

# Central review of bone marrow biopsy specimens from patients with neuroblastoma

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## Abstract

**In order to achieve some uniformity in histological detection of bone marrow infiltration by neuroblastoma and to provide a measure of variation in histological opinions, sections from 712 evaluable trephine biopsy cores from children in a European Neuroblastoma Study Group (ENSG) study were reviewed centrally. Biopsy specimens were graded as tumour positive or negative. Discordance between local and central review opinions was found in 5% of specimens. Only five of 165 children at presentation and nine of 256 re-staging procedures in 126 children, affecting one child each, had their diagnosis upgraded to positive. In six re-staging procedures, affecting one child each, the diagnosis was downgraded. The low discordance rate is encouraging and substantially less important than previously documented difficulties in obtaining adequate specimens.**

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Bone marrow trephine biopsy, in addition to aspiration, is frequently part of the assessment of children with neuroblastoma. No centrally reviewed study of histological diagnoses from such biopsy specimens has been published. We report a large series of bone marrow biopsy specimens from children with neuroblastoma in which sections from all cores have been reviewed centrally. The purpose of the review process was to achieve some uniformity of histological opinion in children entered into European Neuroblastoma Study Group (ENSG) therapeutic trials, but it also provided the first opportunity to obtain some measure of the extent of variation in those opinions in a large series.

## Methods

The study ran from January 1990 to July 1995. Sections of bone marrow biopsy specimens stained with haematoxylin and eosin or Giemsa (and often with a silver stain for reticulin) were examined by both authors. Ninety five per cent of the sections were the original slides that had been examined locally. The remainder were freshly stained sections from the original blocks. Immunohistochemical stains were not reviewed because few centres used them regularly, there is no uniformity in antibodies and

their value remains in doubt.<sup>1</sup> Evaluable biopsy specimens were graded as positive if frankly malignant tumour cells or ganglion-like neural tissue were present. All other evaluable biopsy specimens were graded as negative, irrespective of other possible abnormalities of cellularity, architecture, fibrosis or unidentifiable mononuclear cells. Evaluability has been discussed previously<sup>2</sup> but, briefly, evaluable cores had to contain 0.5 cm or more of well preserved bone marrow after fixation unless definite tumour cells were present. Numbers of positive and negative cores were determined and for each core the reviewers' opinion was compared with that of the local pathologist in order that discordance rates could be calculated. For convenience, discordant results were described as false positive or false negative with no intention to beg the question about the right answer. Therefore, no formal calculation of sensitivity or specificity of local pathologists' reports was made. For each discordant result the impact of the change on the final histological diagnosis of the staging or re-staging procedure was determined. Results were grouped into those from presentation and those after treatment had started. Many children had more than one biopsy specimen taken during each staging or re-staging procedure, and many were re-staged more than once.

## Results

Sections from 712 evaluable biopsy cores from 26 participating centres were graded. Table 1 summarises the results. As expected, most cores at presentation contained tumour while most after treatment had started did not. The discordance rate in both groups is approximately 5%.

In only five of 165 children at presentation was tumour missed by the local pathologist and the histological diagnosis of the staging procedure upgraded to positive. The other eight false negative biopsy specimens were accompanied by another from the same child with locally detected tumour and therefore did not affect the staging. The single false positive biopsy specimen at presentation was from a child with an accompanying, infiltrated biopsy. Changing the result to negative did not affect the staging. Of 256 post-treatment, re-staging procedures the final histological diagnosis was upgraded in nine, and downgraded in six. In each case a different child was affected. The remaining five discordant, post-treatment results did not affect the staging. The reviewers thus disagreed with the local histological opinion in only 3% of presentation and 6% of post-

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Table 1 Summary of reviewers' opinions on bone marrow histology

	Number of stagings		Evaluable biopsy specimens		Discordant biopsy specimens		
	Total	Positive	Total	Positive	False positive	False negative	Total
Presentation*	165	129	279	208	1	13	14 (5%)‡
After treatment†	256	105	433	151	8	12	20 (5%)‡

\*At presentation, 165 children had one staging procedure each.

