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Detection of Bone Marrow Infiltration by Neuroblastoma in Clinical Practice: How Far Have We Come?

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INTRODUCTION

ALTHOUGH A 'rare' disease, neuroblastoma is the commonest extracranial non-haemopoietic tumour of children and in most cases is widely disseminated at presentation. The bone marrow is usually infiltrated by tumour. The importance of this disease lies in the poor outcome of most children with disseminated disease, and the substantial portion of the health care budget of paediatric oncology centres which is expended in its management. The importance of detecting bone marrow infiltration lies in providing cytological or histological evidence of stage 4 disease, thus influencing the choice of appropriate treatment in those with no other evidence of dissemination, and in monitoring the response to that treatment [1]. Ever increasing amounts of human and material resources are being devoted to improving the accuracy and reliability of detecting bone marrow infiltration. It is now appropriate to examine the current state of 'conventional' and alternative methods of tumour detection (rather than identification or diagnosis) in routine clinical practice. The most widely available alternative approach is immunological detection. Bone marrow culture, cytogenetic and molecular biological techniques may have a role to play in the future but are currently too specialised to be carried out routinely.

ASSESSMENT OF BONE MARROW AT PRESENTATION

Conventional techniques

In most cases this is simple. 'Conventional' techniques comprise examination of Romanovsky-stained bone marrow aspirate smears and sections of bone marrow trephine biopsies, usually formalin fixed, decalcified and paraffin embedded. These sections are stained with haematoxylin and eosin, or Giemsa, and a silver stain for reticulin fibrosis may also be useful. The cytological features of infiltration include clumps of tumour cells, syncytia, rosettes and cytoplasmic/stromal fragments [2,3]. Rosettes are present in >60% of those with marrow infiltration and in occasional cases >50 rosettes per smear may be found (unpublished observations). The need for diligent examination of smears cannot be overemphasised, but it is time consuming and lacks glamour. Within the sections of the biopsy varying degrees of primitive cell infiltration, fibrosis or scarring, and other mononuclear cell or fibroblast-like proliferations may be found [3,4]. Sometimes classical Homer-Wright rosettes are also present. Infiltration with non-haemopoietic tumour is by its very nature not uniformly distributed throughout the bone marrow. A good case has been made for sampling more than one site. Internationally agreed criteria require a minimum of two aspirates and two biopsies of marrow at initial staging [1], and revisions of these criteria recommend that at least 1 cm of well preserved bone marrow (as opposed to bone or cartilage) be obtained [5]. These techniques are sufficient to detect infiltration in most cases. European data show that even without adhering to the modern requirements for adequate staging, <10% of children with stage 4 disease have no 'conventionally' detectable infiltration at presentation [6].

Alternative techniques

The major problem lies in those with apparently limited stage disease (stages 1, 2 and 3) and the rare stage 4 cases without obvious marrow involvement. Considerable efforts have been made to develop alternative methods of detecting small numbers of tumour cells in such children. The major stimulus is the understandable assumption that children with apparently limited stage disease, who do in fact have some dissemination, will fare worse than those with uninvolved bone marrow. In clinical practice, immunofluorescent or immunocytochemical investigations of bone marrow aspirates, using a variety of antibodies directed against neuroblastoma-associated antigens, are most widely used, and panels or mixtures rather than single antibodies seem to be favoured [5,7–12]. Claims of the superiority of such approaches to 'conventional' methods are being made [10,12].

How does one assess such claims? If the 'right' answer (or a gold standard) is known, rates of true and false positivity or negativity (and thus meaningful figures for sensitivity and specificity) can be calculated. Experiments *in vitro*, in which normal bone marrow is seeded with neuroblasts derived from cell lines, can go some way to answering these questions. Extraordinarily small numbers of neuroblasts seem to be detectable in such experiments [12]. However, these experiments are intrinsically artificial and take little account of the patchy nature of infiltration *in vivo*, particularly when the number or size of individual metastases is small, or of the tendency of neuroblasts to adhere strongly to each other and to stroma within bone marrow, or of the difficulty of aspirating tumour cells from densely fibrotic areas of marrow. The most superficially convincing multicentre study [12] showed that the outcome of 'conventionally' investigated, limited stage children, and those with stage 4 disease but apparently uninvolved marrow, with immunocytologically detectable infiltration was worse than in similarly staged, immunologically negative patients. This observation suggested that, whatever the niceties of argument about sensitivity and specificity, this immunocytological technique,

