
	9	0.974	0.839	1995	16	Negative, positive	9	u
	71	0.163	0.42	1997	38	Negative, positive	9	u
	49	0.182	0.522	1999	73	Negative, positive	3	u
Meta-analysis: HR = 1.206, 95% CI = 0.787 to 1.848, $p = 0.391$								
S-100	72	–1.066	1.12	1988	14	Negative, positive	9	u
	9	–0.331	1.083	1995	16	Negative, positive	9	u
	71	–0.949	0.451	1997	38	Negative, positive	9	u
Meta-analysis: HR = 0.414, 95% CI = 0.193 to 0.889, $p = 0.024$								
Cytokeratin	9	–0.558	1.078	1995	16	Negative, positive	9	u
	71	–0.398	0.767	1997	38	Negative, positive	9	u
Meta-analysis: HR = 0.637, 95% CI = 0.187 to 2.167, $p = 0.47$								
Leu-7,	50	1.429	0.78	1989	15	Negative, positive	9	u
HNK-1,	9	0.79	0.739	1995	16	Negative, positive	9	u
CD57	71	0.073	0.48	1997	36	Negative, positive	9	u
Meta-analysis: HR = 1.766, 95% CI = 0.809 to 3.852, $p = 0.153$								
MIC-2	29	0.3988	1.0279	1992	43	Negative, positive	9	u
	9	1.3201	1.0698	1995	15	Negative, positive	9	u
	71	0.1963	0.6336	1997	38	Negative, positive	9	u
Meta-analysis: HR = 1.598, 95% CI = 0.622 to 4.106								
FLI-1 or	43	1.401	1.0686	1999	18	Absence, presence†	9	u
ERG	66	0.1823	1.2247	1996	8	Absence, presence†	9	u
Meta-analysis: HR = 2.397, 95% CI = 0.495 to 11.616, $p = 0.278$								
Type 1	82	–1.8559	0.44	1996	55	Other types, type 1	7	u
(DFS, L)	83	–1.3569	0.921	1998	23	Other types, type 1	9	u
Meta-analysis: HR = 0.171, 95% CI = 0.079 to 0.373, $p < 0.0001$								
Type 1	82	–0.27541	0.5045	1996	30	Other types, type 1	7	u
(DFS, M)	83	–1.8547	0.9274	1998	12	Other types, type 1	9	u
Meta-analysis: HR = 0.418, 95% CI = 0.093 to 1.874, $p = 0.254$								

* *Both adjusted estimates were adjusted for metastatic disease, age > 25 years, and central sites in localised disease patients*

† *Absence or presence of marker 'FLI-1' or 'ERG' denotes EWS–FLI or EWS–ERG gene arrangements; 'Type 1' denotes EWS–FLI1 type 1*

Appendix 6

$\log_e$(HR) for LDH by cut-off point, and the meta-regression model line



Appendix 7

Selected papers, with study Nos. (neuroblastoma review)

Owing to their large number, these papers have not been placed in alphabetical order.

2. Kozakewich HP, Perez-Atayde AR, Donovan MJ, Fletcher JA, Estroff JA, Shamberger RC, *et al.* Cystic neuroblastoma: emphasis on gene expression, morphology, and pathogenesis. *Pediatr Develop Pathol* 1998;**1**(1):17–28.

5. Berthold F, Baillot A, Hero B, Schurr P, Nerenz A, Hunneman DH, *et al.* Which cases are found and missed neuroblastoma screening at 1 year? Results from the 1992 to 1995 study in three federal states of Germany. *J Clin Oncol* 1999;**17**(4):1200–7.

6. Schilling FH, Spix C, Berthold F, Erttmann R, Hero B, Michaelis J, *et al.* German neuroblastoma mass screening study at 12 months of age: statistical aspects and preliminary results. *Med Pediatr Oncol* 1998;**31**(5):435–41.

7. Horn M, Blatt J. Continued remission in children with neuroblastoma despite elevations of urinary catecholamine metabolites. *Am J Pediatr Hematol Oncol* 1992;**14**(3):202–6.

8. Berthold F, Trechow R, Utsch S, Zieschang J. Prognostic factors in metastatic neuroblastoma. A multivariate analysis of 182 cases. *Am J Pediatr Hematol Oncol* 1992;**14**(3):207–15.

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Appendix 8

Breakdown of the MEDLINE, EMBASE and CANCERLIT results (neuroblastoma review)

The EMBASE results were conditional on the paper not being found by MEDLINE; and the CANCERLIT results were conditional on the paper not already being found in MEDLINE or EMBASE. The first investigator (1st) classified all the papers as relevant (R), uncertain (?) or not-relevant (NR) by reading the abstract. A second investigator (2nd) checked all 1st-?, about 10% of 1st-R and about 10% of 1st-NR by reading the abstract. Any paper classified as still R after the second investigator assessment was obtained and read fully to decide if it was to be included in the final set of relevant papers ('final yes').

MEDLINE			EMBASE			CANCERLIT		
		Final yes			Final yes			Final yes
1st-R 349	2nd-R 339	278	1st-R 58	2nd-R 58	39	1st-R 39	2nd-R 39	21
	2nd-? 3	3		2nd-? 0	0		2nd-? 0	0
	2nd-NR 7	–		2nd-NR 0	–		2nd-NR 0	–
		281	R		**39**			**21**
1st-? 186	2nd-R 44	29	1st-? 45	2nd-R 12	8	1st-? 38	2nd-R 9	3
	2nd-? 62	26		2nd-? 22	4		2nd-? 20	8
	2nd-NR 80	–		2nd-NR 11	–		2nd-NR 9	–
		55			**12**			**11**
1st-NR 1001	2nd-R 8	3	1st-NR 370	2nd-R 0	0	1st-NR 1329	2nd-R 0	0
	2nd-? 19	6		2nd-? 1	0		2nd-? 1	0
	2nd-NR 974	–		2nd-NR 369	–		2nd-NR 1328	–
		9			**0**			**0**
1536		**345**		**473**	**51**		**1406**	**32**
Total ‘final yes’ = 428								

Appendix 9

Complete list of identified tumour markers, with numbers overall and within each clinical area (neuroblastoma review)

Tumour marker	Overall	Screening	Diagnosis	Prognosis	Monitoring
MYC-N	201	7	148	151	9
VMA	125	44	78	45*	18
HVA	105	38	64	35†	16
DNA index/ploidy/diploidy/triploidy/ aneuploid/hyperdiploidy	56	5	37	44	1
Chromosome 1p or 1p36	47	4	34	40	1
Ferritin or isoferritn	49	3	36	33	5
NSE	45	2	33	28	9
LDH	32	1	22	26	4
Dopamine	24	2	22	10	4
TrkA (nerve growth factor receptor)	25	0	16	16	0
Adrenaline/epinephrine	15	0	15	5	4
Multidrug resistance/associated protein/p-glycoprotein	16	0	7	16	0
Nonadrenaline/noradrenaline/ norepinephrine	13	0	13	5	2
CD44	10	0	7	8	0
Neuropeptide Y	12	0	10	9	0
Tyrosine hydroxylase	12	0	11	3	3
Chromosome 17q	11	0	9	8	0
Ha-Ras P21/H-Ras/c-Ha-Ras	11	0	8	6	1
Telomerase/telomeric repeats	11	0	6	7	0
Chromosome 14q	8	0	6	7	0
Ganglioside GD2	8	0	7	5	2
S-100 protein	7	1	5	5	0
Chromosome 11q	6	0	5	6	1
Low-affinity nerve growth receptor	6	0	3	6	0
Metanephrine	6	0	6	1	1
TrkC	6	0	3	5	0
3-Methoxy-4-hydroxyphenyl glycol	5	1	3	1	0
4-Hydroxy-3-methoxymandelic acid	5	0	5	1	2
Dihydroxyphenylalanine	5	1	5	1	3
Dopamine β-hydroxylase	5	0	3	2	2
Proliferating cell nuclear antigen/ index/Ki67/KiS5 protein	5	0	5	4	0
3-Methoxytyramine	4	0	4	1	0
Ganglioside Bcl2	4	0	2	3	0
Ganglioside GD1a	4	0	3	3	1
Ganglioside GD1b	4	0	3	3	1

continued

continued

Tumour marker	Overall	Screening	Diagnosis	Prognosis	Monitoring
Ganglioside GM1	4	0	3	3	1
Ganglioside GM3	4	0	3	3	1
Leukocytes	4	0	0	4	0
Normetanephrine	4	0	4	1	1
PGP9.5 protein	4	0	4	1	2
Somatostatin	4	0	1	3	0
Synaptophysin	4	0	4	1	0
TrkrB	4	0	4	2	0
3-Methoxytyrosine	3	0	0	1	0
Chromosome 1q	3	0	2	3	0
Chromosome 3p	3	0	3	3	0
Ganglioside GD3	3	0	2	3	0
Ganglioside GM2	3	0	2	3	0
Ganglioside Gt1b	3	0	2	2	1
Haemoglobin	3	0	1	3	0
Platelets	3	0	1	3	0
Vannilactic acid	3	0	3	1	0
3,4-Dihydroxyphenylacetic acid	2	0	2	0	0
α subunit of GTd protein	2	0	1	2	1
Carcinoembryonic antigen	2	0	2	1	0
Chromogranin	2	0	2	1	0
Chromosome 2p	2	0	2	0	0
Chromosome 9p	2	0	0	2	0
c-Myc	2	0	1	2	0
c-Src	2	0	2	1	0
DDX1 gene copy no.	2	0	2	2	0
Desmin	2	0	2	0	0
Dihyrdroxyphenylacetic acid	2	0	2	0	0
Erythrocyte sedimentation rate	2	0	0	2	0
Ganglioside Gq	2	0	1	2	0
Hydroxymandellic acid	2	1	2	0	0
Hydroxymethoxyphenylethylenglycol	2	0	2	0	0
Interleukin-1β enzyme	2	0	2	1	0
Lymphocyte counts	2	0	0	2	0
Mage1	2	0	0	2	0
Mage3	2	0	0	2	0
Metadrenaline	2	0	2	1	0
Metaiodobenzylguapidine	2	0	2	0	0
MHC class I gene expression	2	0	0	1	1
Nb84	2	0	2	0	0
NM23-h1	2	1	2	2	0
Normepinephrine	2	0	2	1	1
N-Ras	2	0	1	2	0
P16 gene/mutation	2	0	0	2	0
P75 gene	2	0	2	0	0
PP60csrcn	2	0	1	2	0
Serum creatine kinase BB	2	0	1	1	0
Sialic acid	2	0	2	1	1
Tyramine	2	0	2	0	0

continued

continued

Tumour marker	Overall	Screening	Diagnosis	Prognosis	Monitoring
Vasoactive intestinal peptide	2	0	2	1	0
1gf1 insulin-like growth factor	1	0	1	0	1
1gf2 insulin-like growth factor	1	0	1	0	1
1gfbp insulin-like growth factor	1	0	1	0	1
27 kDa heat shock protein (hsp27)	1	0	1	0	0
3–*O*-Methyldopa	1	0	1	0	0
3-Methoxy-5-hydroxymandelic acid	1	0	1	0	1
5-Hydroxyindoleacetic acid	1	0	1	0	0
5-Hydroxytryptophen	1	0	1	0	0
AGC granulocyte count	1	0	0	1	0
Agnor protein	1	0	1	1	0
α1-proteinase inhibitor	1	0	1	0	0
αV-β3 integrin	1	0	1	0	0
BDNF	1	0	1	1	0
βAminosobatyric acid	1	0	1	1	0
B-Myb	1	0	0	1	0
Canicular multispecific organic anion transporter	1	0	1	1	0
Caspase 8	1	0	1	0	0
Catecholamines (total amount)	1	0	1	0	0
CD56	1	0	1	0	0
CD95	1	0	0	1	0
Ceruloplasmin	1	0	1	0	0
Ceruloplasmin oxidase activity	1	0	1	0	0
c-Fos	1	0	1	1	0
Choline	1	0	1	0	0
Choline acetyltransferase	1	0	0	0	1
Chromogranin A	1	0	1	0	0
Chromosome 17p	1	0	1	0	0
Chromosome 1q 21–25	1	0	1	1	0
Chromosome 1q 32–43	1	0	1	1	0
Chromosome 3q	1	0	1	1	0
Chromosome 4p	1	0	1	1	0
Chromosome 5q	1	0	1	1	0
CPP 32	1	0	1	1	0
Creatinin	1	0	1	0	0
c-Scrc[3]	1	0	1	1	0
csrcn1	1	0	1	1	0
csrcn2	1	0	1	1	0
Cystathionine	1	0	1	1	0
Cytokeratin	1	0	1	0	0
Dihydroxyphenylethleneglycol	1	0	1	0	0
EPH family receptor tyrosine kinase	1	0	0	1	0
GE-25 peptide	1	0	1	0	0
Gelatinase A	1	0	0	1	0
Glial fibrillary acidic protein	1	0	1	1	0
GST TT genes	1	0	0	1	0
Gt	1	0	1	1	0
Haptoglobin	1	0	1	0	0