†After treatment started, 126 children had one or more re-staging procedures.

‡Discordant results expressed as per cent of total evaluable biopsy specimens.

treatment staging procedures. Annual discordance rates over the five complete years of the study were 9%, 3%, 6%, 5%, and 3%. There were too few discordant results to allow meaningful centre by centre analysis and the 11 centres with no discordance submitted a mean of only four evaluable cores each.

### Discussion

Current recommendations<sup>3</sup> for staging and evaluating response to treatment in neuroblastoma include the use of trephine biopsy specimens as well as aspirates. This advice is based on the commonly experienced difficulty in detecting non-haemopoietic tumour metastases in aspirated bone marrow. Neuroblastoma is no exception.<sup>4,5</sup>

Despite the fact that most children with neuroblastoma are treated in specialist centres, few centres see large numbers of cases. Most pathologists thus have limited experience of detecting this tumour in bone marrow biopsy specimens. If routine histological examination of bone marrow biopsy specimens is important in evaluating the results of a therapeutic trial or in the management of individual patients, it should be of interest to establish the degree of variation in interpretation of this investigation. We have already documented notable variations in the adequacy of the core itself.<sup>2</sup> This report concentrates on the histological opinions.

In most cases interpretation was straightforward. Discordance between the reviewers and local reporters usually involved small deposits of what, in the reviewers' opinion, was tumour that had been missed locally. There is no gold standard and the right answer is by no means clear in every case. There is a trend towards over diagnosing infiltration in the post-treatment biopsy specimens. In some cases we suspect that osteoblastic proliferation was misinterpreted as neuroblastoma but there were too few false positive cases to be certain. Discordance rates were reassuringly low (5%) and affected the final staging procedure in only 3% at presentation and 6% post-treatment. There was no change with time. Discordance did not seem to affect some centres more than others, unlike the variations in adequacy of the biopsy core.<sup>2</sup> Bone marrow biopsy is but one of many tests used to assess extent of disease and response to treatment. The whole weight of correctly staging/re-staging a patient does not

rest on it alone. This low rate of discordance is therefore probably acceptable and unlikely to be improved on. In order to demonstrate convincingly a reduction in the discordance rate to 2.5% we would need to analyse at least 500 more staging/re-staging procedures.

Only crude, positive/negative assessments were analysed because, in effect, that is all that is required by current ENSG studies and internationally agreed criteria.<sup>3</sup> Our study indicates that pathologists in the participating centres use similar, albeit sometimes ill defined and subjective, criteria to detect tumour. Other more subtle abnormalities such as abnormal architecture and pattern of fibrous tissue<sup>4</sup> were also recorded by the reviewers. When the study is mature it may be possible to decide whether such abnormalities are clinically important. Should this be the case some reappraisal of the purpose of and need for central review may be necessary. Currently, the inadequate biopsy rate (average 17%) and its variation between centres<sup>2</sup> is a substantially larger problem than differences in histological opinions about the presence or absence of tumour in the specimens.

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Participating centres: University Hospital of Aarhus; Royal Aberdeen Children's Hospital; Royal Belfast Hospital for Sick Children; Children's Hospital, Birmingham; Royal Hospital for Sick Children, Bristol; Addenbrooke's Hospital, Cambridge; Llandough Hospital, Cardiff; University Hospital, Copenhagen; Our Lady's Hospital for Sick Children, Dublin; Royal Hospital for Sick Children, Edinburgh; Royal Hospital for Sick Children, Glasgow; St James's University Hospital, Leeds; Leicester Royal Infirmary; Gasthuisberg, Leuven; St Bartholomew's Hospital, London; Hospital for Sick Children, London; Alder Hey Hospital, Liverpool; Royal Manchester Children's Hospital; Royal Victoria Infirmary, Newcastle; Queen's University Medical Centre, Nottingham; Rikshospitalet, Oslo; John Radcliffe Hospital, Oxford; Derriford Hospital, Plymouth; Children's Hospital, Sheffield; Southampton General Hospital; Royal Marsden Hospital, Sutton.

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