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applied centrally in a dedicated laboratory, may be a powerful and clinically useful tool. Unfortunately, there were no data on the number or quality of biopsy cores taken during 'conventional' staging, nor was there central review of biopsies from stage 4 patients with 'conventionally' negative bone marrows. This undermines conclusions about the clinical importance of such a centrally executed immunological approach to tumour detection unless one accepts that local hospitals will inevitably fail to apply 'conventional' techniques properly. In addition, the use of immunocytological techniques merely increased the proportion of stage 4 cases with infiltration to the level already achieved by 'conventional' methods in other studies [6]. The argument above is further complicated by the possibility that some European centres might regularly 'understage' or some American centres 'overstage' patients on the basis of infiltration of organs other than the bone marrow.

There have been no comprehensive, large studies of the use of antibodies to reveal 'conventionally' undetectable metastases in sections of bone marrow biopsies. This is an even more difficult task since the range of useful antibodies is smaller, their specificity in doubt and studies on frozen sections of marrow, which might increase the range of antibodies, are dauntingly difficult [13].

ASSESSMENT OF BONE MARROW AFTER TREATMENT

Therapeutic trials in neuroblastoma have shown initial chemosensitivity in most cases. However, the re-emergence of resistant cells is all too familiar to paediatric oncologists, pathologists and haematologists. Laudably comprehensive international criteria for monitoring response to treatment [1] include recommendations about re-examination of bone marrow, but the problem of what constitutes residual neuroblastoma using 'conventional' methods [4] remains. At present there are no uniformly accepted criteria beyond finding, in the opinion of the pathologist/haematologist, indisputably malignant neuroblasts. The justifiable assumption, and the clinical observation, that residual neuroblasts are even more difficult to detect than at presentation has stimulated the use of immunological methods in this area too [8–11,14,15]. However, a new dimension of uncertainty is added to the problems already mentioned: the presence of reactive cells, proliferating osteoblasts and stromal tissue, some of which react with antibodies to neuroblastoma-associated antigens [11,13,14]. In addition, marked hypocellularity of treated bone marrow together with residual fibrosis/scarring may result in hypocellular or heavily blood contaminated aspirates of bone marrow. It is clear that formidable obstacles to assessment of response of bone marrow metastases and comparisons of 'conventional' with alternative approaches remain. Results of several small studies are inconclusive or contradictory but, of the 'conventional' methods, examination of bone marrow histology [11,15–17] is the most rewarding. Revisions of the international response criteria hope that immunocytological detection methods may at some time obviate the need for bone marrow biopsies [5]. However, there is little prospect of meaningful investigation of the value of such techniques until recommended 'conventional' re-staging techniques are routinely and properly executed, and large numbers of uniformly treated children have been adequately investigated by both approaches. Even the clinical importance of the different histological patterns seen in the bone marrow during treatment [4] is at present unknown. It is more likely that immunological methods of detection will complement 'conventional' techniques rather than replace them.

CONCLUSIONS

At presentation, the routine use of alternative methods of detecting bone marrow infiltration in all children with stage 4 neuroblastoma is arguably a waste of resources. 'Conventional' approaches are and may remain the gold standard, provided they are properly carried out. There is considerable potential clinical benefit in the use of immunological techniques in patients with apparently limited stage disease and in the rare stage 4 case with apparently uninvolved marrow. However, studies attempting to demonstrate such benefit will be flawed, waste resources and may contribute to inappropriate therapeutic planning unless 'conventional' techniques are applied and assessed as rigorously as their alternatives. There is no consensus about the relative benefits of 'conventional' versus alternative methods for assessing response of bone marrow metastases to treatment. How far have we come? In routine clinical practice, not very far. We must relearn how to crawl efficiently before deciding that walking, let alone running, will get us safely to our destination.

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