continued

continued

Tumour marker	Overall	Screening	Diagnosis	Prognosis	Monitoring
Hcd10	1	0	1	1	0
Haemosiderin	1	0	1	0	0
HnK1	1	0	0	1	0
Hydroxyphenylethylene glycol	1	0	1	0	0
Hydroxyphenylacetic acid-4	1	0	1	0	0
Ich 1	1	0	1	1	0
Karyotypic	1	0	0	1	0
k-Ras	1	0	1	0	0
L-Amino acid decarboxylase	1	0	1	1	1
L-Dopa	1	0	1	0	0
Leu7	1	0	1	0	0
L-Myc	1	0	1	0	0
Lumbar cerebrospinal fluid	1	0	1	0	0
Manganese superoxide dismutase	1	0	1	0	0
Mart1	1	0	0	1	0
Matrix metalloproteinase 1	1	0	0	1	0
Matrix metalloproteinase 2	1	0	0	1	0
Matrix metalloproteinase 9	1	0	0	1	0
Melatonin	1	0	1	0	0
Midkine	1	0	0	1	0
MMP-2	1	0	0	1	0
N-Acetyl-5-hydroxytryptanane	1	0	1	0	0
Neuroblastoma amplified gene	1	0	1	0	0
Neurofilament	1	0	0	1	0
Neurone specific he1–n1	1	0	0	1	0
Neurone specific huD	1	0	0	1	0
Neuronal Src	1	0	0	1	0
Neurotensin	1	0	1	0	0
NM 23 RNA	1	0	1	1	0
N-Methyl-5-hydroxytryptanine	1	0	1	0	0
Non-neuronal enolase	1	0	0	1	0
Ny-eso1	1	0	0	1	0
Octopamine	1	0	1	0	0
ODC gene	1	0	0	1	0
P110 protein	1	0	1	1	0
P53 gene	1	0	1	1	0
Pancreastatin	1	0	1	1	1
PG21 gene	1	0	0	1	0
Pleiotrophin	1	0	0	1	0
Polysialated neural cell adhesion molecule	1	0	0	0	1
PP60csrc	1	0	1	1	0
Protein kinase C-α	1	0	1	0	0
RRM2 gene	1	0	0	1	0
S1G2M (phase of cell cycle)	1	0	0	1	0
Secretoneurin-IR	1	0	1	0	0
Serum polysialated neural call adhesion molecule	1	0	0	1	1
Syndecan-1	1	0	0	1	0

continued

continued

Tumour marker	Overall	Screening	Diagnosis	Prognosis	Monitoring
Tc-99m methylene disphosphoric acid	1	0	1	0	0
Tetanus toxin	1	0	1	0	0
Tissue inhibitor matrix 2 metalloproteinase	1	0	0	1	0
Tissue polypeptide-specific antigen	1	0	1	0	0
Transferrin	1	0	1	0	0
Translocation (11;22)	1	0	1	0	0
Tryptophen	1	0	1	0	0
Tynurenine	1	0	1	0	0
Vanillic acid	1	0	1	0	0
Vanylacetic acid	1	0	1	0	0
Vanylglycol	1	0	1	0	0
Vanylglycolic acid	1	0	1	0	0
Vascular Parameters	1	0	0	1	0
Vimentin	1	1	0	0	0
White cell count	1	0	1	1	0

[*] *Thirty-six of these studied VMA; 20 studied the VMA:HVA ratio*
[†] *Twenty-six of these studied HVA; 20 studied the VMA:HVA ratio*

Appendix 10

Results at each stage of the process of extracting $\log_e$(HR) and its variance for each of the 13 prognostic markers evaluated (neuroblastoma review)

Flow charts showing the extraction process for each of the 13 prognostic markers studied. An explanation of the flow charts is given in the caption to *Figure 4*.

❶ = attempt 1, ❷ = attempt 2, ❸ = attempt 3, ❹ = attempt 4, ❺ = attempt 5

DNA/chromosome abberations

Figure 10: MYC-N
Figure 11: DNA index
Figure 12: Chromosome 1p

Biological markers

Figure 13: LDH
Figure 14: CD44
Figure 15: NSE
Figure 16: TrkA
Figure 17: Ferritin
Figure 18: Multidrug resistance protein

Urinary catecholamines

Figure 19: VMA
Figure 20: HVA
Figure 21: VMA:HVA
Figure 22: Dopamine

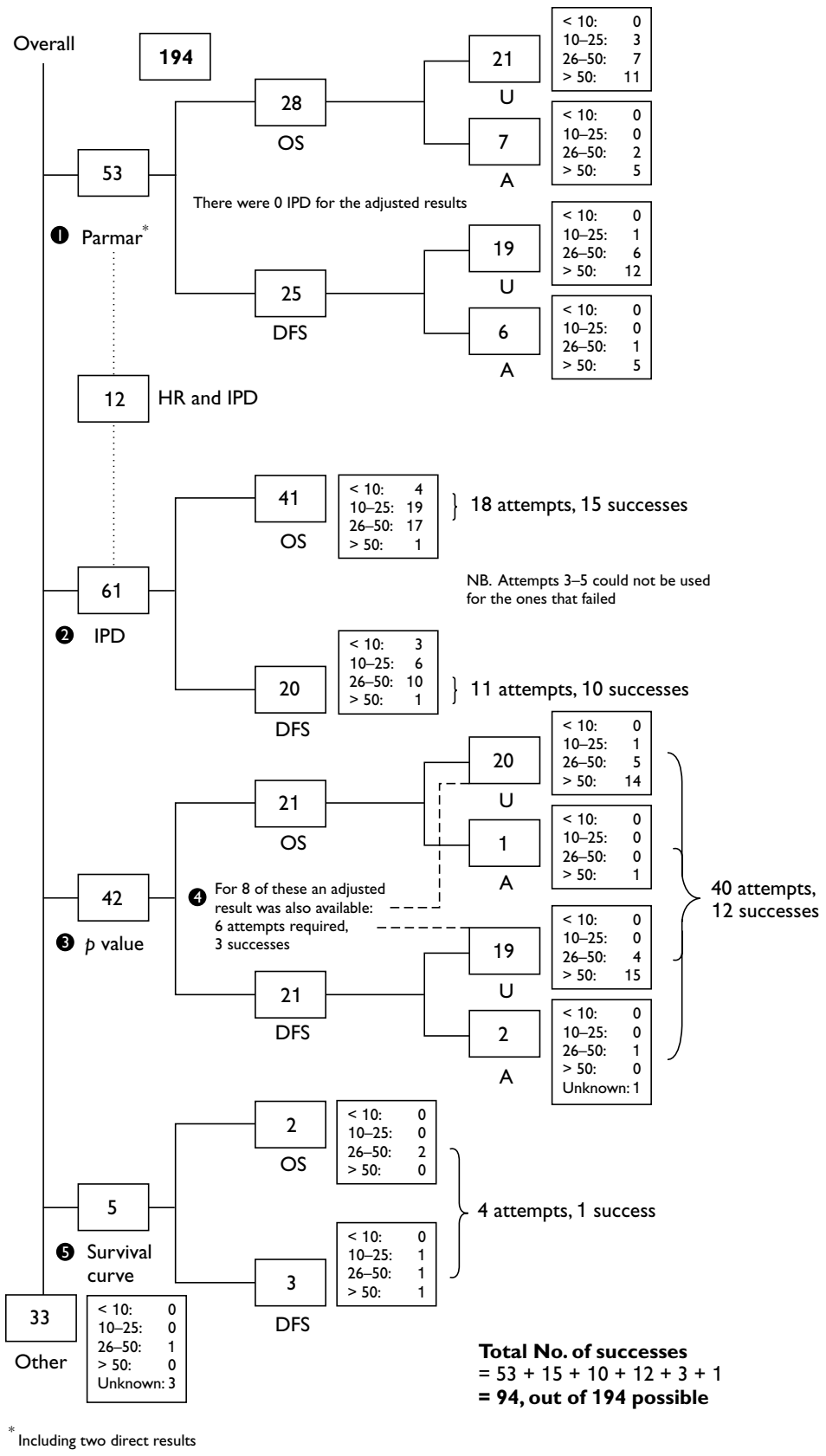

FIGURE 10 *MYC-N*

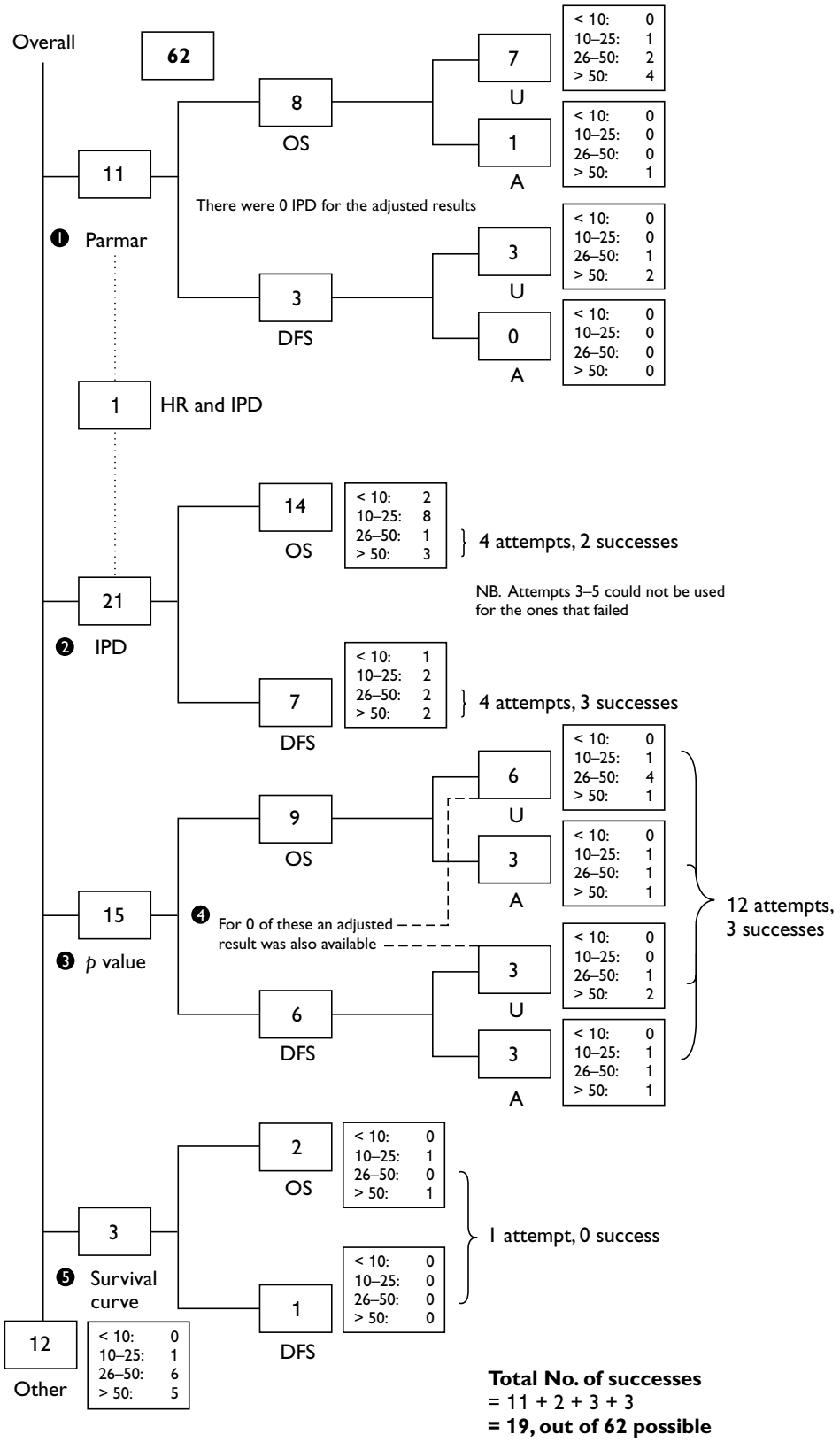

FIGURE 11 *DNA index*

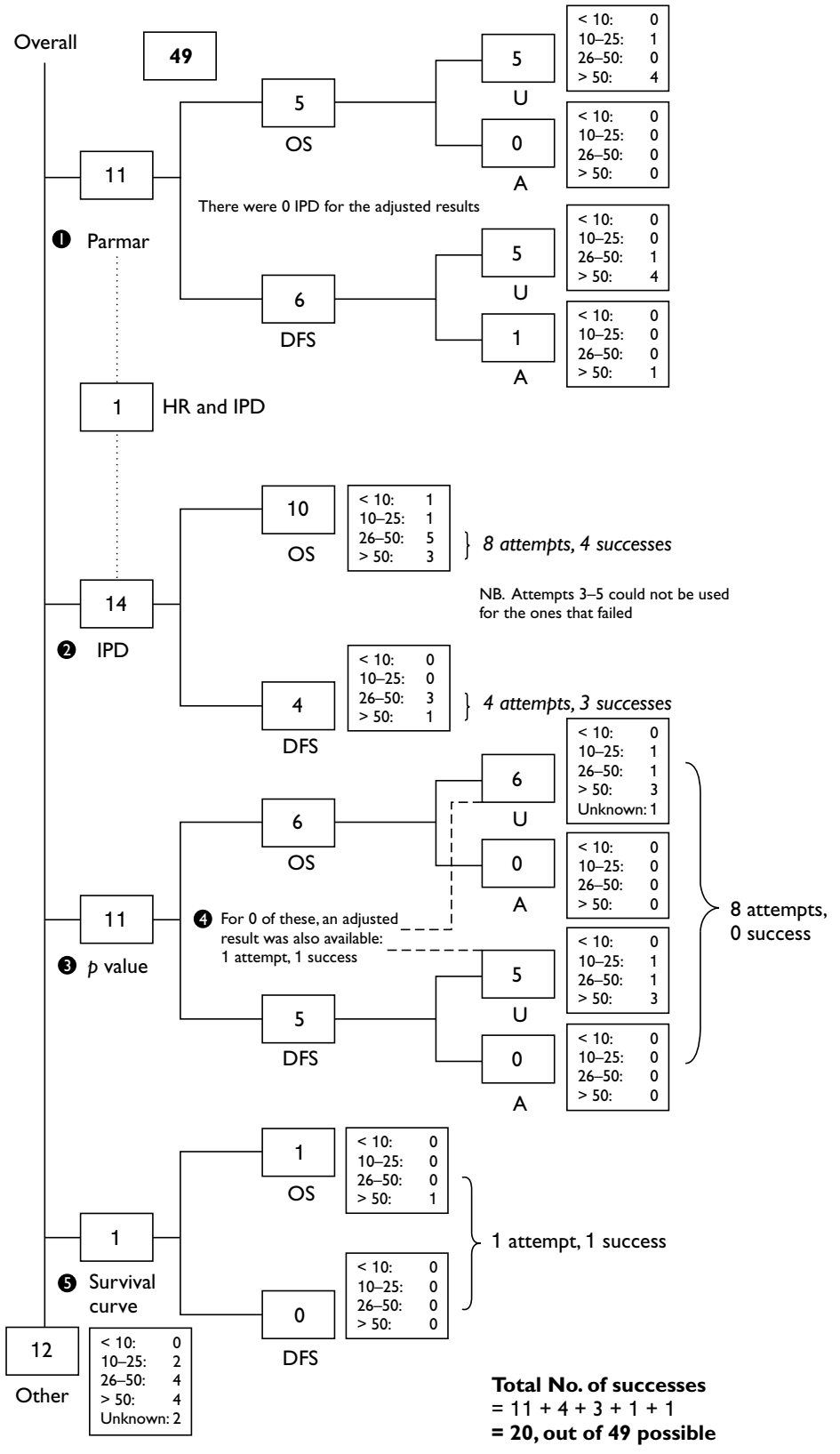

FIGURE 12 *Chromosome 1p*

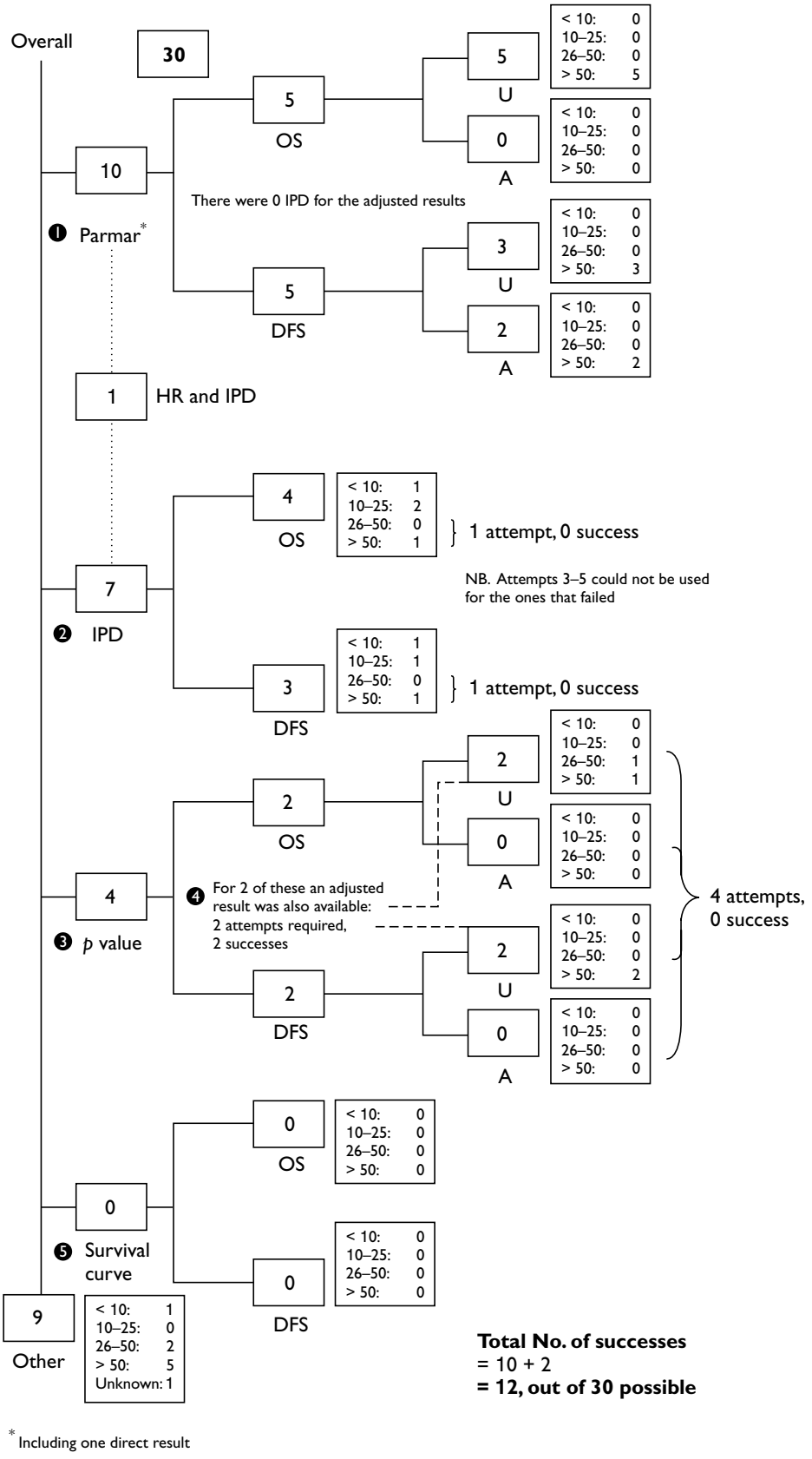

FIGURE 13 *LDH*

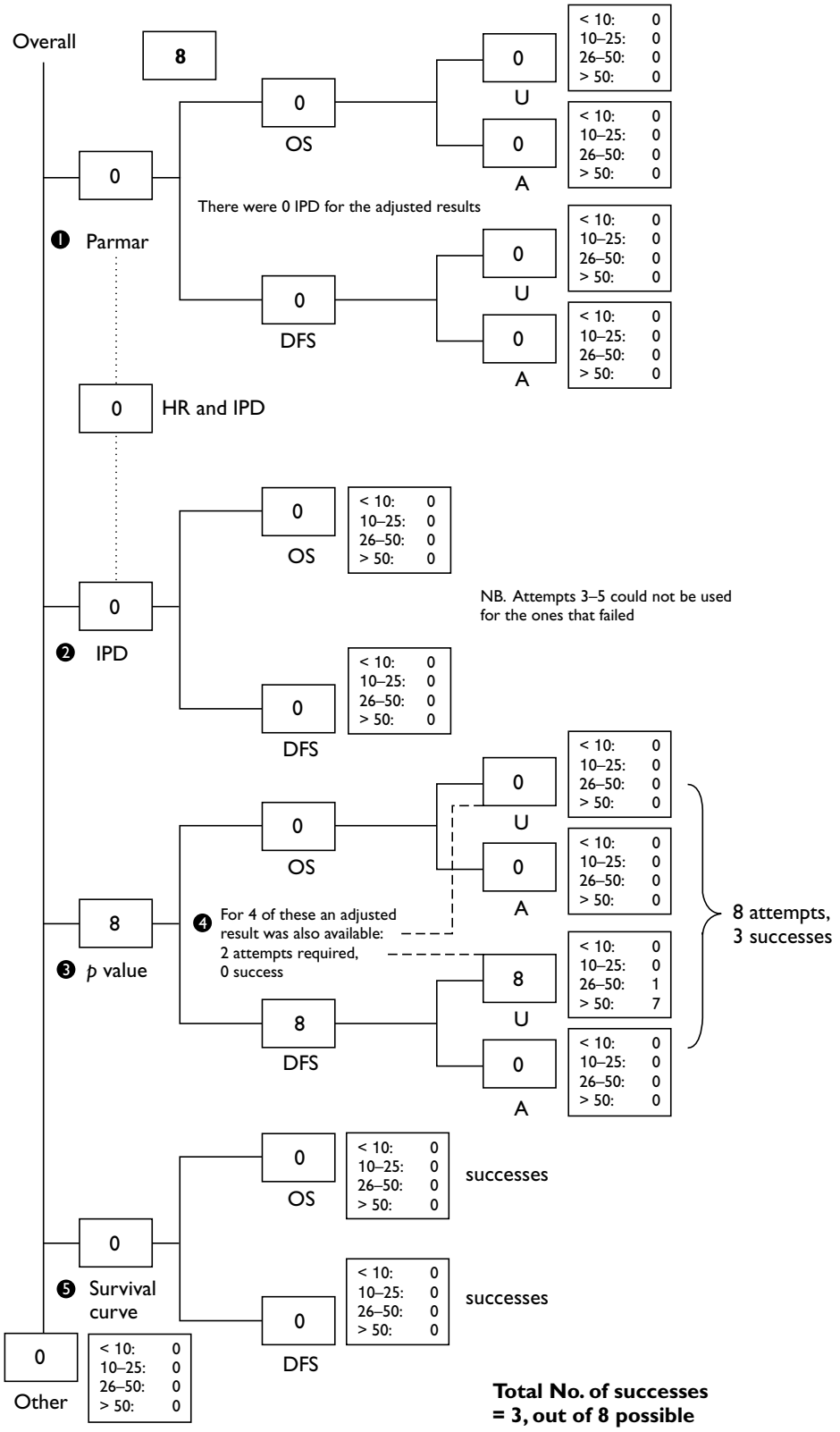

FIGURE 14 *CD44*

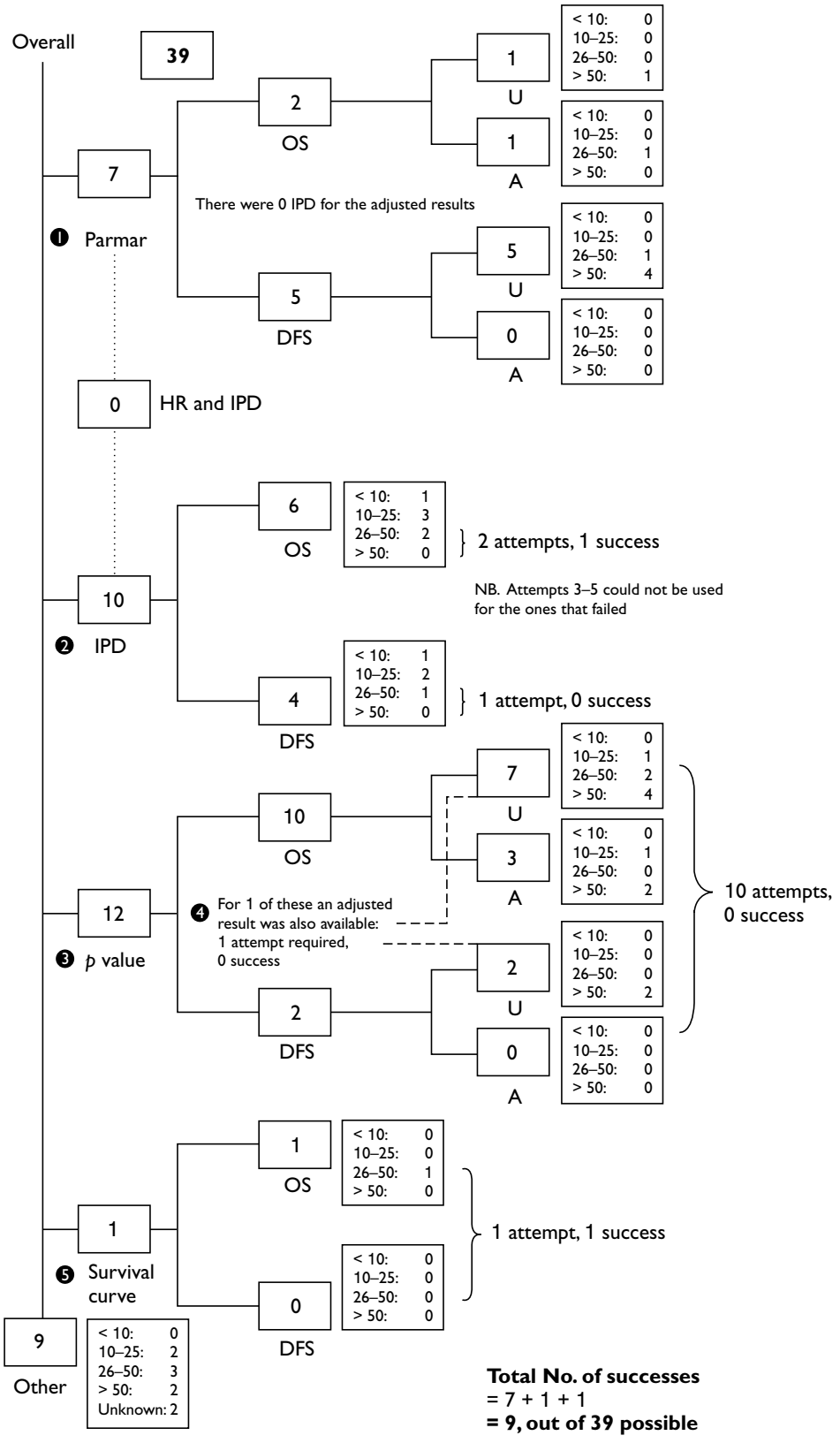

FIGURE 15 *NSE*

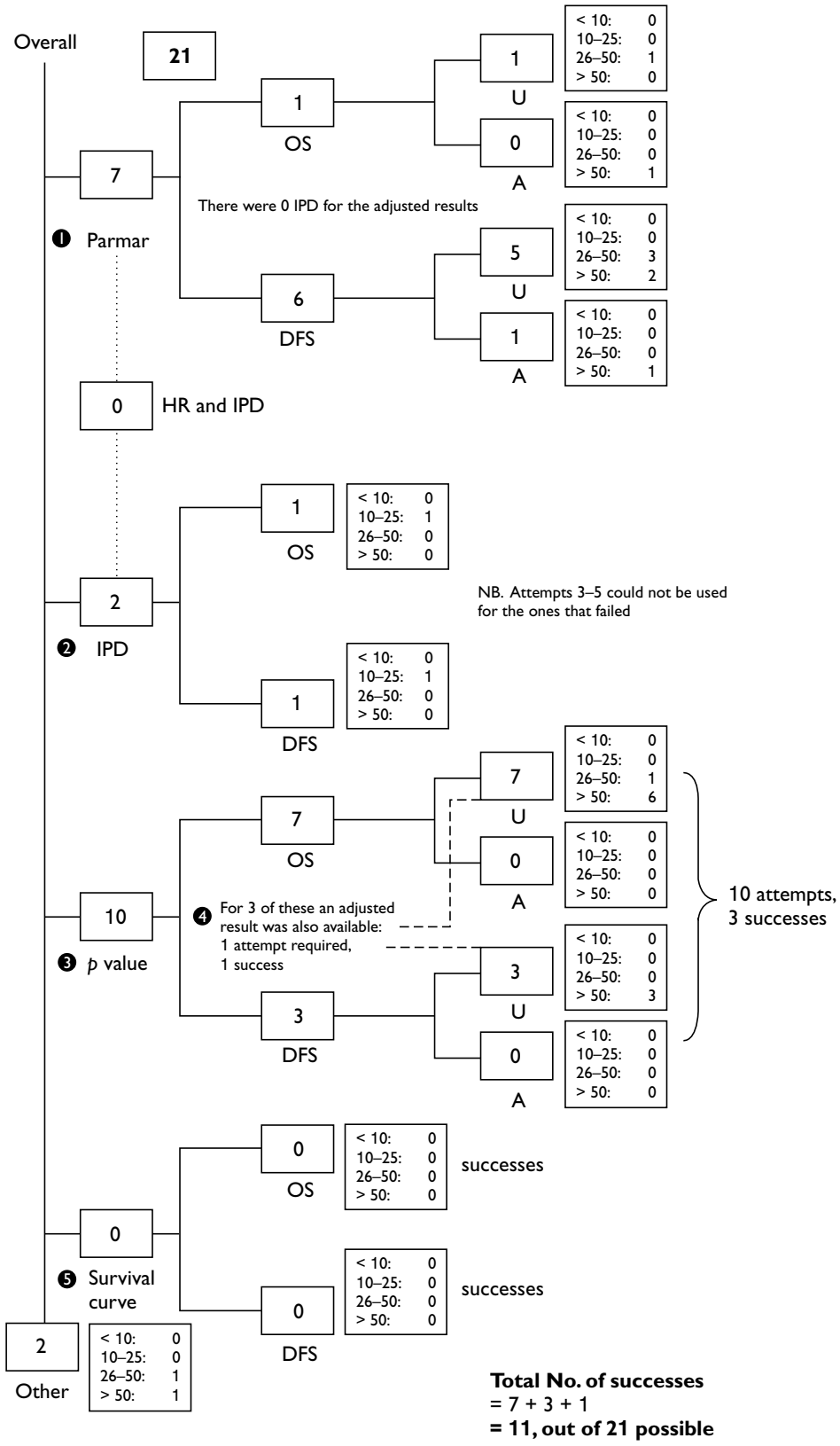

FIGURE 16 *TrkA*

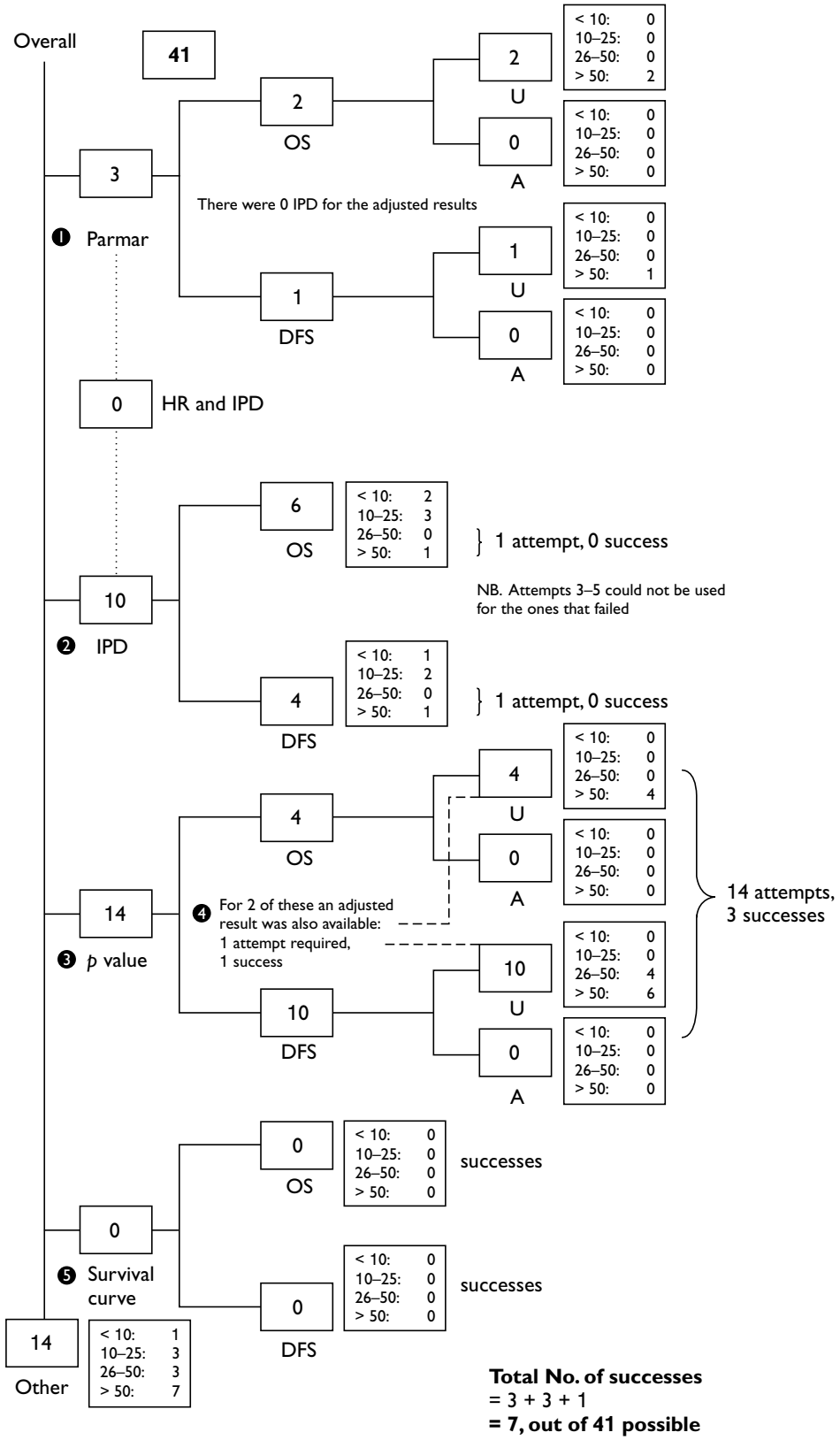

FIGURE 17 *Ferritin*

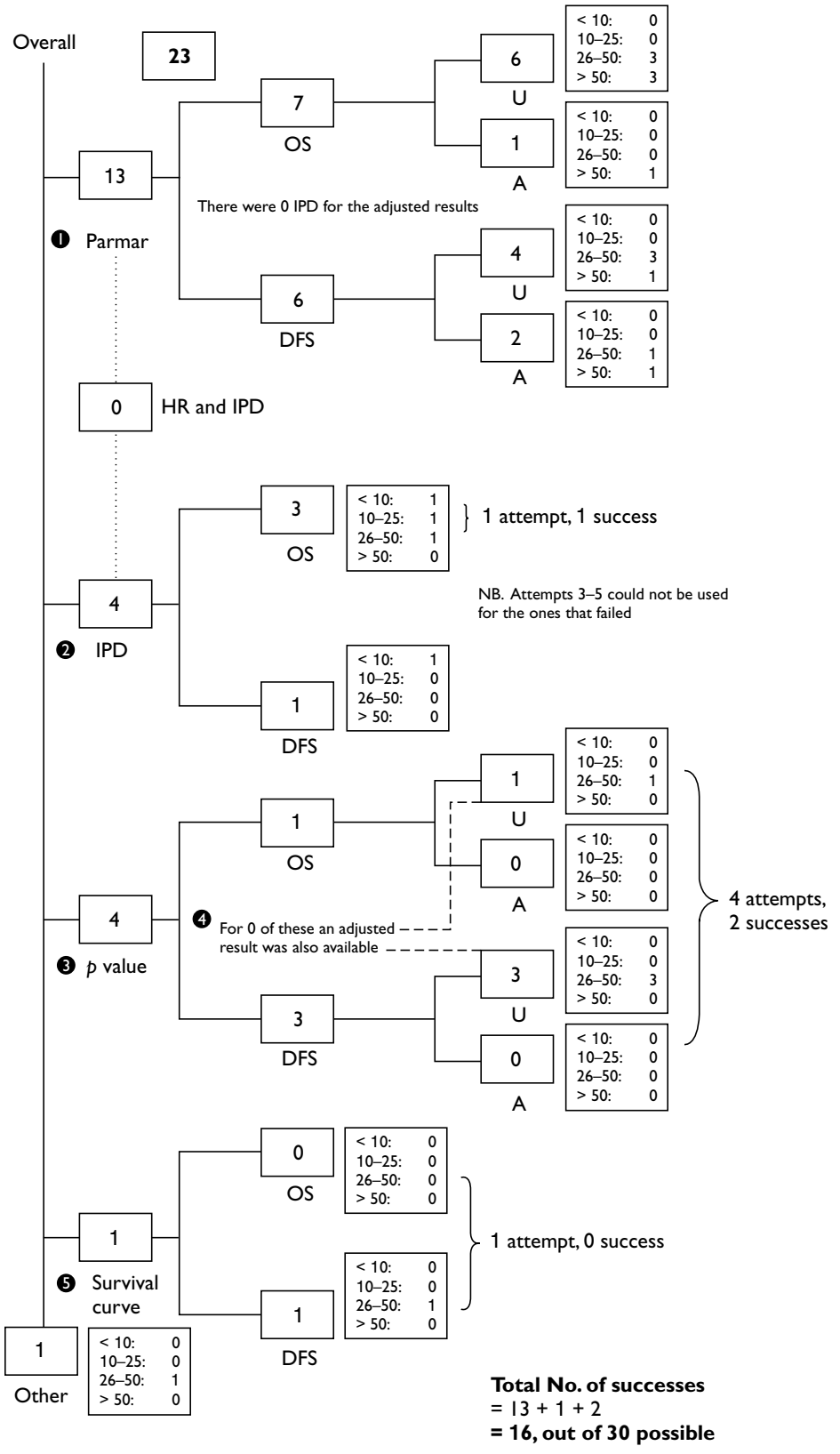

FIGURE 18 *Multidrug resistance protein*

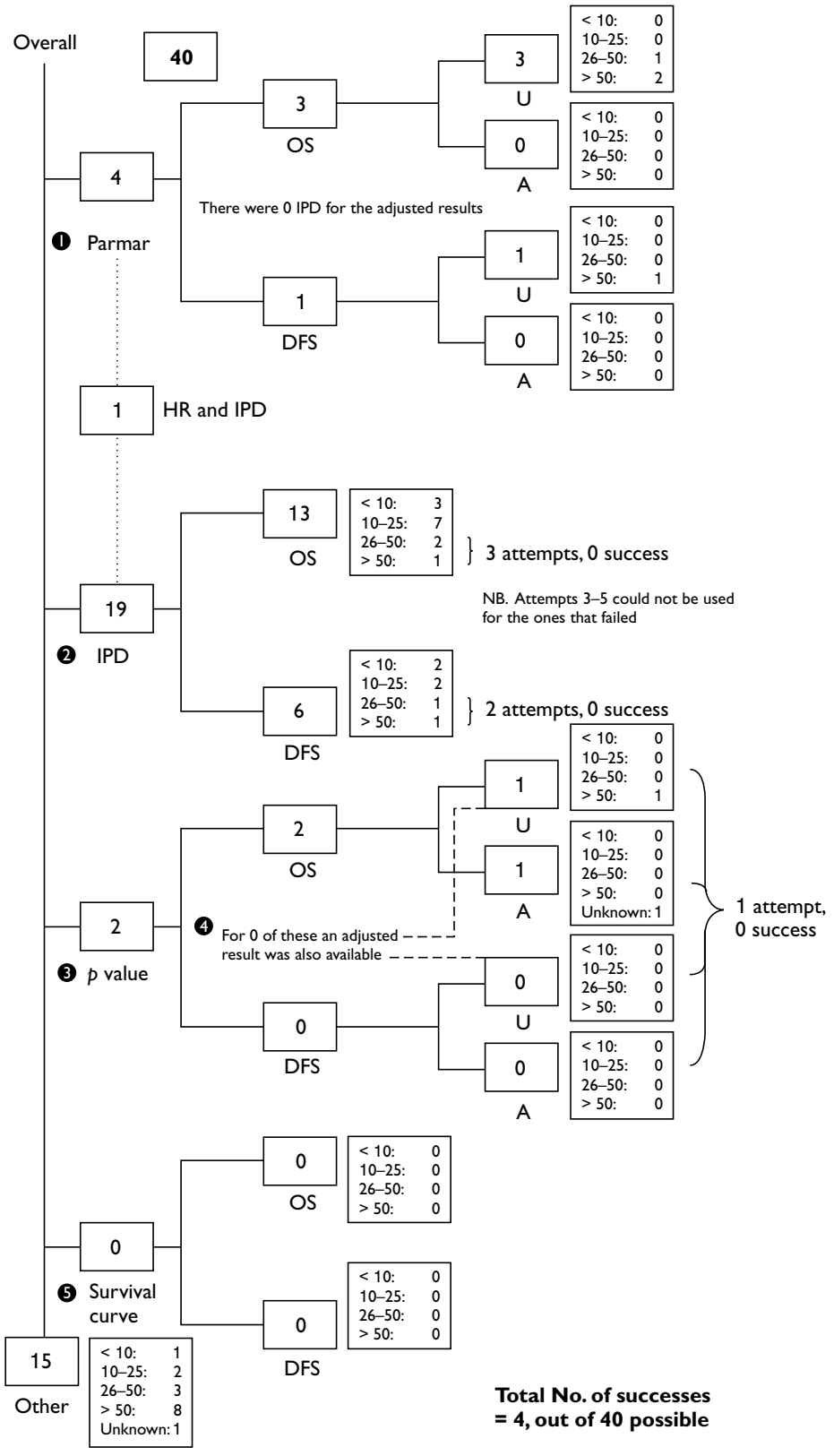

FIGURE 19 *VMA*

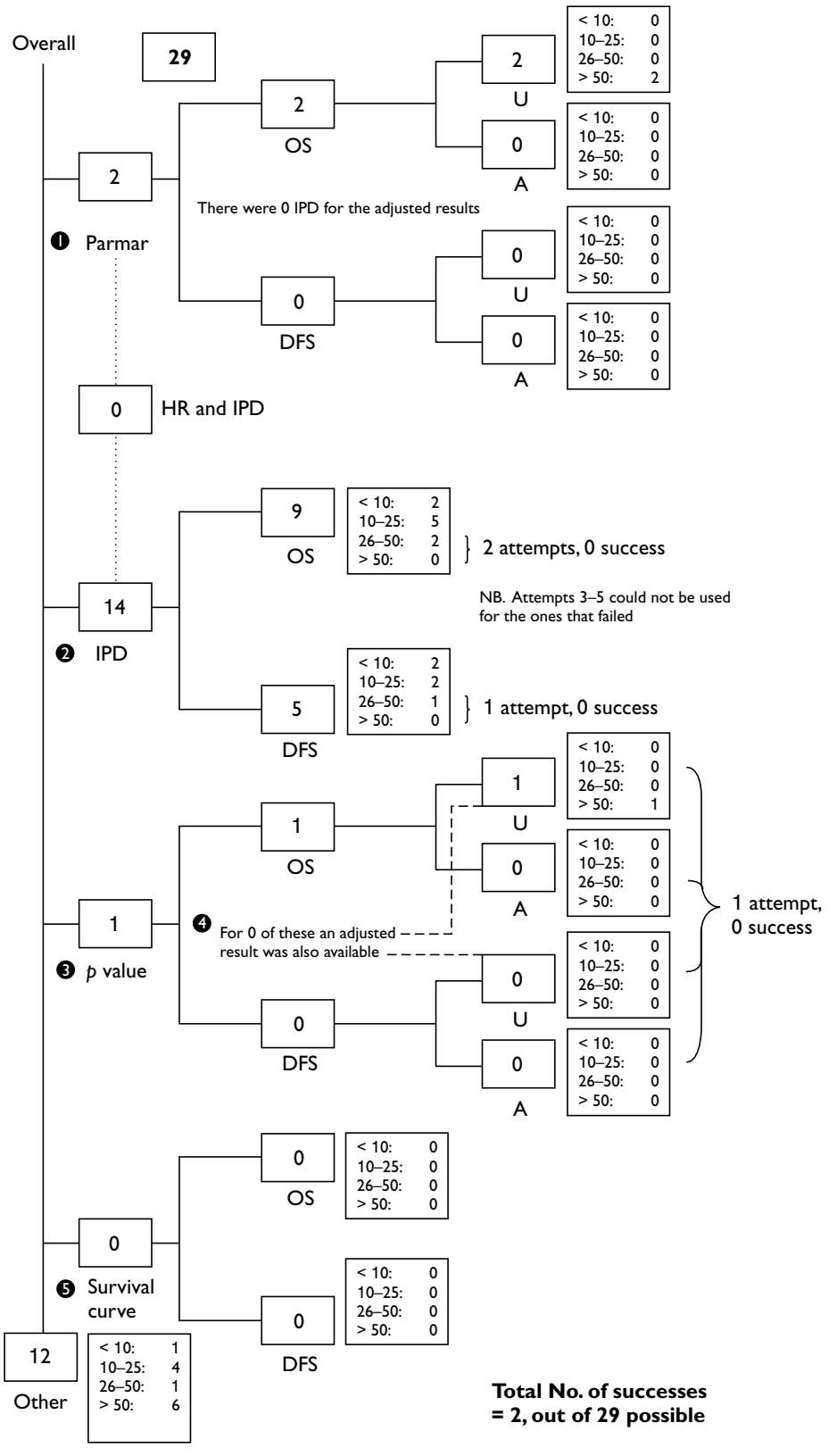

FIGURE 20 *HVA*

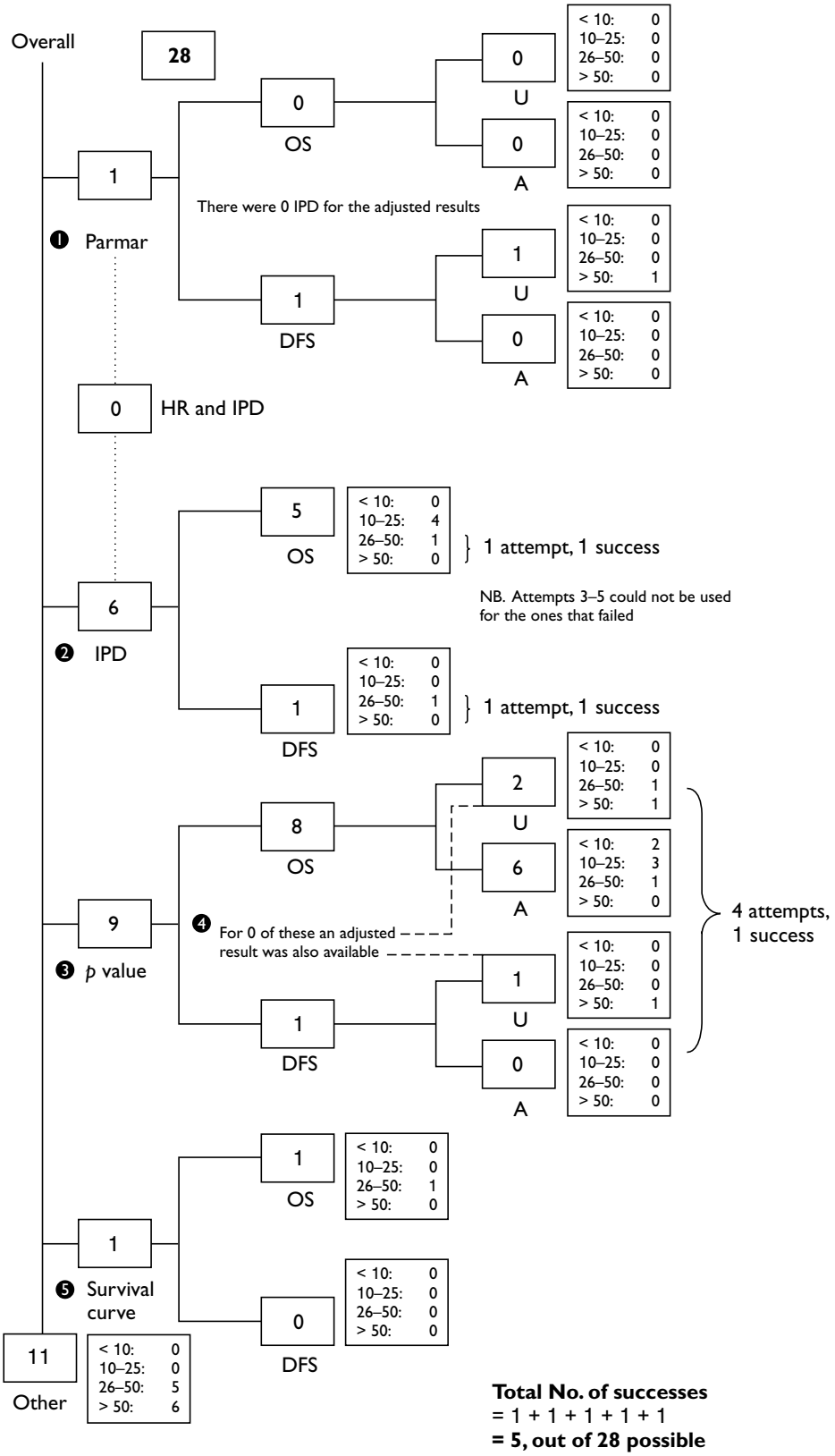

FIGURE 21 *VMA:HVA*

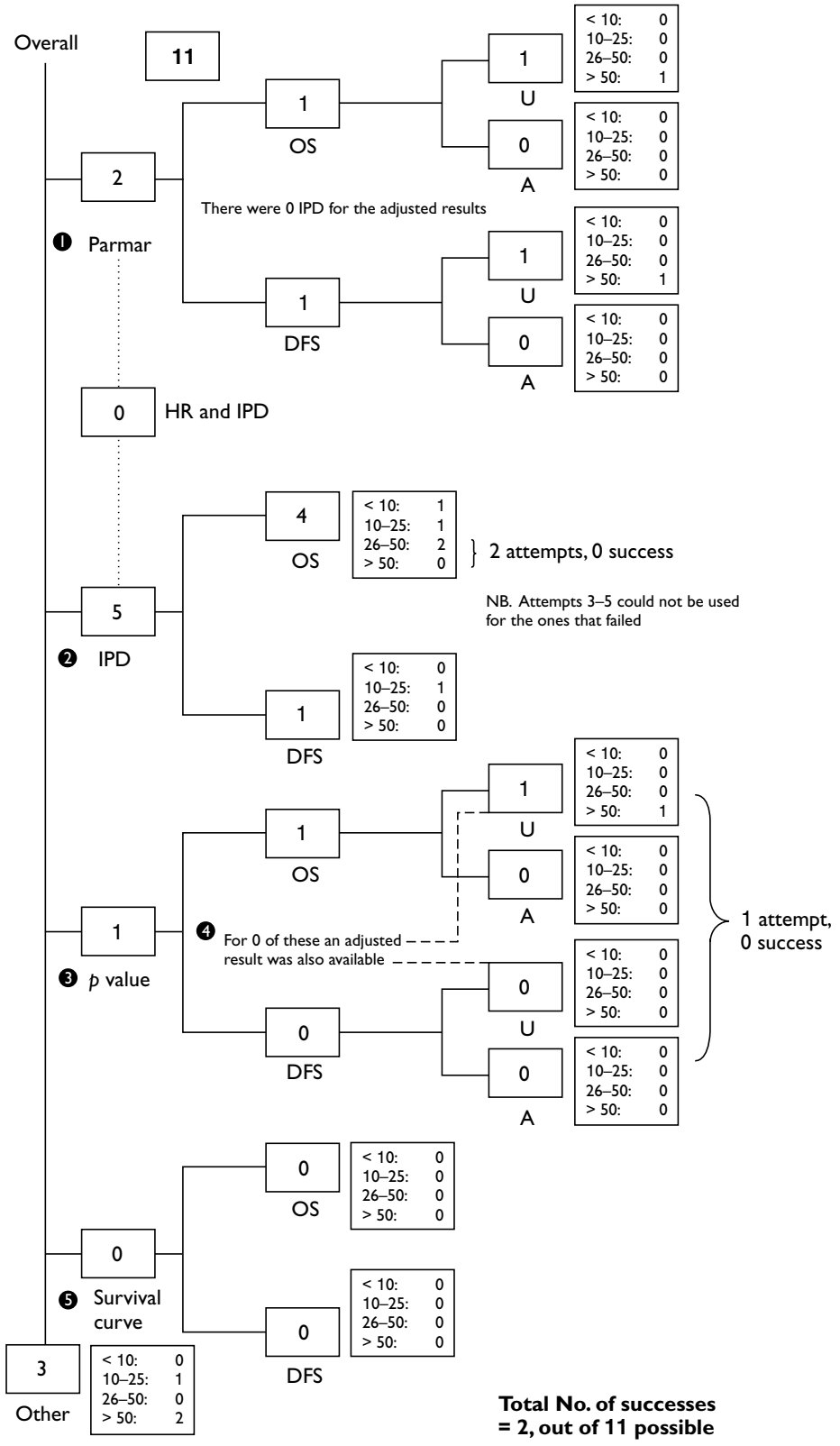

FIGURE 22 *Dopamine*

Appendix 11

Successful estimates made for each marker, with details of important clinical and statistical information relating to each (neuroblastoma review)

Tables 12–24 show the successful estimates of $\log_e$(HR) and its variance obtained through attempts 1–5 for each of the 13 prognostic tumour markers studied together with covariate information. (NB. The number of different estimates for each marker tallies with total successes displayed in *Figures 10–22.*)

DNA/chromosome aberrations:

- *Table 12* (MYC-N)
- *Table 13* (DNA index)
- *Table 14* (chromosome 1p)

Biological markers:

- *Table 15* (LDH)
- *Table 16* (CD44)
- *Table 17* (NSE)
- *Table 18* (TrkA)
- *Table 19* (ferritin)
- *Table 20* (multidrug resistance protein)

Urinary catecholamines:

- *Table 21* (VMA)
- *Table 22* (HVA)
- *Table 23* (VMA:HVA)
- *Table 24* (dopamine)

Table key

Paper No.	Number of the paper from which the estimate was derived.
Attempt	Refers to which of attempts 1–5 the estimate came from.
Parmar No.	If attempt 1 or 3 was used, this specifies which of the methods presented by Parmar and co-workers[55] was used to obtain the estimate (see appendix 14).
No. of patients	Total number of patients in the two groups defined by the level of the tumour marker of interest.
Worse group?	Relates to the cut-off point and refers to which group of patients had the worse outcome, e.g. 'high' is the worse group with levels above the cut-off, 'low' is the worse group with levels below the cut-off, etc.
$\log_e$(HR)	Estimate of $\log_e$(HR); log HR > 0 if the high group had a worse outcome, < 0 if the low group was worse.
var[($\log_e$HR)]	Estimate of the variance of $\log_e$(HR).
u/a	Whether the estimate of the (HR) obtained was unadjusted (u) or adjusted (a).
Age	The age of patients: whether < 1 year of age; > 1 year of age; or all ages (both < 1 and > 1 year of age).
Stage	Stage of neuroblastoma represented by the patients studied; all = all stages (i.e. Stages 1, 2, 3, 4, 4S).
Outcome	Survival: OS or DFS.
Cut-off	Cut-off used to define the two groups (low, high) for the marker of interest.
Adjusted for...	If the result obtained was an adjusted estimate, this explains what the estimate was adjusted for.
Adjusted result might also be available...	If the result obtained was an unadjusted estimate and an adjusted result was also **potentially** available, this column defines what the adjusted result was adjusted for.

TABLE 12 *MYC-N*

Paper No.	Attempt	Parmar No.	No. of patients	Worse group?	$\log_e$(HR)	var[$\log_e$(HR)]	u/a	Age	Stage	Outcome	Cut-off	Adjusted for...	Adjusted result might also be available...
388	1	1	149	High	1.449	0.328	a	All	Stage 1, 2, 3	DFS	4 copies	Age (> 1 year, < 1 year)	
388	1	1	87	High	1.022	0.28166	a	All	Stage 4	DFS	4 copies	No. of symptoms	
27	1	7	66	High	1.27069	1.6462585	a	All	All	OS	10 copies	Age (continuous scale), stage, Shimada, nse	
40	1	7	94	High	2.37108	0.5192455	u	All	All	OS	1 copy		
59	1	7	30	High	1.53749	0.3	a	< 1 year	Stage 2, 3, 4, 4S	OS	3 copies	Diploid	
87	1	3	60	High	2.59002	1.077294	u	All	All	OS	Mean of gene expression		*MYC-N* amplification
87	1	3	60	High	1.63511	0.413884	u	All	All	DFS	Mean of gene expression		*MYC-N* amplification
87	1	3	60	High	0.70804	0.3935495	a	All	All	OS	3 copies	MYC-N gene expression	
93	1	7	21	High	1.75241	0.4083333	u	All	Unknown	OS	1 copy		
102	1	5	59	High	2.04122	0.3848234	a	All	All	DFS	3 copies	Age (< 1 year, > 1 year), stage, DNA index	
108	1	7	77	High	2.62768	0.569877	a	Unknown	All	OS	Unknown	PTN	
109	1	8	59	High	2.99172	0.2637121	u	All	All	OS	Unknown		
111	1	7	153	High	1.25903	0.1464032	u	All	All	OS	3 copies		
122	1	7	29	High mRNA	0.77122	0.4687848	a	Unknown	All	DFS	Unknown	Unknown	
142	1	8	42	High	0.55015	0.1451613	u	Unknown	All	DFS	10 copies		
188	1	3	225	High	2.18605	0.1220517	u	All	Stage 1, 2, 3	DFS	10 copies		
194	1	7	18	Positive	1.71722	0.4444444	u	All	All	OS	Negative or positive protein		
199	1	7	492	High	0.76198	0.038358	u	All	All	OS	Unknown		
200	1	7	41	High	1.05881	0.2918403	u	All	All	DFS	10 copies		
200	1	7	41	Positive	1.4755	0.2010766	u	All	All	DFS	Negative or positive protein		
247	1	7	26	High	2.1285	0.6828283	u	All	Stage 2, 3, 4, 4S	OS	10 copies		
254	1	5	232	High	1.77071	0.207138	u	All	All	DFS	10 copies		
256	1	7	32	High	1.52171	0.213868	u	All	Stage 3, 4	OS	10 copies		

continued

TABLE 12 contd *MYC-N*

Paper No.	Attempt	Parmar No.	No. of patients	Worse group?	$\log_e$(HR)	var[$\log_e$(HR)]	u/a	Age	Stage	Outcome	Cut-off	Adjusted for...	Adjusted result might also be available...
276	1	8	81	High	2.97696	0.3313636	u	All	All	DFS	10 copies		
278	1	5	47	High	2.50144	0.5779104	u	All	All	DFS	10 copies		Stage, age
280	1	3	89	High	1.91692	0.1172873	u	All	All	DFS	1 copy		Stage, ferritin, chromosome 1p
285	1	7	30	High	2.65543	0.4658385	u	All	All	OS	3 copies		
288	1	7	29	High	1.82247	0.5005952	u	All	Stage 3, 4	OS	10 copies		
295	1	7	34	High	2.39393	0.529304	u	All	All	DFS	3 copies		Stage, histology, ploidy, chromosome 1p, TrkA
297	1	3	295	High	1.21788	0.0462941	u	All	All	OS	3 copies		LDH, ferritin, stage, site, age
306	1	3	60	High	0.47	0.2813982	a	All	All	DFS	3 copies	Multidrug resistance protein	
316	1	5	43	High	0.95551	0.2164599	u	All	All	OS	3 copies		
317	1	7	225	High	1.89995	0.3334014	u	All	All	DFS	10 copies		
332	1	7	48	High	1.61859	0.1730769	u	All	All	DFS	10 copies		
373	1	7	68	High	3.54167	0.8286738	u	All	All	OS	Unknown		
376	1	3	237	High	0.92822	0.0721786	a	All	All	OS	Unknown	Age (< 1 year, > 1year), stage, 11q23, loss of heterozygosity	
378	1	7	147	High	0.84244	0.0655474	u	All	All	DFS	5 copies		
386	1	7	167	High	1.59948	0.2362874	u	Unknown	Stage 1, 2, 3	DFS	4 copies		1p
386	1	7	126	High	0.84255	0.074337	u	Unknown	Stage 4	DFS	4 copies		
387	1	3	60	High	2.59002	1.077294	u	All	Unknown	OS	1 copy		
387	1	3	60	High	1.63511	0.414245	u	All	Unknown	DFS	1 copy		
393	1	7	45	High	1.86553	0.3214286	u	All	All	OS	Unknown		
396	1	8	110	High	2.80061	0.2739106	u	Unknown	All	OS	10 copies		
465	1	5	85	High	0.25464	0.0844886	u	> 1 year	Stage 4	DFS			
469	1	5	37	Positive	0.04879	0.1589515	a	> 1 year	Unknown	OS	Negative or positive protein		MYC-N copy
469	1	8	37	High	1.00202	0.2910289	u	> 1 year	Unknown	OS	5 copies		
474	1	7	12	High	2.36561	1	u	> 1 year	Stage 3	DFS	10 copies		
501	1	8	18	High	5.04262	1.2	u	All	All	OS	3 copies		
506	1	3	237	High	0.6668	0.0850725	a	All	All	OS	Unknown	Age (< 1 year, > 1 year), Stage 4, Shimada, 1p36	

continued

***TABLE 12* contd** *MYC-N*

Paper No.	Attempt	Parmar No.	No. of patients	Worse group?	$\log_e$(HR)	var[$\log_e$(HR)]	u/a	Age	Stage	Outcome	Cut-off	Adjusted for...	Adjusted result might also be available...
506	1	3	237	High	0.3001	0.0675255	a	All	All	DFS	Unknown	age (< 1 year, > 1 year), Stage 4, Shimada, 1p36	
544	1	7	81	High	1.7352	0.1989146	u	All	All	OS	Unknown		
548	1	7	319	High	5.70267	3.0035714	u	All	Stage 1	OS	3 copies		Age
548	1	7	319	High	5.70267	3.0035714	u	All	Stage 1	DFS	3 copies		Age
39	2		33	High	3.3329	0.5065169	u	All	All	OS	1 copy		
44	2		48	High	1.5372	0.265225	u	All	All	OS	1 copy		
44	2		50	High	1.6375	0.2626563	u	All	All	DFS	1 copy		
50	2		48	Positive	2.0684	0.4823303	u	All	All	OS	Negative or positive		
80	2		48	Positive	1.5135	0.2344496	u	Unknown	All	OS	Negative or positive		
80	2		48	Positive	1.4552	0.1676903	u	Unknown	All	DFS	Negative or positive		
106	2		48	High	2.3113	0.2516026	u	All	All	OS	1 copy		
107	2		35	High	2.364	0.3201296	u	All	All	OS	1 copy		
109	2		59	High	2.1876	0.176568	u	All	All	DFS	1 copy		
173	2		31	High	0.4291	0.6590192	u	All	All	OS	Unknown		
173	2		30	High	0.4145	0.6689604	u	All	All	DFS	Unknown		
200	2		41	High	1.3233	0.2592846	u	All	All	OS	1 copy		
200	2		41	High	2.1691	0.418609	u	All	All	OS	Negative or positive		
214	2		26	High	0.7332	0.5032484	u	All	Stage 3, 4	OS	1 copy		
216	2		31	High	1.745	0.5194085	u	All	All	OS	1 copy		
239	2		27	High	1.1773	0.3287876	u	All	All	DFS	1 copy		
246	2		38	High	2.8988	1.216609	u	All	All	OS	1 copy		
246	2		38	High	1.8485	0.435864	u	All	All	DFS	1 copy		
260	2		28	High	2.7462	1.2115405	u	All	Stage 1, 2, 3, 4	OS	1 copy		
260	2		28	High	2.9515	1.1752728	u	All	Stage 1, 2, 3, 4	DFS	1 copy		
277	2		48	High	1.827	0.2238236	u	All	Stage 1, 2, 3, 4	OS	10 copies		
277	2		48	High	1.8951	0.2131669	u	All	Stage 1, 2, 3, 4	DFS	10 copies		
288	2		28	High	1.8983	0.7782768	u	All	Stage 1, 2, 3, 4	DFS	1 copy		
540	2		32	High	0.7039	0.3101376	u	All	All	OS	10 copies		
540	2		32	High	0.7648	0.2367796	u	All	All	DFS	10 copies		

continued

***TABLE 12* contd** *MYC-N*

Paper No.	Attempt	Parmar No.	No. of patients	Worse group?	$\log_e$(HR)	var[$\log_e$(HR)]	u/a	Age	Stage	Outcome	Cut-off	Adjusted for...	Adjusted result might also be available...
122	3	7	34	High mRNA	1.34419	0.2568889	a	Unknown	All	DFS	Unknown	Unknown	
145	3	7	28	High	3.58047	2.7222222	u	< 1 year	All	DFS	3 copies		
145	3	7	61	High	0.93392	0.1052915	u	> 1 year	All	DFS	3 copies		
181	3	7	29	Staining	1.2562	0.2378394	u	All	All	OS	No staining/ staining		
193	3	7	40	High	1.63118	0.6926407	u	All	All	OS	4 copies		
306	3	7	60	High	2.08042	0.4532293	u	All	All	OS	3 copies		Multidrug resistance protein
315	3	8	121	High	3.29269	0.2547413	u	All	All	DFS	2 copies		
337	3	7	57	Positive	1.70149	0.1485597	u	All	All	DFS	Negative or positive		
337	3	7	57	Positive	1.38329	0.1383142	u	All	All	OS	Negative or positive		
337	3	7	37	High	0.41085	0.1932524	u	All	All	DFS	1 copy		
356	3	7	58	High	2.24971	0.7628118	u	All	All	OS	10 copies		
505	3	7	102	High	1.42979	0.1350555	u	All	Stage 4	DFS	10 copies		
152	5		89	High	1.5216	0.12119	u	All	All	DFS	1 copy		
335	4	3	Unknown	High	2.55723	0.3010159	a	All	Stage 3	DFS	1 copy	Age (< 1 year, > 1 year), Shimada, ferritin	
495	4	5	122	High	0.51879	0.1712756	a	All	All	DFS	3 copies	Telomerase, stage, LDH, age (continuous scale)	
528	4	5	67	High	0.29267	0.3483816	a	All	All	DFS	3 copies	Telomerase, stage	

TABLE 13 *DNA index*[*]

Paper No.	Attempt	Parmar No.	No. of patients	Worse group?	$\log_e$(HR)	var[$\log_e$(HR)]	u/a	Age	Stage	Outcome	Cut-off	Adjusted for...	Adjusted result might also be available...
280	1	3	89	Diploid worse	–0.78845736	0.133215	u	All	All	DFS	Diploid, aneuploid		Stage, MYC-N, ferritin, chromosome1p
102	1	5	59	Diploid worse	–2.20717491	0.4499407	u	All	All	DFS	Diploid, near-triploid		Age, stage, MYC-N
117	1	5	41	Diploid worse	–0.91629073	0.1013537	u	All	All	OS	Diploid, aneuploid		Age, stage, MYC-N, MDR1
46	1	6	68	No worse	–1.484	0.3489654	a	All	All	OS	Aneuploid: no or yes		Sex, age (1 year), primary site, stage
199	1	7	654	DNA = 1 worse	–0.5173953	0.0176853	u	All	All	OS	Index = 1 versus > 1		
295	1	7	34	Diploid worse	–1.08907665	0.3087607	u	All	All	DFS	Diploid/ tetraploid versus aneuploid		Stage, histology, MYC-N, chromosome 1p, TrkA
393	1	7	45	Diploid/ tetraploid worse	–1.56081127	0.225	u	All	All	OS	Diploid/ tetraploid versus triploid		
93	1	7	21	Diploid, tetraploid	–1.64338774	0.3533654	u	All	All	OS	Diploid, tetraploid versus hyper-diploid, triploid		
40	1	7	92	DNA = 1 worse	–4.5804236	1.9377289	u	< 2 years	All	OS	Index: 1, > 1		
469	1	7	57	1.2 worse	–0.77056647	0.5259524	u	> 1 year	Unknown	OS	Index: 1.2, > 1.2		
234	1	7	54	Diploid worse	–1.058132	0.16875	u	All	All	OS	Diploid, aneuploid		
239	2		27	Diploid worse	–0.9432	0.336	u	All	All	DFS	Diploid, aneuploid		
109	2		51	< 1 worse	–0.9735	0.27092	u	All	All	OS	Index: 1, > 1		
109	2		51	< 1 worse	–0.2976	0.5962928	u	All	All	DFS	Index: 1, > 1		
548	2		31	> 1 worse	0.118	1.143616	u	All	Stage 1	OS	Index: 1, > 1		
548	2		31	< 1 worse	–0.2976	0.596298	u	All	Stage 1	DFS	Index: 1, > 1		
193	3	7	40	Diploid worse	–2.34177039	0.5064894	u	All	All	OS	Diploid, aneuploid		
410	3	7	52	2*n* worse	–2.2182168	0.4544538	u	< 1 year	All	DFS	Diploid, triploid		
410	3	7	43	4*n* worse	1.89401454	0.9338384	u	< 1 year	All	DFS	Triploid, tetraploid		

[*] *The DNA index (DI) of 1 corresponds to diploid level (2c) whereas the DNA index of unnormal population is represented by the mean channel number of the G1 peak of the tumour divided by the mean channel number of the standard G1 peak. Cells with a DI of 1.5 are classified as triploid; tetraploid cells have an index of 2.0. Index values between these numbers represent aneuploid populations*

TABLE 14 *Chromosome 1p*

Paper No.	Attempt	Parmar No.	No. of patients	Worse group?	$\log_e$(HR)	var[$\log_e$(HR)]	u/a	Age	Stage	Outcome	Cut-off	Adjusted for...	Adjusted result might also be available...
188	1	3	91	LOH1p present	3.05870707	0.3503725	u	All	Stage 1, 2, 3	DFS	Loss of heterozygosity: no or yes		Stage, MYC-N, surgery, histology, lymph node
280	1	3	89	LOH1p present	1.90210753	0.1210736	u	All	All	DFS	No loss versus loss		Stage, feritin, MYC-N
338	1	3	53	LOH1p present	2.80336038	0.3275413	u	All	All	OS	LOH1p: no or yes		MYC-N, age, stage (three separate results)
386	1	6	74	LOH1p present	2.1	1.2271598	a	Unknown	Stage 1, 2, 3	DFS	Deletion: no or yes		MYC-N
111	1	7	156	LOH1p present	1.2050837	0.134127	u	All	All	OS	Loss of heterozygosity: no or yes		
109	1	7	59	LOH1p present	1.34137329	0.2637121	u	All	All	OS	Deletion: no or yes		
295	1	7	32	LOH1p present	0.16905866	0.4029909	u	All	All	DFS	Negative or positive		Stage, histology, MYC-N, DNA-ploidy, TrkA
403	1	7	52	LOH1p present	1.48255346	0.203003	u	All	Unknown	DFS	Loss of heterozygosity: no or yes		
544	1	7	58	LOH1p present	0.34926181	0.2681333	u	All	Stage 2, 3, 4, 4S	OS	Loss of heterozygosity: no or yes		
93	1	7	21	LOH1p present	0.82349718	0.4083333	u	All	All	OS	Deletion: no or yes		
506	1	7	238	LOH1p present	0.7892176	0.0411491	u	All	All	DFS	Loss of heterozygosity: no or yes		Stage 4, MYC-N, age
80	2		35	LOH1p present	0.9495	0.2692572	u	Unknown	All	OS	Negative or positive		
106	2		48	LOH1p present	1.3107	0.2459168	u	All	All	OS	Deletion: no or yes		
173	2		30	LOH1p present	0.0322	0.5869092	u	All	All	OS	Negative or positive		
277	2		50	LOH1p present	1.7086	0.2281018	u	All	Stage 1, 2, 3, 4	OS	Deletion: no or yes		
80	2		35	LOH1p present	0.8045	0.190096	u	Unknown	All	DFS	Negative or positive		

continued

***TABLE 14* contd** *Chromosome 1p*

Paper No.	Attempt	Parmar No.	No. of patients	Worse group?	$\log_e$(HR)	var[$\log_e$(HR)]	u/a	Age	Stage	Outcome	Cut-off	Adjusted for...	Adjusted result might also be available...
173	2		30	LOH1p present	0.649	0.705768	u	All	All	DFS	Negative or positive		
277	2		50	LOH1p present	1.6676	0.2056623	u	All	Stage 1, 2, 3, 4	DFS	Deletion: no or yes		
506	4	3	237	LOH1p present	0.385	0.0673	a	All	All	OS	Loss of heterozygosity: no or yes		Stage 4, MYC-N, age
188	5		91	LOH1p present	3.0694	2.143	u	All	Stage 1, 2, 3	OS	Loss of heterozygosity: no or yes		

LOH1p, loss of heterozygosity of chromosome 1p

TABLE 15 *LDH*

Paper No.	Attempt	Parmar No.	No. of patients	Worse group?	$\log_e$(HR)	var[$\log_e$(HR)]	u/a	Age	Stage	Outcome	Cut-off	Adjusted for...	Adjusted result might also be available...
388	1	1	341	High	0.548	0.0469	a	All	Stage 1, 2, 3	DFS	Unknown	Infiltration, site, histology, resectability, age, symptoms	
280	1	3	87	High	1.5260563	0.1564682	u	All	All	DFS	1500 U/l		Ferritin, stage
297	1	3	268	High	1.39376638	0.047319	u	All	All	OS	1000 U/l		MYC-N, ferritin, stage, site, age
495	1	5	122	High	0.79299252	0.2545364	a	All	All	DFS	300 if age < 1 year, 400 if age > 1 year	Telomerase, stage, MYC-N, age	
14	1	7	120	High	2.94965604	0.8035714	u	All	Stage 1, 2, 3, 4S	OS	1500 U/l		
14	1	7	162	High	0.65947127	0.0401674	u	All	Stage 4	OS	1500 U/l		
188	1	7	104	High	2.53773835	0.3997044	u	All	Stage 1, 2, 3	DFS	2 x normal range		
199	1	7	1149	High	0.42124455	0.0117229	u	All	All	OS	1500 U/l		
317	1	7	104	High	2.08031249	0.3997044	u	All	Stage 1, 2, 3	DFS	2 x normal value		
40	1	7	92	High	4.58042362	1.9377289	u	All	All	OS	1500 U/l		
8	4	6	182	High	0.9003	0.0705538	a	All	Stage 4	DFS	Unknown	Resectability, grade, leukocytes, symptoms, condition, age	
9	4	6	96	High	1.0187	0.0974252	a	All	Stage 1, 2, 3	DFS	Unknown	Resectability, weight loss, tumour extension, age, site, pain, condition, No. of symptoms	

TABLE 16 *CD44*

Paper No.	Attempt	Parmar No.	No. of patients	Worse group?	$\log_e$(HR)	var[$\log_e$(HR)]	u/a	Age	Stage	Outcome	Cut-off	Adjusted for...	Adjusted result might also be available...
279	3	7	52	Negative	–1.8157498	0.3045045	u	Unknown	All	DFS	Negative or positive		
315	3	8	121	Negative	–3.929365	0.2360842	u	All	All	DFS	Negative or positive		MYC-N, age, stage, histology
384	3	8	52	Negative	–2.5354032	0.2865925	u	All	All	DFS	Negative or positive		MYC-N, age, stage, histology

TABLE 17 *NSE*

Paper No.	Attempt	Parmar No.	No. of patients	Worse group?	$\log_e$(HR)	var[$\log_e$(HR)]	u/a	Age	Stage	Outcome	Cut-off	Adjusted for...	Adjusted result might also be available...
297	1	3	169	High	1.54756251	0.0812001	u	All	All	OS	100 ng/ml		MYC-N
278	1	5	47	High	1.19392247	0.2633936	u	All	All	DFS	0.4 µg/mg		
188	1	7	155	High	1.27914445	0.1713379	u	All	Stage 1, 2, 3	DFS	Normal versus > 2 x normal range		
317	1	7	155	High	1.27914445	0.1713379	u	All	Stage 1, 2, 3	DFS	2 x normal range		
407	1	7	35	High	2.23638117	0.4537037	a	All	Stage 4	OS	100 ng/ml	Age < 1 year	
482	1	8	63	High	5.32571715	0.7736842	u	All	Stage 1, 2, 3	DFS	80 ng/ml		
482	1	8	78	High	0.77964869	0.085613	u	All	Stage 4	DFS	80 ng/ml		
185	2		40	+++	0.1229	0.2088	u	All	All	OS	–,+,++ versus +++ staining*		
259	5		37	–, ±	–0.121	0.185	u	All	All	OS	–, ± versus +, ++ staining*		

* *Expression: –, none; +++, high. See original paper for further details*

TABLE 18 *TrkA*

Paper No.	Attempt	Parmar No.	No. of patients	Worse group?	$\log_e$(HR)	var[$\log_e$(HR)]	u/a	Age	Stage	Outcome	Cut-off	Adjusted for...	Adjusted result might also be available...
306	1	3	60	Low	–0.6931472	0.4063087	a	All	All	DFS	PCR = 0.1	MRP, age, MYC-N, stage	
295	1	7	31	Low	–1.162861	0.3520147	u	All	All	DFS	PCR = 0.1, > 0.1		Stage, histology, MYC-N, DNA, chromosome 1p
276	1	7	66	Minus worse	–1.88171	0.3324176	u	All	All	DFS	–, 2+*		
276	1	7	27	Minus worse	–2.1720294	0.4005495	u	All	All	DFS	–, 3+*		
276	1	7	78	1+ worse	–1.0405874	0.2368421	u	All	All	DFS	1+, 2+*		
276	1	7	39	1+ worse	–1.8124836	0.4090909	u	All	All	DFS	1+, 3+*		
393	1	8	45	Absence	–2.8106939	0.25	u	All	All	OS	Absence versus presence		
183	3	7	50	Low	–2.3746805	0.3427945	u	All	All	OS	0 density units		
306	3	7	60	Low	–1.8699177	0.4807692	u	All	All	OS	PCR = 0.1, > 0.1		MYC-N, multidrug resistance protein, age, stage
332	3	8	81	Low	–1.7870962	0.1065789	u	All	All	DFS	Staining intensity: negative versus positive		
217	4	3	80	Absence	–2.2082744	0.817038	a	All	All	OS	Absence versus presence	MYC-N, stage	

* *Expression: –, none; 3+, high. See the original paper for details*

TABLE 19 *Ferritin*

Paper No.	Attempt	Parmar No.	No. of patients	Worse group?	$\log_e$(HR)	var[$\log_e$(HR)]	u/a	Age	Stage	Outcome	Cut-off	Adjusted for...	Adjusted result might also be available...
280	1	3	78	High	1.85629799	0.1501135	u	All	All	DFS	142 µg/l		Stage, MYC-N, chromosome 1p
297	1	3	254	High	1.1249296	0.0540265	u	All	All	OS	150 ng/l		MYC-N, LDH, stage, site, age
199	1	7	251	High	0.74821271	0.0475308	u	All	All	OS	Unknown		
196	3	7	77	High	1.46751801	0.1989063	u	All	All	OS	Varies depending on age (see paper)		
337	3	5	39	High	1.00503394	0.2170872	u	All	Stage 3, 4, 4S	DFS	150 µg/l		
505	3	7	115	High	0.99459021	0.1004405	u	< 1 year	Stage 4	DFS	143 ng/ml		
335	4	4	228	High	2.175	0.288	a	All	Stage 3	DFS	143 ng/ml	Age, MYC-N, Shimada	

TABLE 20 *Multidrug resistance protein*

Paper No.	Attempt	Parmar No.	No. of patients	Worse group?	$\log_e$(HR)	var[$\log_e$(HR)]	u/a	Age	Stage	Outcome	Cut-off	Adjusted for...	Adjusted result might also be available...
387	1	3	60	High	2.00148	0.6236541	u	> 1	Unknown	OS	Used median (see paper)		
387	1	3	60	High	2.16332303	0.6105274	u	> 1	Unknown	DFS	Used median (see paper)		
306	1	3	60	Expression worse	1.74046617	0.7225887	a	All	All	OS	Expression: no or yes	MYC-N	
306	1	3	60	Expression worse	1.82454929	0.4845401	a	All	All	DFS	Expression: no or yes	MYC-N	
389	1	3	47	High	1.47017585	0.5056672	u	All	All	DFS	80th percentile PCR		MYC-N
389	1	3	47	High	1.74221902	0.5450615	u	All	All	DFS	90th percentile PCR		MYC-N
58	1	7	44	Positive worse	2.09532884	0.2668872	u	All	Stage 3, 4, 4S	DFS positive	Negative,		
58	1	7	44	Positive worse	2.10491968	0.3202647	u	All	Stage 3, 4, 4S	OS	Negative, positive		
122	1	7	29	Unknown	0.04293363	0.4687848	a	Unknown	All	DFS	Unknown		
309	1	7	64	Negative worse	–0.8005417	0.1668296	u	All	All	OS	Negative, positive		
532	1	7	35	0+, 1+ worse	–1.3419169	0.2552083	u	All	All	OS	0+, 1+, 2+, 3+*		
117	1	11	66	0–30	1.7	0.2400794	u	All	All	OS	0 versus 0–30		Age, stage, DNA index, MYC-N
117	1	11	42	> 30	4	0.2552083	u	All	All	OS	0 versus > 30		Age, stage, DNA-index, MYC-N
107	2		35	0+, 1+	–1.3535	0.30547	u	All	All	OS	0+, 1+, 2+, 3+*		
520	3	7	34	Positive	1.68606613	0.2668872	u	All	Stage 3, 4, 4S	DFS	Negative, positive		
520	3	7	43	Positive	1.17851003	0.3202647	u	All	Stage 3, 4, 4S	OS	Negative, positive		

* *Expression: 0, none; 3+, high. See the original paper for further details*

TABLE 21 *VMA*

Paper No.	Attempt	Parmar No.	No. of patients	Worse group?	$\log_e$(HR)	var[$\log_e$(HR)]	u/a	Age	Stage	Outcome	Cut-off	Adjusted for...	Adjusted result might also be available...
297	1	3	269	Low	–0.2357223	0.0693965	u	All	All	OS	< 2.5 versus > 2.5 standard deviations of normal range		MYC-N
565	1	7	59	Low	–0.7451424	0.3088731	u	< 1	Stage 2, 3, 4, 4S	DFS	Unknown		
544	1	7	74	Low	–0.16533119	0.1841044	u	All	Stage 2, 3, 4, 4S	OS	1 µmol/mmol of creatinine		
256	1	7	32	Low	–1.81873931	0.210526316	u	All	Stage 3, 4	OS	4.6 mg/day of VMA (urinary value)		

TABLE 22 *HVA*

Paper No.	Attempt	Parmar No.	No. of patients	Worse group?	$\log_e$(HR)	var[$\log_e$(HR)]	u/a	Age	Stage	Outcome	Cut-off	Adjusted for...	Adjusted result might also be available...
297	1	3	232	High	0.41210965	0.1593855	u	All	All	OS	< 2.5 versus > 2.5 standard deviations from mean value		MYC-N
544	1	7	73	Low	–0.15360415	0.1589133	u	All	Stage 2, 3, 4, 4S	OS	26 µmol/mmol of creatinine		

TABLE 23 *VMA:HVA ratio*

Paper No.	Attempt	Parmar No.	No. of patients	Worse group?	$\log_e$(HR)	Var[$\log_e$(HR)]	u/a	Age	Stage	Outcome	Cut-off	Adjusted for...	Adjusted result might also be available...
230	1	8	97	Low	–1.5675518	0.1988041	u	Unknown	Stage 1, 2, 3	DFS	0.7		
246	2		32	Low	–0.7454	1.225	u	All	All	OS	1		
246	2		32	Low	–1.1827	0.8428	u	All	All	DFS	1		
337	3	5	52	Low	–0.5128236	0.198736	u	All	Stage 3, 4, 4S	DFS	1		
256	5		31	Low	–0.835	0.242	u	All	Stage 3, 4	OS	0.4		

TABLE 24 *Dopamine*

Paper No.	Attempt	Parmar No.	No. of patients	Worse group?	$\log_e$(HR)	Var[$\log_e$(HR)]	u/a	Age	Stage	Outcome	Cut-off	Adjusted for...	Adjusted result might also be available...
188	1	7	258	High	0.86877942	0.0999677	u	All	Stage 1, 2, 3	DFS	2000 (see paper)		
544	1	7	73	High	1.03737031	0.1530003	u	All	Stage 2, 3, 4, 4S	OS	2.5 (see paper)		

Appendix 12

Assessment of publication bias in the DFS estimates for *MYC-N* (neuroblastoma review)

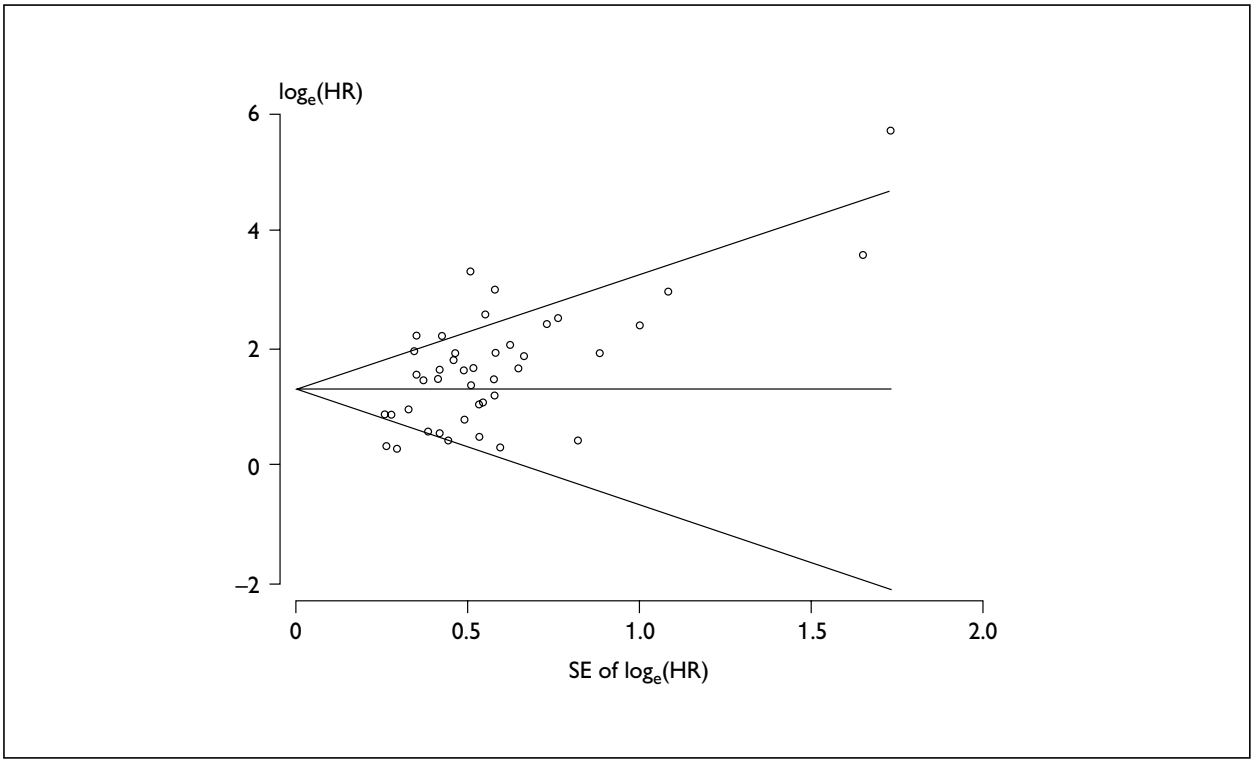

FIGURE 23 *Begg funnel plot of log$_e$(HR) for MYC-N and DFS (pseudo 95% CIs)*

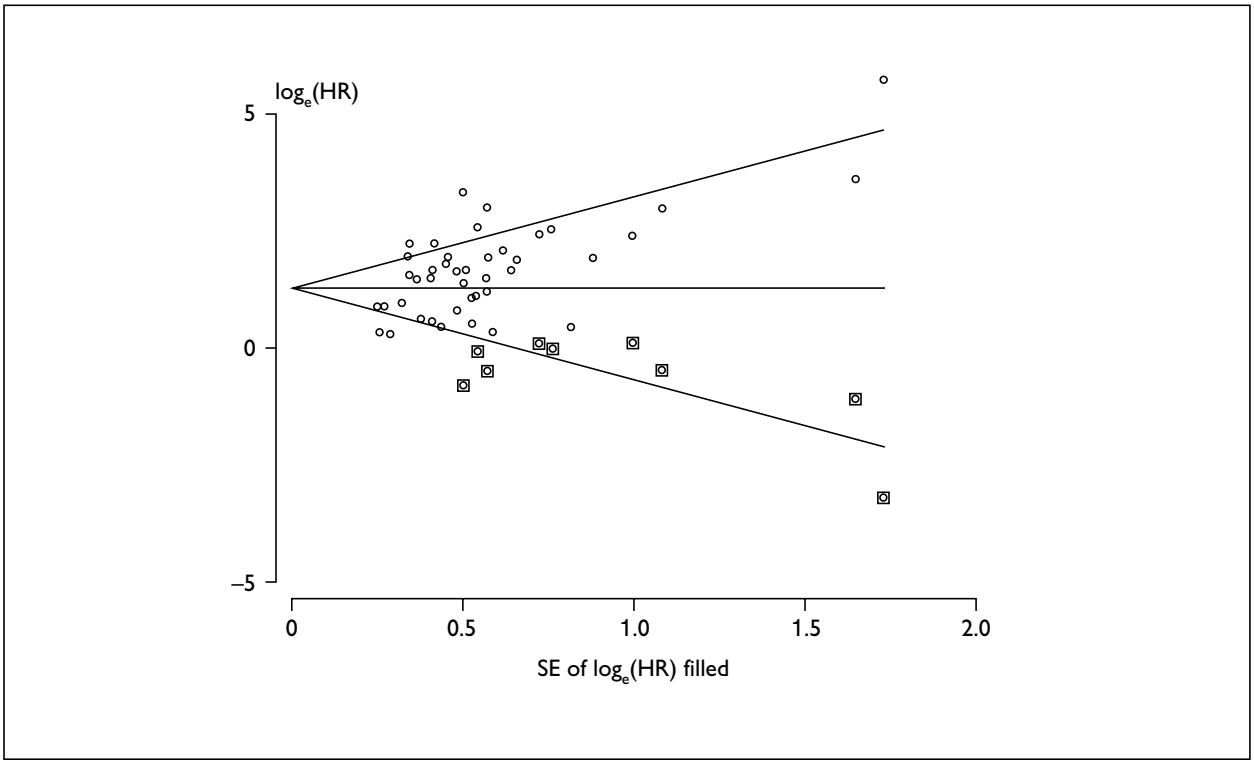

FIGURE 24 *Funnel plot of log$_e$(HR) for MYC-N and OS indicating studies estimated as 'missing' by trim and fill (pseudo 95% CIs)*

Appendix 13

Description of the two papers reporting cost information (neuroblastoma review)

Two papers were identified, and their details are given below.

(1) Sawada T, Todo S, Fujita K, Iino S, Imashuku S, Kusunoki T. Mass screening of neuroblastoma in infancy. *Am J Dis Child* 1982;**136**(8):710–2.

This reports on a study based in Japan in the 1970s and reports on a VMA for screening infants for neuroblastoma. It is not an economic evaluation, and was detected because it contains some limited cost information in the abstract, giving cost figures that are not developed in any further detail in the main body of the paper. Few details are given regarding the costing methodology and it is therefore difficult to judge the reliability of these figures.

Whilst the authors claim to conduct a 'cost–benefit' analysis of the screening programme, the analysis actually consists of a number of cost comparisons. One of these is that, in terms of treatment costs alone, it is less costly in total to treat neuroblastoma cases detected through screening (four patients in this scheme from almost 80,000 screened) than it is to treat those who might be expected to be detected clinically (two patients), since later detection requires more intensive treatment. Furthermore, these savings exist when the costs of the management of the screening programme were included. These were estimated at US $7500 per annum for postal charges, printed materials and the payment of a technical assistant to screen 13,000 infants per annum.

(2) Scriver CR, Gregory D, Bernstein M, Clow CL, Weisdorf T, Dougherty GE, *et al.* Feasibility of chemical screening of urine for neuroblastoma case finding in infancy in Quebec. *Can Med Assoc J* 1987;**136**(9):952–6.

This paper reports on screening infants for neuroblastoma in Quebec, Canada, using chemical screening of urine in addition to an existing urine metabolite screening programme for the period 1979–1985. Chemical screening in this paper refers to testing for elevated levels of HVA and VMA in urine. It is not an economic evaluation but does include cost information. Costs are reported in Canadian dollars (1985 value). These covered all investigative and treatment procedures for patients with an early diagnosis (before 1 year) and late diagnosis (later than 1 year) at Montreal Children's Hospital. Also costed were screening costs, capital costs, additional costs of testing above those already incurred as part of the current urine metabolite screening programme, confirmatory gas chromatography and follow-up investigations for those testing positive (e.g. physical examination).

The study reports that the treatment of patients diagnosed relatively early is less expensive per patient ($14,000) than the more intensive treatment required for those diagnosed later ($61,000), although few details are given about the methods by which these costs were calculated. In fact, they acknowledge in the paper that the analysis is 'not rigorous'. Based on the expected numbers of cases detected, the screening programme as a whole was estimated to be cost-saving and would also generate better expected health outcomes for positive patients due to early treatment. In a 100,000 population, total costs under the screening regime were estimated at $210,700 per annum. Without screening the total annual costs were estimated at $492,000. This results in a cost-saving of approximately $280,000, with eight lives saved.

Appendix 14

Statistical methods used to obtain estimates of $\log_e$(HR) and its variance

The following describes the methods that were used to obtain estimates of $\log_e$(HR) and its variance (var[$\log_e$(HR)]) from all the occasions desired. The methods are based on those of Parmar and co-workers,[55] and each method number corresponds to those cited throughout the report and in the tables presented in appendices 5 and 11.

(1) Given $\log_e$(HR) and var(HR):

Extract these direct estimates

(2) Given HR and var(HR):

Calculate $\log_e$(HR)
Calculate a 95% CI for HR = HR ± 1.96 × SE(HR)
Calculate a 95% CI for $\log_e$(HR) = $\log_e$ (95% HR CI)
Then use (4)

(3) Given HR and an α_i % CI:

Use logs to obtain $\log_e$(HR) and its 95% CI
Then use (4)

(4) Given $\log_e$(HR) and an α_i % CI:

Calculate

$$\text{var}[\log_e(\text{HR})] = \left(\frac{\text{upper CI}_i - \text{lower CI}_i}{2\Phi^{-1}(1-\alpha_i/2)} \right)^2$$

(5) Given HR and a p value (p_i):

Calculate $\log_e$(HR) and use (6)

(6) Given $\log_e$(HR) and a p value:

Calculate

$$\text{var}[\log_e(\text{HR})] \approx \left(\frac{\log_e(\text{HR})}{\Phi^{-1}(1-\alpha_i/2)} \right)^2$$

(7) Given a p value (p_i) for log(HR) or HR, and the total number of deaths/recurrences (O_i) and the group sizes are unequal with sizes n_1 and n_2:

Calculate

$$\log_e(\text{HR}) \approx \frac{\left[\sqrt{O_i n_{1i} n_{2i}}/(n_{1i}+n_{2i})\right]\Phi^{-1}(1-p_i/2)}{O_i n_{1i} n_{2i}/(n_{1i}+n_{2i})^2}$$

$$\text{var}[\log_e(\text{HR})] \approx \frac{(n_{1i}+n_{2i})^2}{O_i n_{1i} n_{2i}}$$

(8) Given the χ^2 statistic from the log-rank/ Mantel–Haenszel test or Cox regression or Wilcoxon test comparing two groups of patients defined by marker status and the total number of deaths/recurrences (O_i) in each group:

Use (7), since

$$\sqrt{\text{log-rank statistic}} = \Phi^{-1}\left(1-\frac{p_i}{2}\right)$$

(9) Given IPD that include initial tumour marker value, follow-up time and final known status:

Calculate the direct estimate by using a Cox proportional hazards model

(10) Given a survival curve with censoring points on it:

Estimate the observed number of events and patients at risk from each group at each event time, and use these to estimate the expected number of events for each group. Then

$$\log_e(\text{HR}) = \frac{O_1/E_1}{O_2/E_2}, \qquad \text{var}[\log_e(\text{HR})] = \frac{1}{E_1} + \frac{1}{E_1}$$

where O_i is the total number observed events in group i (i = 1, 2) and E_i is the total number expected events in group i (i = *1, 2*)

(11) Given an HR and only group numbers and group events:

Calculate $\log_e$(HR)

Calculate var[$\log_e$(HR)] from (7)

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We look forward to hearing from you.

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